PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM AND WORKSHOP

ON

NATIE SEEDS IN RESTORATION OF DRYLAND ECOSYSTEMS

NOVEMBER 20 – 23, 2017

STATE OF KUWAIT

Organized by

Kuwait Institute for Scientific Research (KISR)

International Network for Seed-based Restoration (INSR)

Sponsored by

Kuwait National Focal Point (KNFP)

Kuwait Foundation for the Advancement of Sciences (KFAS)

Islamic Development Bank (IDB)

PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM AND WORKSHOP

ON

NATIE SEEDS IN RESTORATION OF DRYLAND ECOSYSTEMS

NOVEMBER 20 – 23, 2017

KISR

معهد الكويت للأبحاث العلمية

KUWAIT INSTITUTE FOR SCIENTIFIC RESEARCH

2017

Disclaimer

The contents provided in individual papers are the opinion of the author (s) and Editors and KISR hold no responsibility for views expressed.

Submissions have been included in the e-version of the proceedings with only minor editing. However, these submissions will be subjected to in-depth technical review/ editing prior to their inclusion in the printed version of symposium proceedings.

Table of Contents

Preface

Kuwait's Native flora comprising 256 annual, 83 herbaceous perennial, 34 shrubs and one tree species is of significant scientific and economic value as it contains genes that impart resistance to major abiotic stresses such as drought, heat and salinity and contain several bioactive molecules with great potential for medicinal and agricultural applications. These plants are distributed in six terrestrial ecosystems, namely, coastal plain and lowland, desert plain and lowland, alluvial fan, escarpment, ridge and hilly, wadi and depression, and burchan sand dune. Each of these ecosystems hosts a dominant plant species with several associated species. Natural events (prolonged droughts, strong winds, dust storms and unexpected weather events) and human activities (overgrazing, off-road traffic movement, gravel quarrying, irrigated agriculture development, etc.) have resulted in deterioration of vegetation cover in Kuwait. The Iraqi invasion of Kuwait and subsequent military activities have fast-tracked the land degradation process and vegetation loss, disrupted natural plant succession process, caused widespread contamination of soil with chemical pollutants, and substantial changes in physical and chemical soil properties. For several decades, the Kuwait Institute for Scientific Research (KISR) is actively engaged in the characterization of terrestrial ecosystems, propagation of native plants, and restoration of drylands ecosystems impacted by many of the above causes. Additionally, KISR has successfully restored the *Rhanterium epapposum* community at its study site in the southwest of Kuwait (Kabd). Findings of these studies are being used in executing large-scale remediation and restoration projects, known as the Kuwait Environment Remediation Program (KERP) covering almost 1670 km^2. This program relies heavily on ecological restoration of damaged areas as this approach using native seeds and plants.

Recently, the State of Kuwait has developed the Kuwait Vision for 2035 which relies on achieving balanced economic and human development based on conservation and sustainable utilization of natural resources. Acknowledging the alarming rate of degradation of terrestrial ecosystems and its eminent potential for extinction of several of Kuwait's unique flora and fauna, the Kuwait Vision for 2035 places significant emphasis on the development of suitable management strategies for conservation and sustainable utilization of native biodiversity. In this regard, KISR in cooperation with the Ministry of Planning has initiated a government initiative project to develop techniques and establish facilities for mass production of seeds and desert plants.

Ecological Restoration of damaged ecosystems has become truly multi-disciplinary international effort and its success requires an integrated approach. While restoration and management of ecosystems face several challenges, it also presents opportunities for cooperation in research, and implementation of large-scale restoration and community outreach programs. This also requires appropriate policies and their timely enforcement.

Most countries in the region are also faced with similar challenges in conserving and managing native biodiversity. Because restoration projects are long-term, highly resource intensive activities, exchange of ideas and sharing of experiences among experts, restoration practitioners, and policy makers will undoubtedly be of great value in ensuring success of such projects. In view of these facts, KISR in cooperation with the International Network for Seed Based Restoration (INSR) of the Society for Ecological Restoration (SER) jointly organized the "International Symposium and Workshop on Native Seed in Restoration of Dryland Ecosystems" in Kuwait from November 20 - 23, 2017. The symposium was a great success because of the encouragement and financial support from our sponsors (Kuwait National Focal Point, Kuwait Foundation for the Advancement of Science and Islamic Development Bank) and wholehearted cooperation from participants.

We are delighted to receive overwhelming response from experts from both from the Arab region and other parts of the globe. Leading restoration experts from Australia, Europe, USA, India, Gulf Cooperation Council countries and other Arab countries presented their research findings and shared their experiences on various topics related to native plant conservation, selection of native plants for different applications, see farming, seed storage, seed quality testing, seed-based restoration and impact of climate change on terrestrial ecosystems. Some of the papers presented in the symposium were ecological restoration case studies from different parts of the world. Based on the deliberations in various technical session, a Panel of experts developed a roadmap and a comprehensive action plan, which will serve a guidelines for future ecological restoration projects.

It is our sincere hope that the proceedings will serve as a valuable source of information for all those concerned with conservation and sustainable utilization of native plants for the benefit of the present and future generations.

Again, we would like to thank our sponsors, participants and all those who contributed for the success of this international event.

Kuwait

November 2017 **Editors**

Symposium Committees

I. International Advisory Committee

Dr. Samira A. S. Omar	KISR	Chairperson
Prof. Kingsley W. Dixon	Society for Ecological Restoration – INSR	Co-chairperson
Dr. Abdul Nabi Al-Ghdaban	KISR	Member
Dr. Afaf Al-Nasser	KISR	Member
Prof. Faisal K. Taha	KISR	Member
Dr. Narayana R. Bhat	KISR	Rapporteur

II. Organizing Committee

Prof. Faisal K. Taha	KISR	Chairperson
Dr. Mohammed Albaho	KISR	Co-chairperson
Dr. Tareq A. Madouh	KISR	Member
Dr. Ali M. Quoreshi	KISR	Member
Dr. Fadila Al-Salameen	KISR	Member
Ms. Sherifa Al-Falah	KISR	Member
Ms. Sanabel Al-Musallam	KISR	Member
Ms. Shaurouq Al-Enezi	KISR	Member
Mr. Humoud Al-Khater	KISR	Member
Ms. Noura H. Hammad	KISR	Member
Ms. Fatima Abdulmalek	KISR	Member
Mr. Badar Al-Shuwail	KISR	Member
Ms. Muneera Al-Khaled	KISR	Member
Ms. Laila Al-Mulla	KISR	Rapporteur
Ms. Wafaa Al-Saleh	KISR	Member

III. Scientific Committee

Dr. Narayana R. Bhat	KISR	Chairperson
Dr. Majda Khalil	KISR	Co-chairperson
Dr. Matra Al-Mutairi	KISR	Member
Dr. Anisul Islam	KISR	Member
Dr. Sudarshan Chellan	KISR	Member
Dr. Ashok Alva	KISR	Member
Mr. Thomas M. Thomas	KISR	Member
Mr. Binson M. Thomas	KISR	Member
Ms. Ouhoud Al-Ragam	KISR	Rapporteur
Ms. Rose J. Ferrao	KISR	Member

SECTION 1

Native Plant Conservation and Selection

Assessing the *Ex-situ* Seed Bank Collections in Conservation of Endangered Native Plants in Kuwait

Tareq A. Madouh

Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, P. O. Box 24885 Safat 13109, Kuwait
e-mail: tmadouh@kisr.edu.kw

Abstract

The Kuwait's desert flora represents a unique diversity of species and habitat types, and serves as precious genetic resources for both restoration of damaged ecosystems and crop improvement research. *Ex-situ* conservation of seeds in seed banks has become increasingly attractive worldwide due to a number of factors that are causing destruction of native biodiversity. The Global Strategy for Plant Conservation (GSPC) suggested that countries should preserve at least 75% of the total number of threatened species in *ex-situ*. Similarly, they suggested to make available at least 20% of the threatened species for recovery plans and restoration programs. In Kuwait, however, the native biodiversity is gradually becoming extinct due to various natural and manmade causes, like drought, salinity, overgrazing and other destructive human activities. These factors resulted in severe land degradation and habitat destruction leading to disruption in *ecosystem balance*. Seed banks are a vital component of an integrated plant conservation strategy, mainly due to their relatively low cost, as well as massive storage capacity for genetic resources. The use of native plant species in ecological restoration has gradually become very common in Kuwait and other GCC countries and is expected to further increase for controlling progression of desertification, climate change, habitat degradation and decline in natural populations. Future research should focus on plant production systems for native plant species to maximize genetic diversity. This will allow introduced seeds and plant materials to have the sufficient flexibility to survive under severe environmental stresses. Therefore, assessing the current status of Kuwait - KISR *ex-situ* conservation seed bank is necessary to identify gaps and assess the baseline situation.

Keywords: Seed storage, ecological restoration, Global Strategy for Plant Conservation (GSPC), biodiversity conservation, habitat destruction.

Introduction

Native seed collection and seed banking schemes are undoubtedly the starting point for meeting regional and national restoration objectives, providing the genetic resources and consultancy that may determine the contribution of restored plant communities to its functional ecosystem (Espeland et al., 2017). Seed banks are valuable for preservation and conservation of genetic diversity of native flora endangered by ecosystem and land use changes, overgrazing, invasive exotic species, pollution and climate change (Millennium Ecosystem Assessment, 2005, cited by León-Lobos et al., 2012). While these causes of biodiversity loss are increasingly intensifying globally and specifically in Kuwait, seed banking is a necessary and cost-effective complement to *in-situ* conservation of native desert plants, and it provides a vital source of seed material to assist in ecological restoration of damaged and degraded habitats (Maunder et al., 2004, cited by León-Lobos et al., 2012) due to their moderately low cost and significant long-term storage capacity for genetic resources and *ex-situ* plant conservation (León-Lobos et al., 2012).

The Kuwait's desert flora is imperiled to gradual extinction from various destructive activities of both natural and unnatural causes. Drought, overgrazing and other human activities are the main causes of land degradation which has led to habitat disturbance and a disruption in the balance of the ecosystem. The use of native plant species in ecological restoration has become gradually predominant in the Kuwait and the GCC countries and expected to further increase; to control the progression of desertification, climate change, habitat degradation and species population decline.

Restoration of functional habitats ecosystems as well as other potential projects dealing with the native flora relies heavily on the use of native plant materials and seeds. Additionally, there is growing recognition that the success of restoration plantings faced with climate change depends significantly on the inherent adaptive genetic variability in the source material (Davis and Shaw, 2001; Jump and Peñuelas, 2005). Latterly, the state of Kuwait has established the Kuwait vision for 2035 which emphasis on attaining balanced economic and human development based on conservation and sustainable utilization of natural resources. Although, the terrestrial ecosystem degradation and the unbalanced ecological function at its uttermost, the Kuwait vision for 2035 placed significant importance on improvement of appropriate management strategies for conservation and sustainable of biodiversity. Deploying well-adapted and ecologically suitable plant materials and seeds are the main core component of successful restoration projects (Bower et al., 2014). Furthermore, limited availability of native seeds and genetically appropriate material may results in a high probability of species extinction and gene pool loss. In this regard, the seed bank at KISR with its valuable collections and the regional leader in this kind of *ex-situ* conservation of native flora, requires further development and enrichment of seeds and genetic resources with priority given to collections of rare species and common native plants species that are most threatened and potentially most suitable for research projects, gene bank preservation, revegetation and restoration programs.

The Global Strategy for Plant Conservation (GSPC) suggested that countries should preserve a minimum of 75% of the total number of threatened species in *ex-situ*. Likewise, they suggested to make available at least 20% of the endangered species for reclamation schemes and restoration programs (Ferrando-Pardo et al., 2016). The forthcoming investigation of the seed bank of Kuwait should emphasis on plant production systems for native plant species to enhance genetic diversity. This will allow introduced seeds and plant materials to have the sufficient flexibility to survive under severe environmental stresses. Therefore, assessing the current status of Kuwait - KISR *ex-situ* conservation seed bank is necessary to identify shortage and assess the baseline condition.

Materials and Methods

A number of experiments were conducted on several native plant species to assess the germination and moisture content percentage of a single year accession i.e. 2014 collection and the difference in seed storage time (first and second stored seed within 6 months and first and second stored seeds more than 6 months). Seeds were collected at ripen and maturity stage for all these selected species i.e. *Cleome ambylocarpa, Gynandriris sisyrinchium, Reseda arabica, Horwoodia dicksoniae, Rumex vesicarius, Savignya parviflora* and *Ephedra alata*. Only undamaged, (free of insect injury), and mature seeds were used. The seeds were germinated in a 9 cm Petri dish; a double layer of Whatman No. 3 filter paper disks. A random sample of seeds from each species was obtained. A count of 75 seeds were selected for each species test and divided into three replicates. Seeds were then placed in petri dishes and approximately 5 ml distilled water was added to moisten the seeds. The petri dishes were then placed in a germination cabinet with 12 h of fluorescent light (200 µE m^2 s^{-1}) and an optimum constant temperature of 25 °C. The dishes were arranged in a completely randomized design with three replicates of 25 seeds in each dish. Germination counts were taken every 72 hours for 42 days. Seeds with a radical extension equal to or greater than 2 mm were considered as germinated, counted and then discarded. Seeds that developed fungal growth were removed from the dishes and considered dead. Percentage germination was calculated based on the total number of germinated seeds at each time interval (calculated as $\chi/25*100$).

The moisture content was determined using the gravimetric oven method. Two replicates of about 2 to 5 g of seeds were used for determination of moisture content. The quantity of seeds used for the procedure depended mainly on the availability of the seeds. Glass petri dishes were weighed before and after placing seeds sample. The dishes were then placed in the oven uncovered at 130 °C to 133 °C. An hour later, the samples were covered and placed in a desiccator to cool for 30 to 45 minutes. The moisture content of all samples were calculated using the following formula:

$$\text{Percentage of moisture content} = M_2 - M_3 * \frac{100}{M_2 - M_1} \qquad \text{……………………………….. (1)}$$

Where, M_1 is the weight in grams of the container and its cover; M_2 is the weight in grams of the container, its cover, and its contents before drying; and M_3 is the weight in grams of the container, cover, and contents after drying.

Results and Discussion

The recommended standard procedures for seeds process before storing and seed banking are generally drying, cleaning, seed health, moisture content and germination testing are extensively identified (Genebank Standards, 1994; FAO, 2013 cited in Hay and Probert, 2013). These organizations further suggested that seed banks directive to germplasm conservation are expected to implement the international standards for gene banks. Seed banks with limited resources to evaluate whether they positively are optimum for maintaining and managing collections may experience difficulties in the future (Hay and Probert, 2013). This article, however, aims to assess the current status of Kuwait - KISR *ex-situ* conservation seed bank in term of the effects of the collection date and storage times on the germination percentage and moisture content percentage to identify and determine standard procedure of desert native plants to enhance storage longevity.

The moisture content percentage is determined in the initial step, few days after seed collection. If the sample moisture percentage is high, the freshly harvested sample needs to be dried outside or in a temperature controlled drier with forced air circulation. Long term seed viability of conventional seed is related to seed moisture content and temperature. Orthodox seeds survive low moisture from (3 to 7%) moisture content and tolerate subsequent rehydration without significant loss of viability, and as moisture content and temperature decreases their longevity increased (Roberts, 1973). The generally accepted rule is that seed longevity increases significantly with every 1% reduction in moisture content and storage temperature are reduced (Ellis and Roberts, 1980). For various taxa, lowering the moisture content up to 7-10% will have much greater impact on longevity than storing at very low temperatures. If seed is to be stored at very low temperature, as planned for KISR Seed Bank, the seed must be dehydrated to at least 5% moisture or there is a risk of damage to the seed.

Differences in moisture content percentage were observed between all native plant species. Results showed in figures 1 & 2 a significant effect of time of stored seeds on moisture content in each of the seven species after collection. The differences between first and second stored seeds within and more than 6 months were significantly reduced for *Gynandriris sisyrinchium*, *Reseda arabica*, *Savignya parviflora* and *Ephedra alata*. Whereas for *Cleome ambylocarpa*, *Horwoodia dicksoniae*, and *Rumex vesicarius* had increased. This increased moisture content could be due to the shortage of time limit between stored dates (data not shown). With the exception of *Horwoodia dicksoniae* (Fig. 1), all other species maintained a relatively higher moisture percentage above 7% at both stored time i.e. within and more than 6 months. Highest percentage moisture recorded at 9.7, 9.2 and 8.8% for *Ephedra alata*, *Savignya parviflora* and *Gynandriris sisyrinchium*, respectively (Figs. 1 & 2).

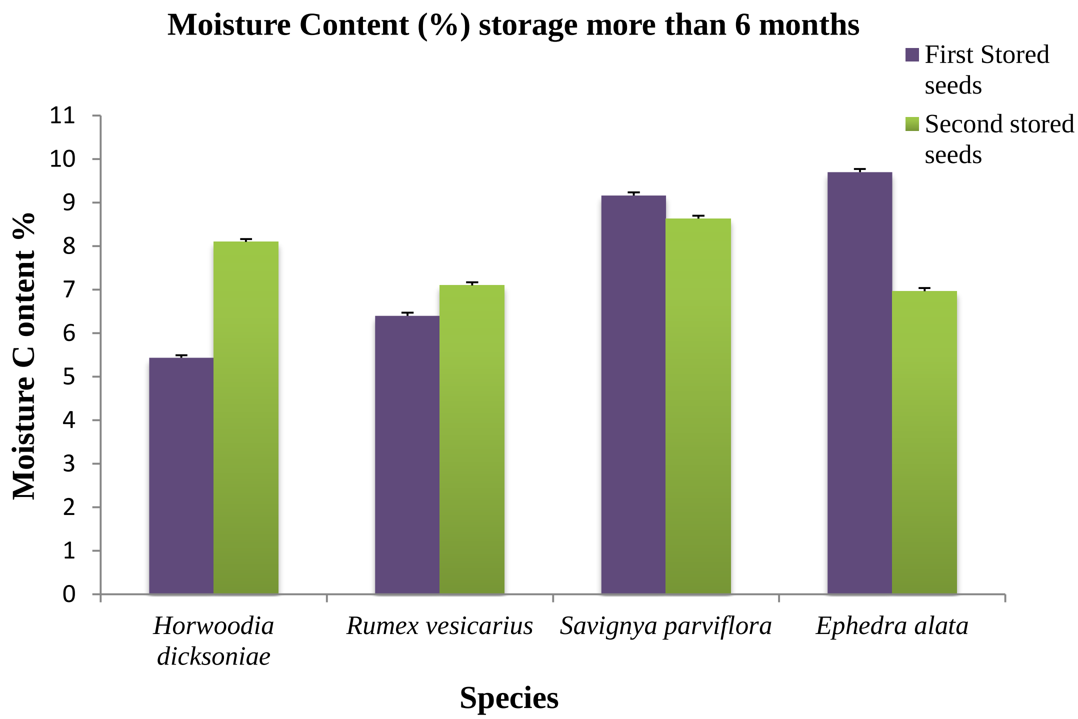


Figure 1. Moisture Content (%) storage more than 6 months. Treatments, First and second stored interval levels are significant at P<0.001. d.f.=5.

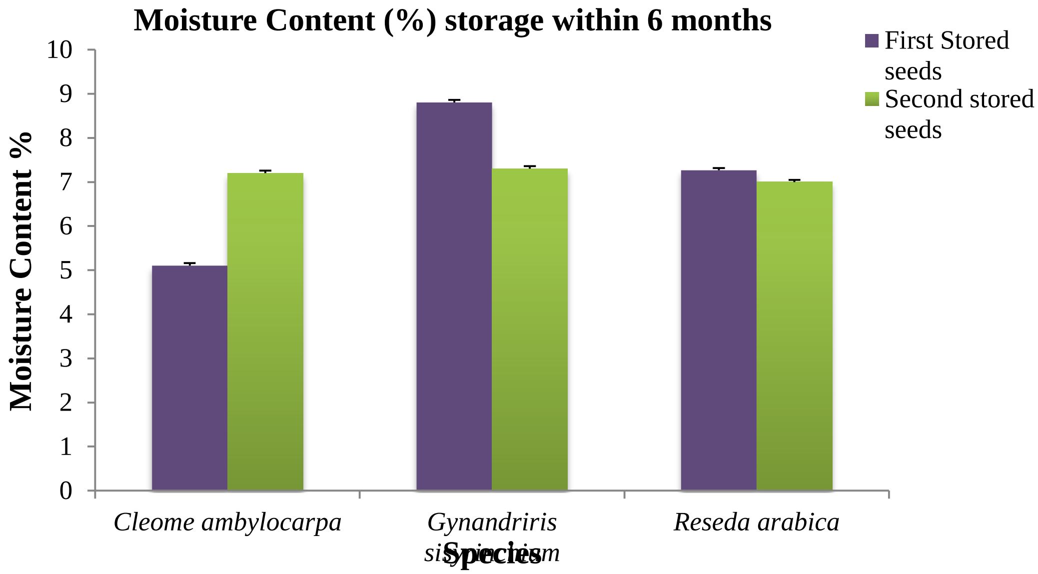


Figure 2. Moisture Content (%) storage within 6 months. Treatments, First and second stored interval levels are significant at P<0.001. d.f.=5.

Drying harvest seed samples outside in the shade is recommended to insure much drier samples and more suitable. The outside relative humidity and temperature is more favorable to significantly lowering the moisture content of the seeds. This higher moisture content observed in 4 of the 7 species during the second period suggested that might be due to the relative humidity in the air-conditioned laboratory could be as much as 25% with a temperature of only 18-20ºC. This type of humid environments may significantly slow the drying process and increase the moisture content of the seeds particularly for those seed species dried under laboratory conditions. This further suggests that desert native seeds should be dried outside in conditions similar to their natural environment to achieve the recommended percentage for conservation between (3 to 7%) and to promote longevity at –20ºC for long term conservation.

Delay in stored time had a significant effect on germination percentage in each of the seven species (Figs. 3 & 4). As the delay in storing seed period increased, percentage germination was substantially reduced for several species. At the storing time of more than 6 months, a significant (P<0.05) reduction in germination percentage was observed in three out of four species. However, only *Horwoodia dicksoniae* maintained germination percentage of 50% in both tested times while *Rumex vesicarius*, *Ephedra alata* and *Savignya parviflora* had the lowest germination with a decreased percentage of 14, 19, 3%, respectively (Fig. 3). In contrast, the stored date within the 6 months results showed no significant differences between times for *Cleome ambylocarpa, Gynandriris sisyrinchium* and *Reseda arabica* (Fig. 4). The different seed stored timing greatly affected species with more than 6 month period but not significantly so within the 6 months period.

Wood *et al.*, 2000, suggested that the procedure for the germination test must overcome any dormancy in many seeds and any physical effects that could have risen during drying or storage and have an adverse impact on germination (Hay and Probert, 2013). The decline in germination during the second period for the various seed species tested may be due to the improper hydration at laboratory conditions (Figs. 3 & 4).

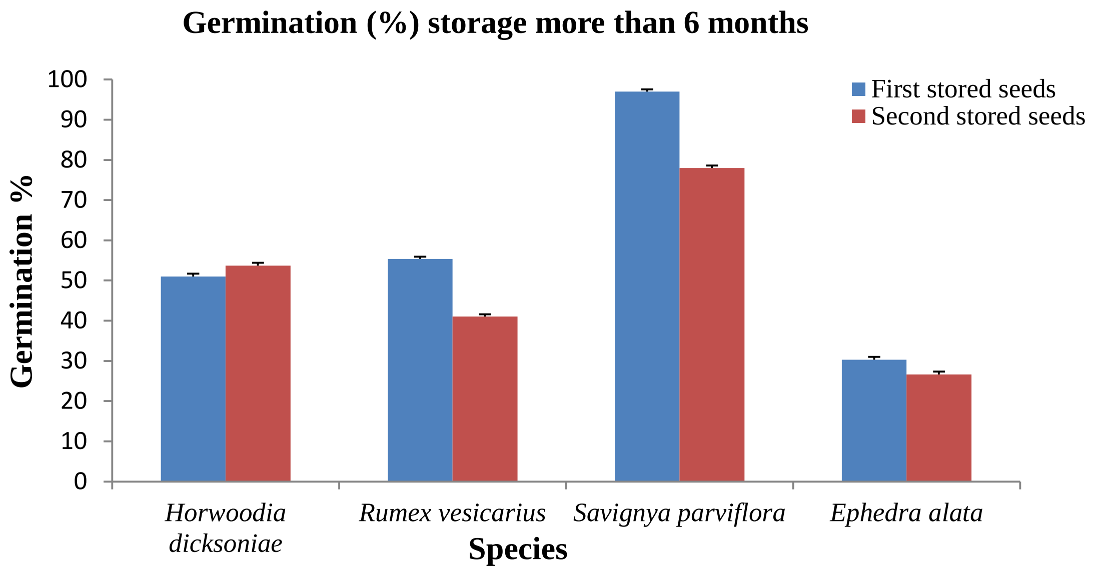


Figure 3. Germination (%) storage more than 6 months. Treatments, First and second stored interval levels are significant at P<0.001. d.f.=5.

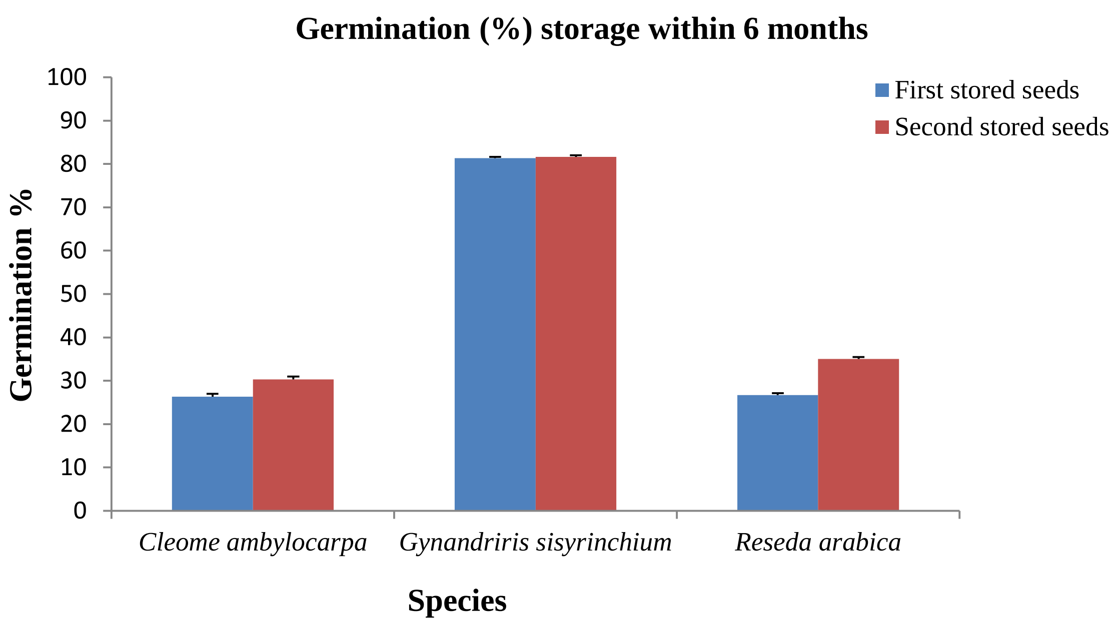


Figure 4. Germination (%) storage within 6 months. Treatments, First and second stored interval levels are significant at P<0.001. d.f.=5.

Another possible explanation in the drop and inconsistent germination among species maybe due to that these desert native species have certain physiological causes of germination inhibitors which are frequently found in many of desert plants. The lower germination percentage may be poor among the species investigated for a number of causes and thus the association of germination percentage and moisture percentage is very low and inconsistent.

Conclusions

Several seed bank investigator and researchers (Manger et al., 2003; Terry et al., 2003), have recommended and emphasized the importance of developing accurate procedures that are effective and practical for seed banks that are routinely storing seeds of wild species and the research necessity to those areas where information is insufficient (Ali et al., 2007; Pérez-García et al., 2009; Probert et al., 2009; Smith et al., 2011).

Thus the use of dehydrating at closed environments or laboratory conditions may not be recommended for native desert species to estimate high quality germination and the required moisture content percentage for longevity and long term conservation. Every effort should be made to dehydrate those native seeds in an environment similar to the original collection site. This should ensure lowering the moisture content to a level that may trigger higher germination rate. This may be very critical for the extreme desert taxa. Further research and investigation is needed to standardize the conservation procedures for arid desert species.

Acknowledgements

The authors wish to acknowledge Kuwait Institute for Scientific Research (KISR) for providing the facilities and importance for preserving and conservation of natural resources and native biodiversity. Also, special thanks for Kuwait Foundation for the Advancement of Science (KFAS) for their generosity for funding projects that are associated with protecting improving the environments.

References

Ali, N.; R. Probert; F. Hay; H. Davies; and W. Stuppy. 2007. Post-dispersal embryo growth and acquisition of desiccation tolerance in *Anemone nemorosa* L. seeds. *Seed Science Research* **17:** 155-163.

Bower, A. D.; J. B. St.Clair; and V. Erickson. 2014. Generalized provisional seed zones for native plants. *Ecological Applications* **24(5):** 913-919.

Davis, M. B. and Shaw, R. G. 2001. Range Shifts and Adaptive Responses to Quaternary Climate Change. *Science* **292:** 673-679.

Ellis, R.H. and E. H. Roberts. 1980. Improved equations for the prediction of seed longevity. *Annals of Botany* **45**: 13-30.

Espeland, E. K.; N. C. Emery; K. Mercer; S. A. Woolbright; K. Kettenring; P. Gepts; and J. R. Etterson. 2017. Evolution of plant materials for ecological restoration: insights from the applied and basic literature. *Journal of Applied Ecology* **54:** 102-115.

FAO . 2013. Draft genebank standards for plant genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations, Rome.

Ferrando-Pardo, I.; P. Ferrer-Gallego; and E. Laguna-Lumbreras. 2016. Assessing the conservation value of *ex situ* seed bank collections of endangered wild plants. *Israel Journal of Plant Sciences* **63(4):** 333-346.

Genebank Standards. 1994. Food and Agriculture Organization of the United Nations, Rome, International Plant Genetic Resources Institute, Rome.

Hay, F. R. and R. J. Probert. 2013. Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology* **1**: 1-11.

Jump, A.S. and Peñuelas, J. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. *Ecology Letters* **8:** 1010-1020.

León-Lobos, P.; W. Michael, P. D. Aranda; and M. Lima-Junior. 2012. The role of ex situ seed banks in the conservation of plant diversity and in ecological restoration in Latin America. *Plant Ecology and Diversity* **5:** 245-258.

Manger, K. R.; J. Adams; and R. J. Probert. 2003. Selecting seed containers for the Millennium Seed Bank project: a technical review and survey. In Smith, R. D.; J. B. Dickie; S. Linington; H. W. Pritchard; and R. J. Probert (Eds.). Seed Conservation – Turning Science into Practice. Royal Botanic Gardens Kew, Kew, p 637-652.

Maunder, M.; E. O. Guerrant; K. Havens and K. W. Dixon. 2004. Realizing the full potential of *ex situ* contributions to global plant conservation. In Guerrant, E. O.; K. Havens; and M. Maunder (Eds.). *Ex Situ* Plant Conservation: Supporting Species Survival in the Wild. Island Press, Washington (DC), p. 389-418.

Millennium Ecosystem Assessment. 2005. Ecosystems and human well-being: synthesis. World Health Organization. Washington, DC: Island Press.

Pérez-García, F.; C. Gómez-Campo; and R. H. Ellis. 2009. Successful long-term ultra dry storage of seed of 15 species of Brassicaceae in a genebank: variation in ability to germinate over 40 years and dormancy. *Seed Science and Technology* **37:** 640-649.

Probert, R. J.; M. I. Daws; and F. R. Hay. 2009. Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Annals of Botany* **104**: 57- 69.

Roberts, E. H. 1973. Predicting the storage life of seeds. Seed Science and Technology, 1: 499-514.

Smith, P.; J. Dickie; S. Linington; R. Probert; and M. Way. 2011. Making the case for plant diversity. *Seed Science Research* **21:** 1-4.

Terry, J.; R. J. Probert; and S. Linington. 2003. Processing and maintenance of the Millennium Seed Bank collections. In Smith, R. D.; J. B. Dickie; S. Linington; H. W. Pritchard; and R. J. Probert (Eds.). Seed Conservation – Turning Science into Practice. Royal Botanic Gardens Kew, Kew, p 307-325.

Wood, C. B.; H. W. Pritchard; and D. Amritphale. 2000. Desiccation-induced dormancy in papaya (*Carica papaya* L.) is alleviated by heat shock. *Seed Science Research* **10:** 135-145.

Steps Towards *In-situ* Conservation for Threatened Plants in Egypt

Karim Abdelhai Omar
Freelance environmental Consultant, IUCN Red List Global Assessor, Egypt
e-mail: kariemomar@gmail.com

Abstract

Conservation of globally endangered plant resources is a critical ecological, cultural, and economic issue. Considerable growing attention has been given in the recent years to ecological and economical *in situ* conservation for sustainable use of wild rare plants. In order to develop an efficient and effective conservation strategy using complementary *in situ* and *ex situ* techniques, we must have a clear understanding of geographical distribution of target species, its habitat preferences and requirements, its population characteristics, threats, and taxonomy. The details of the localities where past collection have been made, the so-called passport data, associated with herbarium and germplasm collections are a key source of information to guide future conservation activities. Good nature-conservation management requires a basic understanding of ecological science at all levels, especially focusing on the landscape ecological aspects. It can be difficult to determine which areas to restore, what species and/or vegetation communities to target in restoration programs, and what threatening processes need to be mitigated. Focusing on the community level can help fill the gap between species and ecosystem approaches to plant conservation. Plant communities are in fact, basic components of the landscape, and their extent and arrangement have consequences both to species survival and ecosystem processes. Decision making is an element, inherent in all stages and areas of conservation, which is directly related to the cost-efficiency of the process. The decision to be made varies; sometimes, it is a question of whether or not a species should be moved to the endangered list; in other cases, it may be how to best allocate resources; or it can be deciding whether or not a remedial action is necessary after landscape degradation.

Keywords: Endangered plants, nature preservation, ex *situ* conservation, ecosystem processes.

Introduction

Conservation of globally endangered plant resources is a critical ecological, cultural and economic issue. Considerable and growing attention has been given in the recent years to issues surrounding the in-situ conservation, and ecologically and economically – based sustainable use of populations of wild rare plants. Most conservation focus has been given to individual internationally and regionally economically significant over-exploited endangered medicinal plant species.

In order to develop an efficient and effective conservation strategy using complementary in situ and ex situ techniques, we must have a clear understanding of *target species* geographical distribution, its habitat preferences and requirements, its population characteristics, threats and taxonomy. The details of the localities where Plant collection have been made, the so-called Passport data, associated with herbarium and germplasm collections, are a key source of information to guide future conservation activities. The ecological, geographic, genetic, reproductive biology and taxonomic data are collectively referred to as eco-geographic data and their analysis is a necessary prerequisite for efficient conservation. Good nature-conservation management requires a basic understanding of ecological science at all levels, especially focusing on the landscape ecological aspects. Species and community ecology can easily be dealt with at a local level. If, however, an attempt is made to develop an understanding of ecological infrastructure, the ability to document information and develop models over large areas is vital. This is where geographical information systems (GIS) and their power and potential come into play (Bridgewater, 1993).

It can be difficult to determine which areas to restore, what species and/or vegetation communities to target in restoration programs, and what threatening processes need to be mitigated. According to De la Cruz- Rot (2001), focusing on the community level can help fill the gap between species and ecosystem approaches to plant conservation. Plant communities are in fact basic components of the landscape and their extent and arrangement has consequences both for species survival and for ecosystem processes[1].

Extinction and declines in plant diversity are due to a range of factors, including population growth, high rates of habitat modification and deforestation, over-exploitation, the spread of invasive alien species, pollution and climate change. The Millennium Ecosystem Assessment noted that approximately 60% of the ecosystem services evaluated are being degraded or used unsustainably (www.milleniumassessment.org). The degradation of ecosystem services often causes significant harm to human well-being and represents a loss of a natural asset or wealth of a country.

The threat of climate change include the direct impacts on habitat, ecosystem functioning and populations of higher concentrations of carbon dioxide; altered rainfall and temperature patterns; and more frequent extreme storms, floods and heat waves. Many species are highly sensitive to changes in climate and weather-related patterns and events. These can disrupt seasonal food supplies and other resources, life cycle events, development, mortality, breeding and fertility, such that entire reproductive strategies become less successful. Expected direct impacts on species populations include: changes in species abundance, changes in distribution, and changes in genetics over the long term as species adapt (Commonwealth of Australia 2009). The ability of species to adapt to changing conditions and recover after extreme climatic events will be compromised by the legacy of fragmentation, habitat loss and other pressures that have collectively reduced overall diversity, population sizes, and resilience in many species.

A focus on species conservation is readily comprehensible, since most people find it easy to empathize with biodiversity inherent in species, especially if they are charismatic or flagship species. Moreover, such a focus may well serve the interests both of conservation and of those who exploit species (Hutton and Leader- Williams 2003). The question that has to be addressed is whether a species-based approach to *in situ* conservation is feasible or even desirable. It is often stated that such an approach to conservation is not possible because of the sheer numbers of entities involved and the continuing rise in the numbers of threatened species (Ricklefs *et al*. 1984), whereas a habitat/ecosystem-based approach allows a large number of species to be given some form of protection at the same time. There is, moreover, an increasing tendency today to shift the focus away from species and to view biodiversity conservation and sustainable use through the lens of the ecosystem, with an emphasis on maintaining the healthy functioning of the system.

The number of wild plant species requiring specific conservation efforts is far too numerous to include all of them in conservation programmes (Sutherland 2001). Even within the main groups of target species of economic importance, the number of species to consider is greatly in excess of any reasonable expectation of conservation possibilities. If a conservation strategy depends, as it often will, on the results of ecogeographical surveys and analyses of genetic and biological variation, all of which require considerable investments of time, money and expertise, not to mention any management interventions and monitoring, then effective action will not be possible for most of the species identified.

In this paper we will present a case study about establishment and implement a Long term conservation program for threatened plant species in St. Catherine Protected Area (SCPA) through Community-based management approach depend on research, building capacities and participation. This study depended on raw data extracted field work that have been done in 2015 (supported by IUCN Med) to identify the geographical range and the current ecological status for 10 restricted plants within the borders of SCPA in order to determine the effect of environmental factors on the distribution and determine the current status of the protection and its requirements in accordance with the standards of the Red List of the International Union for Conservation of Nature (IUCN) to be a first step towards complete conservation program by restoration in the future.

Materials and Methods

Study Area: St Catherine was declared as a Natural Protected Area in 1988 under Law 102/1983 and also established an Executive Council, headed by the Governor of South Sinai, to manage the Protectorate. Full protected-area status was given to approximately 4,350km² of largely mountainous terrain in South Sinai, Egypt. The area includes the highest peaks in Egypt and contains a unique assemblage of natural resources, notably high altitude ecosystems with surprisingly diverse fauna and flora and with a significant proportion of endemic species.

Target Species: A total of ten species were chosen for this study based on their distribution range, availability of historical data, accessibility, economic importance, the current status and threats degree. Five of them are endemic (*Anarrhinum pubescens* Fresen, *Bufonia multiceps* Decne, *Euphorbia obovata* Decne. *Phlomis aurea* Decne, and *Rosa arabica* Crép.), and the others are near endemic (*Polygala sinaica* Botsch., *Nepeta septemcrenata* Ehrenb. ex Benth., *Salvia multicaulis* Vahl., *Hypericum sinaicum* Hochst. ex Boiss., *Origanum syriacum* L.).

Conservation status assessments were done for those species based on IUCN Red List Categories and Criteria. For each species, geographical range, population characteristics, habitat and ecology, threats, conservation actions were determined and presented in the form of Maps, Tables and charts to facilitate the decision making to the maximum. The needed data for this assessment were collected from 2015 field work, reports, published articles in the same area of the study.

To fit to the IUCN Red List Assessment requirements we need to study and discuss the Geographic Range, Population Characteristics, Habitat and Ecology, Threats, Uses and trade, and conservation actions for the target species.

IUCN Red List Assessment

1- Geographic Range

To determine the Geographic Range of these species we collected sufficient data about the following:

- Distribution of *target species* within the target PA during the field survey was record. A GPS fix was recorded in decimal degrees and datum WGS84 using Garmin 12 XL receiver. The fix was recorded to the fifth decimal digit. Arc View GIS 10.3 was used to plot the study sites.
- Number of locations where the target species occurs, Extent of Occurrence (EOO), Area of Occupancy (AOO), and its decline trend were recorded and measured according to IUCN guidelines, 2014. For more clarification,
- Extent of Occurrence measured by drawing a polygon PAs through the distribution points from outside. GIS then determined the area of this polygon in km^2.
- Area of Occupancy also measured though GIS; the distribution map was converted to grids each one cover 2 km^2, each occupied cell was then extracted and the total size were collected and presented in the form of km^2.

2- Population Characteristics

To understand the population characteristics of these species we collected sufficient data about the following:

- Number of *species* populations and subpopulations, number of total individuals were recorded within field visits, number of mature individuals, population structure and dynamics were determined according to IUCN (2014). Population trend, fluctuations, fragmentation, and decline trend were recorded and measured according to IUCN guidelines (2014) using historical data about population size, number of individuals, occurrences from former studies.

3- Habitats and Ecology

To determine the Habitats and Ecology of these species we collect sufficient data about the following:

- Preferable habitat and microhabitat of the target species and its decline trend within the field survey, according to IUCN Habitats Classification Scheme were recorded (IUCN 2014).
- Life form and species correlation were recorded according to field observation.
- Climatic features (Max. Temp., Min. Temp., and Perception) were extracted from BIOCLIM data using DIVA-GIS.
- Soil properties (Physical and chemical) were extracted from several studies held in the area.
- Vegetation characteristics of target species like density, cover, and associated species within each site were recorded.
- Plant species in each given quadrant has been tentatively recorded in the field and put in tabulated form, giving the authentication of their identification with the help of the local floristic workers (Boulos, 1999; 2000; 2002 and 2003).

4- Threats

Using the IUCN threats classification scheme, version 3.1 and based on fieldwork observation and previous work, we used a systematic sampling approach to capture local environmental gradients, placing more than 200 circles with 25 and 50 m diameters at equal distances apart to cover most area of targets which containing the hottest spots for vegetation inside target PA. Within each circle, we record any sign that may be a threat to the plant community.

5- Conservation actions & requirements:

- Timing, scope, severity, and impact score for each threat were determined according to IUCN Threats Classification Scheme (IUCN, 2014).
- In this part, we will collect any information about past, ongoing, and future activities to protect *target species* in-place or outside-place. Conservation actions that will take place on land or that needed in the near future will also recorded. Researches needed according to IUCN Scheme were recommending (IUCN 2014).

Results and Discussion

Geographical Range. On Global scale the five target plant species (*Bufonia multiceps, Euphorbia obovate, Phlomis aurea, Rosa arabica, and Anarrhinum pubescens*) are endemic to the St. Catherine Protected Area (SCPA) in southern Sinai, Egypt, recorded to have a narrow altitudinal range between 900 and 2,630 m asl (1730 m range – twice the height of the Burj Khalifa), and presents about 20% of the total global attitudinal range (0-8848 m asl). The same altitudinal range was also recorded for the five near endemics but only on national scale (information about global scale are inadequate). The highest altitudinal range was recorded to *Anarrhinum pubescens* (1400 m) and lowest was to *Rosa arabica (650 m) (Table 1).*

Regarding to the Extent of Occurrence (EOO), in case of endemics (Gobal Scale), their ranged from 40 km^2 (*Rosa arabica*) to 339 km^2 (*Phlomis aurea*), and their Area of Occupancy (AOO) ranged from 36 km^2 (*Rosa arabica*) to 136 km^2 (*Phlomis aurea*). In case of near endemics (National scale), the EOO ranged from 17 km^2 (*Salvia multicaulis*) to 307 km^2 (*Polygala sinaica*), and their AOO ranged from 24 km^2 (*Salvia multicaulis*) to 108 km^2 (*Nepeta septemcrenata, Origanum syriacum*). In total, the distribution of all species cover about 687 km^2 (EOO) and AOO about 184 km^2 (Figs. 1 and 2).

These target species are clearly distributed in one to two locations (High Mountains Area and Serbal Mountain Area). Farsh Elromana is the only site that own the full 10 species, eight species from our target were recorded in each of Abu Tweita, Elgabal Elahmar, El-Zawitein, Elmesirdi, Farsh Emsilla, Abu Kasaba, Elsomra, Zeaater, and Elmaein, other sites are ranged from 7 to 1 species in each.

Table 1. Geographical Distribution Range of Target Species inside St. Catherine Protected Area

Species	EOO	AOO	Alt. Range	No. Sites	Highest Frequency Sites	No. of locations
Endemic						
Buf.	337	120	1300-2630	34	W. Elfaraa, Gabal Catherine , Abu Tweita, Abu Walee, Elsomra	2
Euph.	179	68	1000-2050	24	Farsh Elromana, El-Zawietin, Abu Walee, W. Elrahaba, Erheibet Nada	2
Phlo.	339	136	1300-2400	53	Shak Musa, Farsh Elromana, W. Alarbein, Elgabal Elahmar, Abu Tweita	2
Rosa	40	36	1700-2350	14	Abu Tweita, Gabal Catherine , Elgabal Elahmar, W. Tenia, Zeaater	1
Anarh.	303	112	1000-2400	40	Elgabal Elahmar, Abu Tweita, Abu Geifa, W. Alarbein, Elmisirdy	2
Near Endemic						
Hype.	187	84	900-2200	29	W. Tenia, Elgabal Elahmar, Abu Tweita, Shak Itlah, Farsh Emsilla	2
Nep.	281	108	1400-2630	45	Shak Musa, Elgabal Elahmar, Gabal Catherine , W. Alarbein, W. Elfaraa	2
Poly.	307	96	990-2250	26	Gabal Catherine , Elgabal Elahmar, Zeaater, El-Zawietin, Elsomra	2
Salvia	17	24	1600-2230	9	Abu Tweita, Farsh Emsilla, Elsomra, Elmaein	1
Org.	287	108	1000-2200	45	Shak Musa, Elmesirdi, Shak Itlah, Elgabal Elahmar, W. Eltalaa	2

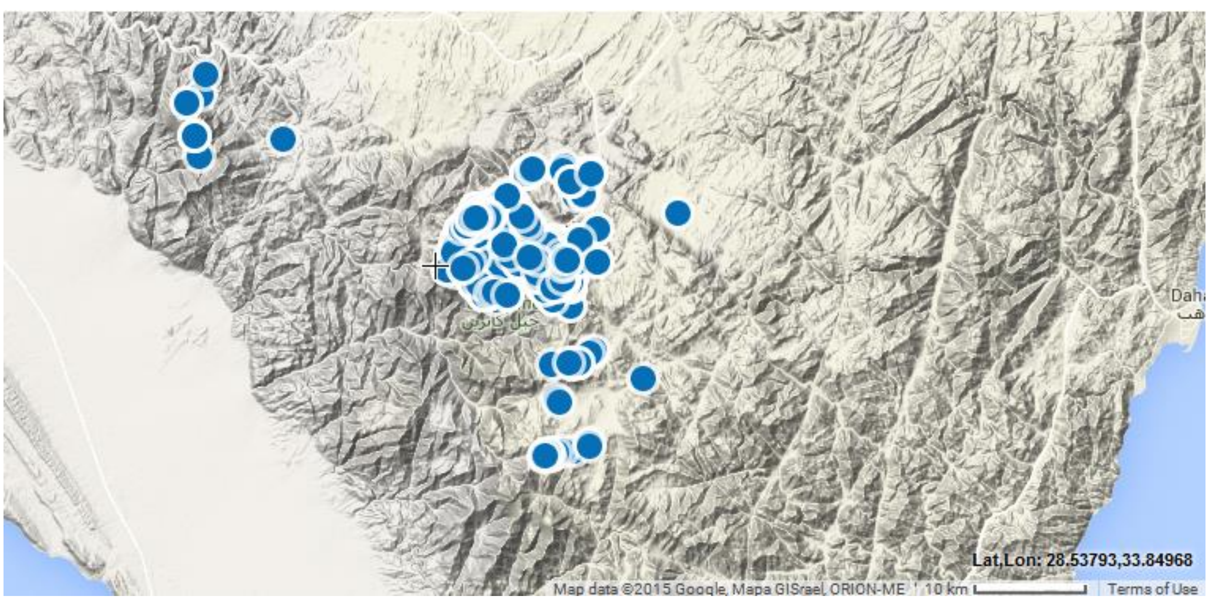


Figure 1. Distribution Range map of *Target species inside St. Catherine Protected Area.*

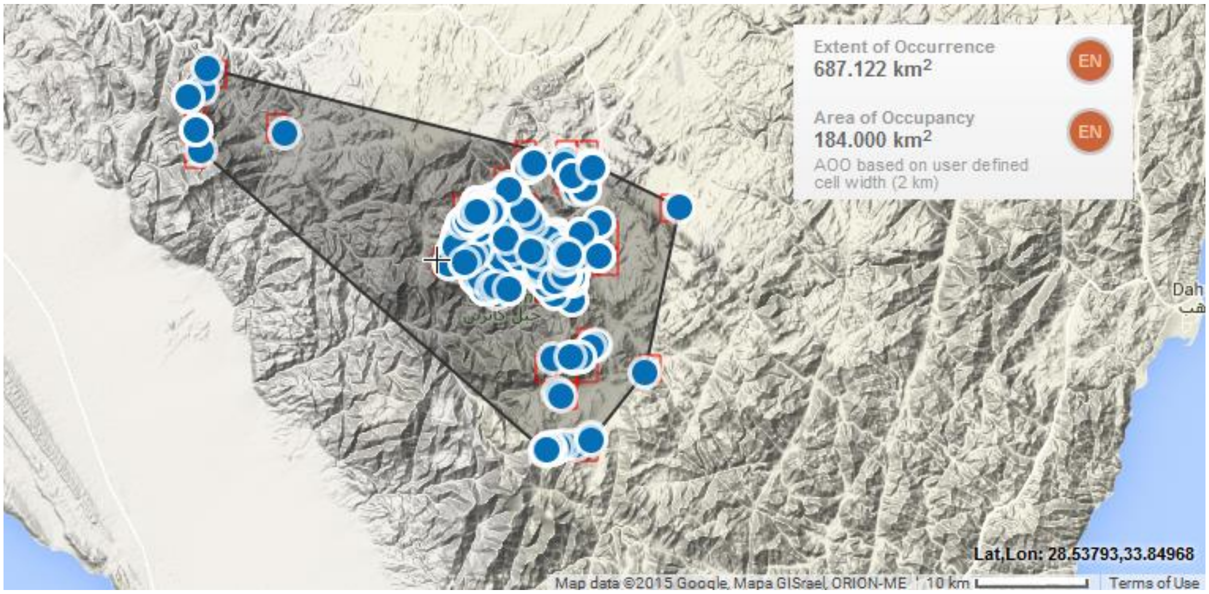


Figure 2. Geographical Range map of Target species inside St. Catherine Protected Area.

Population Characteristics. Most of the Target species (endemics and near endemics) subpopulations are small to very small, with individual plants occurring sporadically in space in little groups where the soil is gravelly and rocky (Fig. 3). The number of mature plants has been observed to decline as a result of several threats mainly, drought, over collection, over grazing, feral donkeys etc. The total global population size estimate for endemics ranged from 90 (*Rosa arabica*) to 5,000 mature individuals in case of *Phlomis aurea*. While the total national population size estimate for near endemics ranged from 1,000 (*Polygala sinaica*) to 4,500 mature individuals in case of *Origanum syriacum*. There are clearly separate subpopulations. During the last 10 years these subpopulations have been observed to have large changes in the total number of individuals, cover and density, due to over grazing by domestic and feral donkeys.

The population of the target species is considered severely fragmented as the mountainous habitat acts as a barrier between the small subpopulations, and as many of these subpopulations have low viability due to destructive overgrazing causing loss of reproductive organs in case of *Polygala, Anarrhinum,* and *Bufonia.* Some work have been done by Mahmoud *et al.* (2008), Moursy (2010), Omar (2010, 2013), Shabana (2014) on the genetic variability of some endemic and near endemic species in the same area, these studies concluded that there are a great polymorphism between different subpopulations may come from the variation in topography and climatic conditions and confirm the presence of isolation between different subpopulations that cause the real fragmentation.

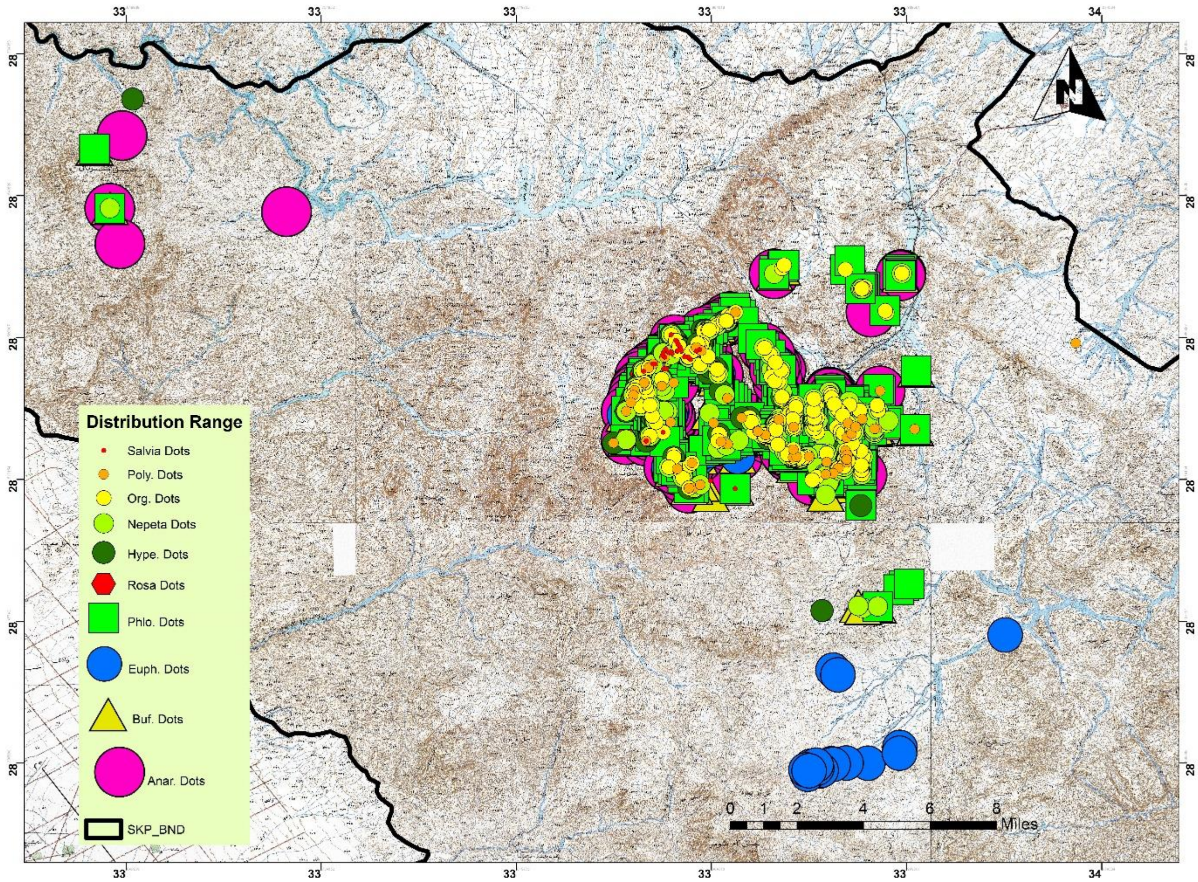


Figure 3. Population structure of target species inside St. Catherine Protected Area.

The species density varies from site to site based on target species but it's found that the highest density for all target species are concentrated in the high mountain areas especially Wady Gebal area, Elgabal Elahmar and Gabal Catherine area (Fig. 4). A total of three main locations were recorded (High Mountain area, Serbal Mountain area, and Elrahaba area). The effects of flooding (the most serious, plausible threat) will be felt separately in each area: thus these species are effectively in three clear locations.

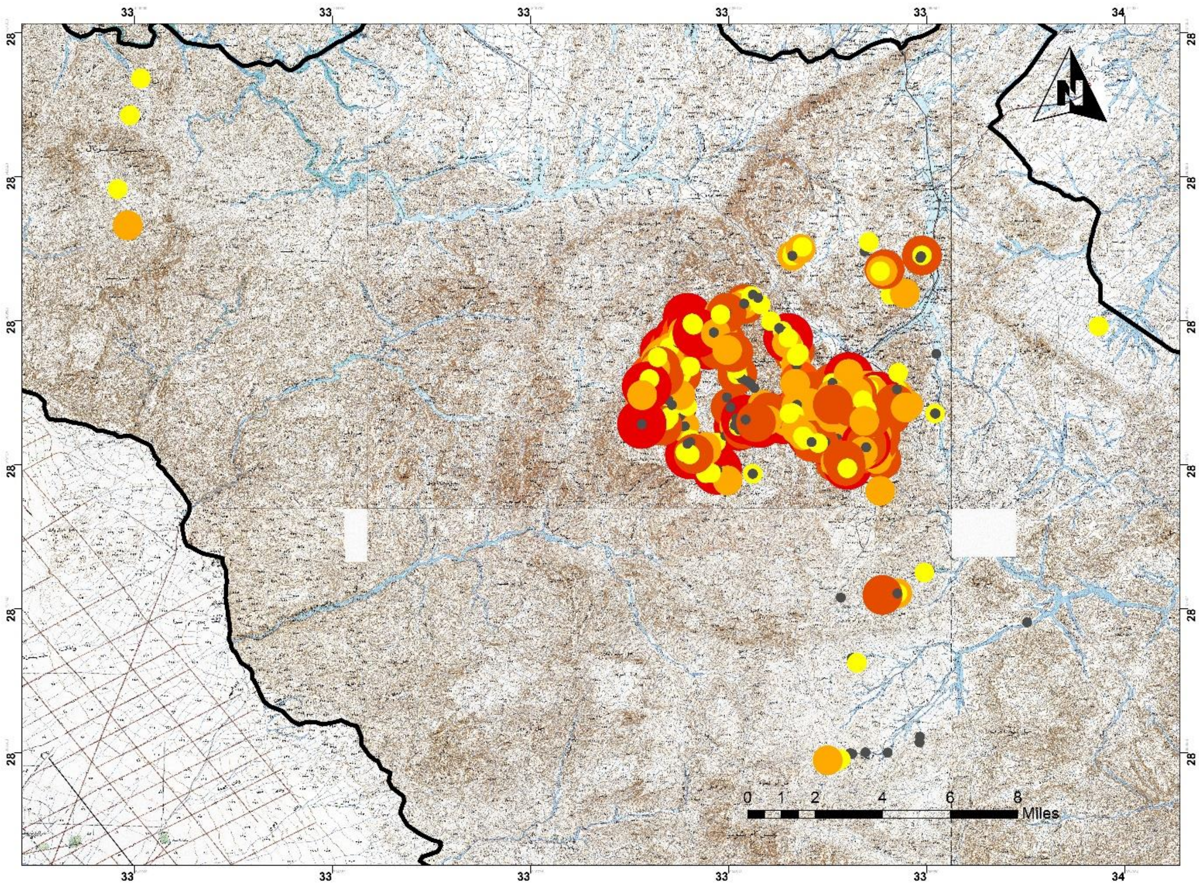


Figure 4. Density of all target species inside St. Catherine Protected Area.

Habitat and Ecology. All target species are perennial with a woody base. All of them are herbs except *Phlomis aurea* (shrub) and *Rosa arabica* (tree). It has been observed that flowers mostly appear in late spring (in case of *Rosa arabica* it may be delayed), and reproduction is by seed in late summer. Target species are restricted to montane wadis with granite rocky ground of mountain areas especially at gorges, slope, wadi bed, and cliffs with steep slopes of up to 90°. Slope Aspect is another factor affecting the distribution dynamic of the target species, most of species were recorded in North, Northeast, Northwest, and West directions. The cold winter climate (minimum temperature can reach -10°C) and cool summers (maximum temperature of c. 29°C) of the high elevations of Mt. St. Catherine are the coolest on the peninsula (Omar *et al.* 2013). The arid climate has a mean annual rainfall of about 37.5 mm (between 1971 to 2015), some in the form of snow, but there is great inter-annual variation with up to 300 mm in any one year, usually between October and May. Relative humidity is low, ranging from 10-35%.

As mention above and recorded by Schlesinger *et al.* (1996) and Durnkerley and Brown, (1997) that soils of South Sinai are desert soils (Aridisols). This agrees with the observations of Kamh *et al.* (1989), Balba (1995), Moustafa and Zayed (1996) and Omar *et al.* (2013) that soils of the target area are gravelly in wadis and plains, rocky at mountains in the surface, sandy to loamy sand in texture, alkaline and non-saline to slightly saline. Its characterized by low content of essential nutrients and cation exchange capacity (CEC).

The optimum density and frequency for the target species (endemics and near endemics) were recorded at elevation between 1600 to 2000 m asl. This can be explained as the topography is a principal controlling factor in vegetation growth and the type of soils as recorded by O'Longhlin (1981); Wood et al. (1988) & Dawes and Short (1994). Elevation, aspect, and slope are the three main topographic factors that control the distribution and patterns of vegetation in mountain areas (Titshall et al. 2000). Among these three factors, elevation is most important (Day and Monk 1974 & Busing et al. 1992). Elevation along with aspect and slope in many respects determines the microclimate and thus large-scale spatial distribution and patterns of vegetation (Day and Monk 1974; Allen and Peet 1990 and Busing et al. 1992).

Threats. St. Catherine Protected Area is one of very few protected areas that have local communities work and live inside its boundaries. These interactions sometimes cause conflicts and threats on the natural resources of the PA. It was observed that donkey's distribution affected by vegetation cover (donkeys concentrated on areas with high vegetation cover) which actually affected by good water supply and showed negative relation with Bedouin community distribution (distributed away from human presence). It was recorded that hotspots areas for target species that located within elevations range from 1800 m to 2000 m such as Abu Tweita, Wadi Gebal, Farsh Elromana and Farsh Emsila showed the highest presence for feral donkeys. Grazing by these usually causes uprooting of the plants as indicated by Bedouins and field observations and this prevents plant regrowth. Soil compaction is associated with use by these animals and causes destruction to a variety of plant species through continuous trampling (Khafaja et al., 2006, Omar et al. 2013). Salvia multicaulis, Polygala sinaica, Bufonia multiceps, *Anarrhinum pubescens,* and Rosa arabica are the most species negatively affected by this threat (Figure 5). However all these effects, much more research is needed on these Feral Donkeys especially regarding distribution dynamics, hotspots and direct and indirect effect on plant species distribution.

Sites like Abo Hebik, Elgalt Elazrak, Abu Tweita, Sherige, Shak Musa, Elmesirdi and W. Eltalaa are most targeted for plant collection. These sites are characterized by water supply and high plant biomass; however, plant collection increases with precipitation and is usually heavly between March and December each year (flowering season). It was observed that plants collection may be affected by economic factors. In other words, when tourism levels fall, Bedouin themselves start to collect plants for income. Local communities mentioned that women are the most common collectors of plants, and they collect 5 times per season. Although the reasons for collecting these plants are always for trade or personal use as fuel, the use of plants as fuel has decreased sharply with the advent of butagaz (Assi, 2007, Omar et al. 2013). Results showed that Origanum syriacum, and Salvia multicaulis are the most collected species for trade within study area because of their medicinal value (Assi, 2007). Rosa arabica also collect by local community for medicinal use but not for trade.

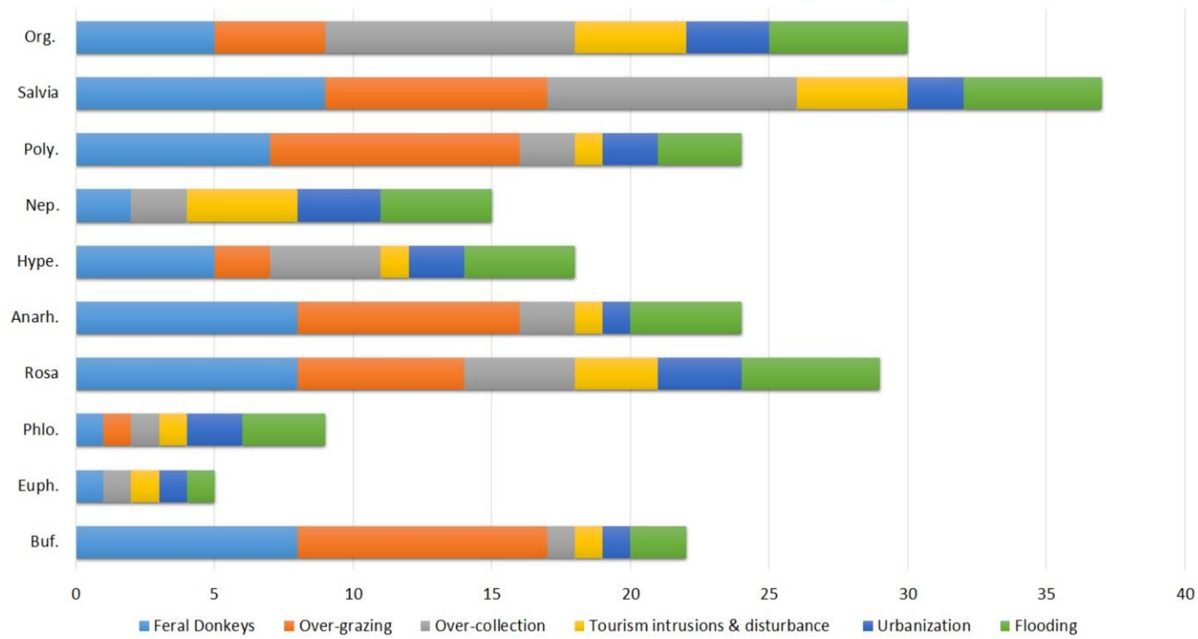


Figure 5. Threats levels on target species inside St. Catherine Protected Area.

Gabal Musa, Gabal Catherine, Wadi Gebal, Farsh Elromana, Elgalt Elazrak, Abu Tweita, Wadi Tenia, W. Sherige and W. Eltalaa are the most sites presented the highest presence for tourism activities. About 3 million person from 51 nationalities visit SCPA from 2003 to 2011 with mean 335.000 people per year (SCPA Management), most of them focused on northern part of SCPA specially world heritage site. Many of the tourists do safari and camping in remote areas; usually safaris extend for many days using different camping points; the most camping points are in Farsh Elromana, W. Tenia and W. Gebal. Some of the negative impacts come from tourists in the way of collecting medicinal plants as a souvenir from the SCPA beside collection for fuel. Soil compaction of trespassing leads to poor vegetation cover comes from continuous walking. On other hand camping take place in shelter sites which give water source for tourists and this led to water consuming and direct effect on vegetation community. However all this the affect level for this activities are consider low, mush more researches are needed in this field.

Ungulates play a major role in regulating primary production (energy produced by photosynthesis) in grazing ecosystems (Huntly 1991). Defoliation can promote shoot growth and enhance light levels, soil moisture, and nutrient availability (Frank *et al.* 1998). Overgrazing, however, can significantly reduce biomass production. Grazing animals can decrease flower and seed production directly by consuming reproductive structures, or indirectly by stressing the plant and reducing energy available to develop seeds. Grazing animals can also disperse seeds by transporting seed in their coats (fur, fleece, or hair), feet, or digestive tracts (Wallander *et al.* 1995, Lacey *et al.* 1992). For some plant species, grazing ungulates may facilitate seed germination by trampling seed into the soil.

Regarding to grazing activities inside SCPA Omar *et al.* (2013) found that Elmesirdi, Sheiage, Elgabal Elahmar and Shak Musa are the most sites represented the highest presence for goats which can explain by it are the closest sites to local communities' settlements. Elawitein, W. Gebal, W. Tenia, Abu Tweita and Farsh Elromana are the most sites represented the highest presence for camels which can explained by the easily accessible and heavily used by tourists for camping which camel take place in transportation. Results showed that Tebook, Abo Twita, Ain Shekia, Shak Sakr and Elmesirdy represent the highest number of grazed individuals among the different locations, because these locations are stressed by tourism and human activity which are combined by the presence of camels and donkeys as transportation tools to and from historical sites, Bedouin communities are also settled beside these locations and this gives goats high presence in these locations. *Bufonia multiceps, Anarrhinum pubescens, Polygala sinaica, Salvia multicaulis, Hypericum sinaicum and Rosa arabica* are the most target species negatively affected by grazing.

Very few sites were affected by collection for scientific researches (Herbarium, phytochemistry and genetics), the most affected research was the collection of specimen for herbarium because the collectors sometimes collect a big amount of plants with flowering parts and roots which may lead to decrease of future population. Also collection for phytochemistry requires more than kilo for good extraction. Results showed that the most affected sites were Kahf Elghola, Wadi Alarbein, Wadi Tennia, Abu Tweita, Elmesirdi, Abu Kasaba, Shak Musa, Shak Elgragenia, and Elgalt Elazrak. *Salvia multicaulis, Hypericum sinaicum, Rosa arabica, Bufonia multiceps, Anarrhinum pubescens, Polygala sinaica, Origanum syriacum, and Nepeta septemcrenata* are the most targets for this activities.

Due to climate change, the wild population of these species could be in extreme danger in the relatively near future. The most important natural threats are the long-lasting droughts, the difficulties of some species to reproduce new generations as a result of long seed dormancy (*Rosa arabica*), or overgrazing by herbivores that even eat the reproductive organs and decrease the chance for the possibility for creating new generation (observed- *Bufonia multiceps, Anarrhinum pubescens, and Polygala sinaica*), the very scarce irregular precipitation during the year, the fragmentation inherent to its habitat, and the possibility that rare floods may cause harm such as uprooting (1-5% loss observed). Water is being relocated in some localities from elevated wadis which are rich in water to supply to low wadis. This activity leads to consumption of water from wells and results in habitat deterioration and declines in population size. This are observed clearly in Wadi Gebal, Farsh Elromana, Wady Tenia and Abu Tweita. *Hypericum sinaicum* is the most target species negatively affected by water lose. However, for these direct effects, there is an urgent need to focus and work studying the direct and indirect effects of Dams & water management/use inside SCPA.

Most of threats root cases comes from lack of awareness, weak law enforcement, lack of suitable strategies, weak financial support and lack of stakeholders cooperation. Since human activities have a strong effect on biodiversity, a population/community level approach is considered to be the level that can help in exploring the responses of the whole ecological system to various kinds of disturbance as reported by Hanski and Gilpin, (1991) and Barbault and Hochberg, (1992).

In general, these species is severely threatened by both natural (aridity of the area and climate change-flooding) and human factors (over-grazing by domestic animals and feral donkeys, over-collection, and unmanaged tourism activities). All these factors are pushing *Target species* to the brink of extinction. From Endemics *Rosa arabica, Anarrhinum pubescens*, and *Bufonia multiceps* are the most negatively affected species by these threats (Figure 6).

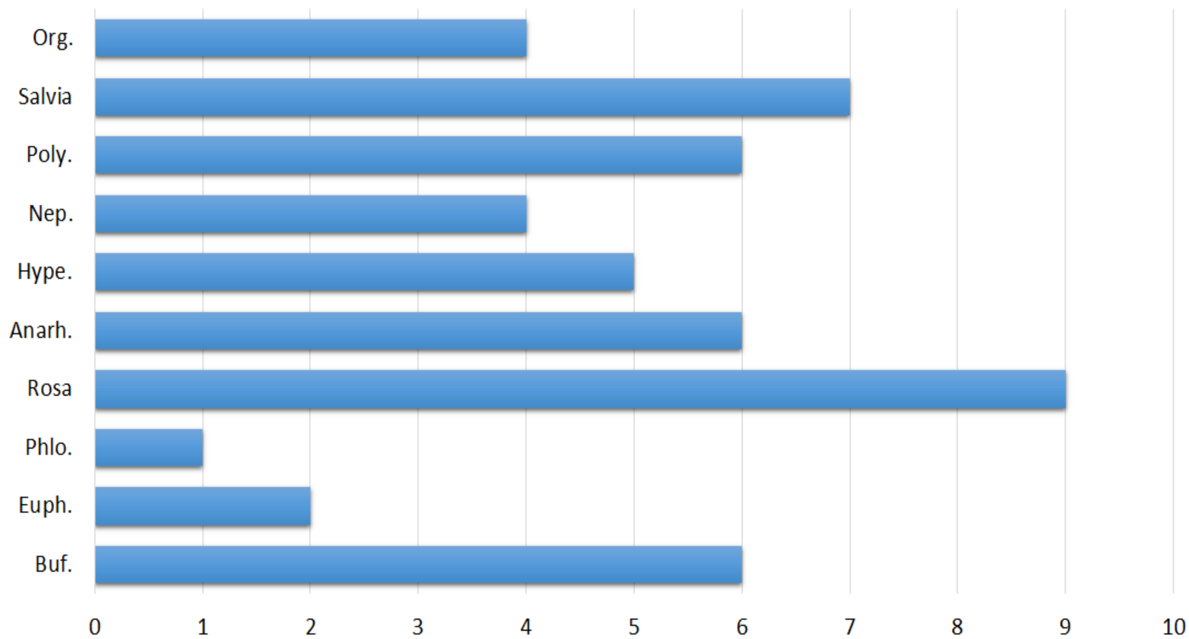


Figure 6. Target species sensitivity to different threats; 1=Very low and 10= Very high.

IUCN Red List Assessment Rationale. Based on the previous analysis especially the geographical range (EOO and AOO), No. of locations, population size and species - habitat trend, target species could be classified based on IUCN Red List categories and criteria as presented in Table 2. On global scale one species listed as Critically Endangered (Rosa arabica) and the other four species listed as Endangered (the four species may be listed as Vulnerable if the two main locations treated as one). On national scale, S. multicaulis can be listed as Critically Endangered, the other four species listed as Endangered. Data about Origanum is inadequate and the situation may be change when sufficient data available.

Conclusions

Many laws and regulations were set to conserve threatened plants in Egypt, although of all these laws and regulations, the species is still in extreme danger and one of the most important threats that may lead to extinction is collecting. Theirs urgent needs to held specific Critically Endangered species conservation convention, Influencing legislations appropriations, harsher punishment for endangered plant species assembly without clear permission from the main authorities. There is an urgent need to integrate the knowledge derived from ecological, demographic and geographical approaches extracted from this study to species conservation in order to be able to formulate management strategies that take into account all different considerations. It's highly recommended to use the information extracted from this study when conservation process take place trough In-situ or Ex-situ techniques.

Table 2. IUCN Red List Status of Target Species Inside St Catherine Protected Area

Species	EOO	AOO	Population Size	No. of locations	Red List
Endemic – Global Assessment					
Buf.	337	120	1300-2400	2	EN
Euph.	179	68	2500-4000	2	EN
Phlo.	339	136	2500-5000	2	EN
Rosa	40	36	90	1	CR
Anarh.	303	112	1800-3000	2	EN
Near Endemic - National Assessment					
Hype.	187	84	2000-3500	2	EN
Nep.	281	108	1500-3000	2	EN
Poly.	307	96	300-1000	2	EN
Salvia	17	24	700-1900	1	CR
Org.	287	108	2500-4500	2	EN

Acknowledgements

Special thanks are due to my great honest team; Dr. Ahmed Abduallah, Dr. Abduallah Nagi, and Mr. Ibraheim Elgamal the team, whose bearing the difficulties of more than superhuman to complete the study. We would like to express our sincere thanks towards volunteer researcher who devoted his time and knowledge in the implementation of this study: Mr. Amir Shalouf. I would like to thank with all the love our professional Bedouin guide Seleim Mehana for his hard work and professionalism within our fieldwork in St. Catherine Protected Area.

References

Allen, R.B. and Peet, R.K. 1990. Gradient analysis of forests of the Sangre de Cristo Range, Colorado. Canadian Journal of Botany, 68: 193-201.

Assi, R. 2007. MP Threat Analysis and Threat Reduction Assessment Report. Conservation and sustainable use of medicinal plants in arid and semi-arid ecosystems project, 61 pp.

Balba, A.M. 1995. Management of problem soils in arid ecosystems. Lewis Publishers, New York.

Barbault, R. and Hochberg, M.E. 1992. Population and community level approaches to studying biodiversity in international research programs. Acta Oecol., 13: 137-146.

Boulos, L. 1999. *Flora of Egypt*. Al hadara publishing, Cairo, Egypt, Vol. 1: 419 pp.

Boulos, L. 2000. *Flora of Egypt*. Al hadara publishing, Cairo, Egypt, Vol. 2: 392 pp.

Boulos, L. 2002. *Flora of Egypt*. Al hadara publishing, Cairo, Egypt, Vol. 3: 373 pp.

Boulos, L. 2003. *Flora of Egypt*. Al hadara publishing, Cairo, Egypt, Vol. 4: 450 pp

Bridgewater, P.B. 1993. Landscape ecology, geographic information systems and nature conservation. In: Haines-Young, R., Green, D.R., Cousins, S.H. (Eds.), *Landscape Ecology and GIS*. Taylor and Francis, London, pp. 23-36.

Busing, R. T., White, P. S., and MacKende, M. D. 1992. Gradient analysis of old spruce-fir forest of the Great Smokey Mountains circa 1935. Canadian Journal of Botany, 71: 951-958.

Commonwealth of Australia, 2009. Australia's Terrestrial Biodiversity 2008. *Chapter 5 Threats to Australian biodiversity.* Australian government, Department of Environment, Water, Heritage and the Art. 149-212.

Dawes, W.R., and Short, D. 1994. The significance of topology for modelling the Surface hydrology of fluvial landscapes. Water Resources Research, 30: 1045- 1055.

Day, F.P., and Monk, C.D. 1974. Vegetation patterns on a Southern Appalachian watershed. Ecology, 55: 1064-1074.

De la Cruz-Rot, M. 2001. Manejoy conservacio´n de comunidades vegetales. II Seminario de Biologı´a de la Conservacio´ n de Plantas Amenazadas. Universidad Polite´cnica, Madrid.

Durnkerley, D.L., and K.J. Brown. 1997. Desert soils. In D.S. Thomas, (ed.) Arid zonegeomorphology: Process, form and change in drylands, second edition. John Wiley and SonsLimited. 55-68 pp.

Frank, D.A.; McNaughton. S.J.; Tracy, B.F. 1998. The Ecology of the Earth's Grazing Ecosystems. *BioScience*, Vol. 48, No. 7., pp. 513-521.

Hanski, I. and Gilpin, M. 1991. Metapopulation dynamics: brief history and conceptual domain. *Biological J. Linean Soc*. 42:3-16.

Huntly. N. 1991. Herbivores and the dynamics of communities and ecosystems. *Annual Review of Ecology and Systematics* 22: 477-503.

Hutton, J.M. and Leader-Williams, N. 2003. Sustainable use and incentive-driven conservation: realigning human and conservation interests. Oryx 37(2):215– 226.

IUCN. 2014. Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. Downloadable from http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Kamh, R.N., El-Kadi, M.A., El-Kadi, A.H., and Dahdoh, M.S.A. 1989. Evaluation of Mn forms in selected soils of Sinai. Desert Institute Bulletin, A.R.E. 39(1):183-197.

Khafaja T, Hatab A and Dsouki A. 2006. Report on the current situation of the plant vegetation cover in the high mountain area of St. Catherine. Plant Conservation and Monitoring Programme, St. Catherine Protectorate.

Lacey, J.R., R. Wallander, and K. Olson-Rutz. 1992. Recovery, germinability, and viability of leafy spurge (*Euphorbia esula*) seeds ingested by sheep and goats. Weed Technology 6:599-602.

Mahmoud, M.A.M., Zalat, S.M., El-Akkad, S.S., Gilbert, F. 2008. Genetic variability in the endemic bee *Anthophora pauperata* among wadisin the St Katherine Protectorate. *Egyptian Journal of Biology*, Vol. 10, pp 77-86.

Moursy, M.M., 2010. Ecophysiological studies on the endemic plant *Euphorbia sanctae-catharinae* Fayed, in St. Catherine Protectorate, South Sinai, Egypt. *M.Sc., Thesis*, Fac. Sci., Al-Azhar Univ 350 pp.

Moustafa, A.M., and Zayed, A.M. 1996. Effect of environmental factors on the flora of alluvial fans in southern Sinai. Journal of Arid Environments, 32: 431-443.

O'Loughlin, E. M., 1981. Saturation regions in catchments and their relations to soil and topographic properties. Journal of Hydrology, 53: 229-246.

Omar, K. 2010. Eco-geographical study on *Nepeta septemcrenata* in St. Catherine protectorate, South Sinai, Egypt. *M.Sc., Thesis*, Fac. Sci., Al-Azhar Univ., 350 pp.

Omar, K., Khafagi, O. and Elkholy, M.A. 2013. *Geomatics and plant conservation: GIS for best conservation planning*. Lambert Academic Publishing.

Ricklefs, R.E., Naveh, Z. and Turner, R.E. 1984. Conservation of Ecological Processes.vIUCN Commission on Ecology Paper 8. IUCN, Gland, Switzerland.

Schlesinger, W.H., Raikes, J.A., Hartley, A.E. and A.F. Cross 1996. On the spatial pattern of soil nutrients in desert ecosystems. Ecology 77: 364-374.
Shabana, H., Moursy, M.M., Omar, K. 2011. Survey of the Flora Enclosures of St. Katherine protectorate "in-situ conservation". Plant Conservation and Monitoring Programme, St. Catherine Protectorate.
Shabana, H.A.M. 2014. Variability among *Phlomis aurea* Decne. populations in Southern Sinai. M.Sc., Thesis, Fac. Sci., Tanta Univ. 260 pp.
Sutherland, W.J. 2001. The Conservation Handbook: Research management and policy. Blackwell Science, Oxford, UK.
Titshall, L.W., O'Connor, T.G., and Morris, C.D., 2000. Effect of long-term exclusion of fire and herbivory on the soils and vegetation of sour grassland. African Journal of Range and Forage Science, 17: 70–80.
Wallander, R.T., B.E. Olson, and J.R. Lacey. 1995. Spotted knapweed seed viability after PAsing through sheep and mule deer J. J. Range Manage. 48:145-149.
Wood, E. F., Sinpalan, M., Beven K., and Band L., 1988. Effects of spatial variability and scale with implications to hydrological modelling. Journal of Hydrology, 102: 29-47

Conservation and Management of *Prosopis cineraria* (L) Druce in the State of Qatar

Elsayed Mohamed Elazazi[1,3], Mohamed Seyd[2], Rashed Al-Mohannadi[1], Maryam Al-Qahtani[1], Nafeesath Shahsi[1], Aisha Al-Kuwari[1], Masoud Al-Marri[1]

1. Genetic Resources Department, Department of Agricultural Research, Ministry of Municipality and Environment State of Qatar
2. Environmental Protection and Wildlife Department, Ministry of Municipality and Environment, State of Qatar.
3. Egyptian Deserts Gene Bank, desert Research Center, Cairo, Egypt.

e-mail: emazazi@mme.gov.qa

Abstract

The Al-Ghaf tree (*Prosopis cineraria* (L.) Druce*)* is one of the most important endangered native trees in the State of Qatar. Currently, it is present only in a few pockets in the State of Qatar and Arabian Peninsula, where it is used as forage for livestock, firewood, and medicine. Climate change, human activities, overgrazing, and the spread of exotic invasive trees like *Prosopis juliflora* [(Sw.) DC.] have led to gradual decline in the distribution and abundance of native plants and trees, including. The research reported in this presentation were aimed at evaluating the status of Al-Ghaf trees at three sites in the State of Qatar and implementing conservation practices, such as control of *Prosopis juliflora,* seed collection, testing seed viability, and *ex-situ* preservation of seeds in the Qatar gene bank. Established methods were used for conserving seeds in the seed bank, DNA bank, and herbarium. The results of the study indicated that the *Prosopis cineraria* distributed in three main sites in the State of Qatar. The site in Al-Ghaf at "Rawdat rashed" represented a stand of seven trees that were endangered by the hyperarid conditions and overgrazing. The second site in the North of Qatar (Rawdat makin or ghafat makin) containing 11 trees distributed sporadically were in good growing condition, but were threatened by human activities and invasive species (*Prosopis juliflora*). The third site located along the road from the North road to Al Zubarah historical site, 5.4 km away from Al-Ghwoyriyah, contained seven small trees. These trees were highly threatened by the *Prosopis juliflora* community. The results showed that seeds from the "Rawdat rashed" site were of high quality as evident from their larger size, 100 seed weight, number of seeds/pod, and their ability to germinate (98%) following mechanical scarification to remove the hard seed coat. Monitoring of vegetation at the "Rawdat makin" or "Ghafat makin" site from 2012 - 2016 revealed a general decline both in coverage and frequency of some native species, including Al-Ghaf trees with rapid increase in the distribution of *Prosopis juliflora*. Therefore, control of invasive P*rosopis juliflora* trees was implemented at this site and monitored for six months. The results revealed a high growth rate of Al-Ghaf trees along with recovery of tree cover and fruiting following implementation of conservation measures.

Keywords: Al-Ghaf, biodiversity conservation, gene bank, invasive species, *Prosopis juliflora* (sw.) DC.

Introduction

Qatar is small in size with maximum length of 185 km and maximum width of 85 km. Qatar is an oblong -shaped peninsula lying on a north-south axis alongside the center of eastern coastline of Saudi Arabia, separated from it by Salwa Bay on the eastern side and attached to it by a total distance of 60 km and at its most southern end (Abdel-Bari, 2012). There are 10 trees in Qatar; *Prosopis* *cineraria, Acacia ehrenbergiana, Acacia tortilis, Acacia nilotica Ziziphus nummularia, Ziziphus spina-christim, Cocculus pendululs, Ephedra foliata, Avicennia marina and Prosopis juliflora.* ABDEL-BARI (2012). *Prosopis cineraria* is one of the most important native tree among few trees growing in the state of Qatar (E. Abdel Bari *et al* 2007)., The P. cineraria trees number in Qatar reported more than 50 trees in many publications, *Prosopis cineraria* is a slowly growing tree in the dry and arid regions of Arabia and India and is beneficial for the growth and development of the other species Abdel Bari et al., (2007).

The native *Prosopis cineraria* and exotic invasive *P. juliflora* are present in arid habitats of Qatar. The genus of Prosopis is one of the commercially important genera of legumes in arid climatic zones with multifarious benefits to humankind. *Prosopis* species are used for agroforestry, apiculture, fodder timber, fuel, shade, firewood as well as affecting soil improvement and sand dune stabilization Leakey and Last 1980; Sharma et al., 2010).

In 2004 it was rated one of the world's top 100 least wanted species (Invasive Species Specialist Group of the IUCN, 2004). *Prosopis juliflora* (Sw.) DC is an evergreen tree native to South America, Central America and the Caribbean. In the United States, it is well known as mesquite. Pasiecznik et al., (2001). It is fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils.

In contrast, the native *Prosopis cineraria* or Ghaf tree, which is on the edge of its range in Qatar, is undergoing a serious declined, all published survey confirmed that there are two main areas of distribution in central and north of Qatar. At the Al Ghafat area at Rawdat Rashed there are eight trees and at the Alghaf are near Madinat AlShamal there are 11 trees. All are mature specimens, which have an estimated age of 90 – 150 years or more Abdel Bari et al (2007). Over the country as whole there are likely to be no more than 50 native trees Norton et al., (2009).

There are two strategies for conservation plant genetic resources, there are (ex situ and in situ) conservation and they are defined in Article 2 of the Convention on Biological Diversity Secretariat (1992). Thus "Ex situ conservation means the conservation of components of biological diversity outside their natural habitats" and "In situ conservation means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticates or cultivated species, in the surroundings where they have developed their distinctive properties. "Secretariat (1992).

In Qatar, as in many other countries, biodiversity is facing threats from a range of human activates, populations growth rapid urbanization, together with the effect of global climate change and global warming, have put pressure on the delicate balance of natural environments

Materials and Methods

This research aimed at surveying *Prosopis cineraria* in the state of Qatar, management, dormancy pre-treatments and conservation of genetic material in gene bank.

- Ecological surveying and plant material collecting, through year 2012 to 2016 during collecting, surveying and inventory activities for flora in Qatar applied the Genetic Resources Department, Department of Agricultural Research, Ministry of Municipality and Environment, Qatar. We collected plant genetic resources of *Prosopis cineraria* as "herbarium samples, seeds, DNA samples" for conservation in gene bank (Table 1) and maps according to Rao et al., (2006).
- Seeds viability test, by estimated germination test according to Rao et al., (2006).
- Plant photographic profiling.

- Conservation plant genetic resources materials in gene bank.
- Management and controlling *P. juliflora*

Table 1: Gene bank accessions of plant genetic resources and locations of P. cineraria

No.	Accession no in gene bank			Location data		
	Herbarium	DNA Bank	Seed Bank	Latitude	Longitude	Location names
1	QAT0007	QAT0007	QAT000158	25.235267^{O}	51.149167^{O}	Rawdat Rashed
2			QAT000190			
3	QAT000159	QAT000159	QAT000191	26.000731^{O}	51.189414^{O}	AlGhaf, Ghafat Makin, Umm Al Hefain
4	QAT000235	QAT000235	QAT000313			
5	QAT000321	QAT000321	QAT000216	25.866631^{O}	51.221674^{O}	Al Ghuwariyah

Seed Collection, Cleaning and Drying. Seeds of *P. cineraria* cleaned after the seeds has cleaned, they were dried in a standard cold drying room, according to protocols described elsewhere Rao et al., (2006).

Seed Sampling and Seed Storage Conditions. Seeds of *P. cineraria* stored in gene bank under optimal conditions for short term conditions (+4 ºC & 40 % RH) and -22 ºC for long term conservation.

Viability Tests. Seed viability was assessed by germination tests following the approach described in Rao et al., (2006). Germination was evaluated before seeds were placed in storage

Germination Studies. Germination tests were carried out in sterilized sand, 25 treated seeds were germinated in Petri dishes in germination incubator with 20°/30°C (12h/12h) following the guidelines of the Association of Official Seed Analysis.

Ecological Studies. The structure of vegetation of *P. cineraria*, was analyzed sociologically according to procedures described Braun, (1932). The component species in the community were listed and counted in a series of 5 quadrates, 20 ×20 m each quadrate.

Density: Density denotes average number of individuals of a given species out of the total of samples examined in a study area however, the species may or may not occur in all the quadrates Rastogi (1999).

$$\text{Density} = \frac{\text{Total number of plants of individual species in all quadrates}}{\text{Total number of quadrates studied}} \quad \text{.... (1)}$$

Management and Controlling of *P. juliflora*. We applied Physical controlling methods for remove *P. juliflora* including uprooting and cutting to control of trees and seedlings of *P. juliflora* that is so quick. We have uprooted large plants with picks or winches (Electric saws, Winches, Loaders, and Dredges).

Results and Discussion

Morphological growth. Most of the existing *P. cineraria* native trees in the state of Qatar have a different morphological form in AlGhaf, Ghafat Makin, Umm Al Hefain trees we recorded the largest and oldest trees in the state of Qatar trees its regeneration their vegetation growth every year (Figure 2). On the other hand, at Rawdat Rashed there are 8 old trees that has been dry branches and shaped by over grazing and limitation of the ground water (Figure 3). El-Keblawy et al., (2009) reported that overgrazing is a major problem for the establishment of different plant growth in deserts and arid locations.

Table 2. Characteristics of *Prosopis cineraria* and *Prosopis juliflora* Populations in Different Sites used for this Study

Group Locations	No. trees measured	Density (trees / ha)
Prosopis cineraria		
Rawdat Rashed	7	1.4
Al Ghuwariyah	8	1.6
AlGhaf, Ghafat Makin, Umm Al Hefain	11	2.2
Prosopis juliflora		
Rawdat Rashed	0	0
Al Ghuwariyah	20	5
AlGhaf, Ghafat Makin, Umm Al Hefain	513	102.6

Our study in first site its unfencing location at north of Qatar (AlGhaf, Ghafat Makin, Umm Al Hefain) reported that there are no vegetative shoots were recorded at the tree base, but we found vegetative shoots after 1 to 1.5m from the tree base. In addition, we did not find any single *P. cineraria* "Ghaf" seedling. This attributed to the lack of safe sites, overgrazing by herbivores, human activities, and effect of invasive species (*P. juliflora*).

Our survey to old local community, they pointed out that the age of the Ghaf trees exceeds 150 years, on the other hand, the man who planted the *P. juliflora* trees was found 25 years ago and had no more than 20 trees for animals shading. By the year 2016, increased to more than 1,000 trees and shrubs in approximately 20 ha.

Figure 1. Distribution map of *P. cineraria*.

Figure 2 *P. cineraria* tree in AlGhaf, Ghafat Makin, Umm Al Hefain.

Figure 3. *P. cineraria* tree in Rawdat Rashed after fencing 2013.

We found the seeds and vegetative parts of the *P. cineraria* are very palatable by goats, sheep, and camels, compared to *P. juliflora* its Low palatability by animals.

The presented study registered many populations within the state of Qatar, can be loosely categorized into two distribution types, open forest and individual tree. Open forests are observed on (Rawdat / Rodat) very compact soils composed of fine sand, silt or loam, soil have higher quantity of organic matter than other deserts areas with flooded during rains.

Data in Table 2 characteristics of *Prosopis cineraria and Prosopis juliflora* populations in different sites, the higher number of *P. cineraria* were recorded in AlGhaf, Ghafat Makin, Umm Al Hefain with 11 trees, on the other hand the greatest density of *P. cineraria recorded In* Al Ghuwariyah 3.5 trees / ha. Contrariwise we didn't record any trees or shrub of *P. juliflora* In Rawdat Rashed, and the greatest density (102.6) recorded for *Prosopis juliflora In* AlGhaf, Ghafat Makin, Umm Al Hefain

Data in Table 3 trunk diameter and tree height showed significant differences among locations, tree height and trunk were particularly uniform within populations. Wee found that the highest average of trunk diameter and tree height (212.3 cm, 12.95 m) respectively at AlGhaf, Ghafat Makin, Umm Al Hefain. On the other hand, the lowest average of trunk diameter and tree height (104 cm, 2.6 m) respectively at Al Ghuwariyah. Through the results in Table 3 we concluded the distribution of *P. cineraria* in Rawdat Rashed and AlGhaf, Ghafat Makin, Umm Al Hefain the most of the trees are similar age, if the trunk diameter and height of trees correlated with age, then the AlGhaf, Ghafat Makin, Umm Al Hefain populations contains trees that are much older. Then the tree in Rawdat Rashed, the youngest trees at Al Ghuwariyah.

Table 3. Mean Trunk Diameter, Mean Tree Height, Longest, Shortest Tree and Gegeneration Status

Group Locations	Tree number	Average of trunk dia. (cm)	Average of tree height (m)	Longest tree (m)	Shortest tree (m)	Regeneration status
Rawdat Rashed	7	201	8.31	12.6	5.67	Root Sprouts (15 cm), vegetative shoots (25 - 100 cm) saplings (20cm) seedlings
Al Ghuwariyah	8	104.	5.5	7.93	2.6	vegetative shoots (25 - 50 cm)
AlGhaf, Ghafat Makin, Umm Al Hefain	11	212.3	9.13	12.95	6.8	Root Sprouts (15 cm), vegetative shoots (25 - 100 cm) saplings (20cm) seedlings

Regeneration / Reproduction. Our study indicated that there are almost no *P. cineraria* seedlings before protection the study sites This might be attributed to the lack of safe sites, *P. cineraria* naturally reproduces through seeds, or vegetatively through root suckers. Flowering and fruiting under Qatar conditions appear in April and May, and seed drying in Jun. at AlGhaf, Ghafat Makin, Umm Al Hefain flowers and seeds production was very height during the study, the dried legumes or seeds pods used for feed livestock. After fencing site for one year we recorded many seedlings. On the other hand, Rawdat Rashed fencing from 2013 so many individual shoots, shrubs were observed from root system and seeds. Rawdat Rashed old mother trees not healthy and dried branches, it was dramatically lower the canopy that might cause the groundwater supplies have been lower. A study in UAE by Gallacher and El-Keblawy (2016) found recruitment of *P. cineraria* by seed was not observed, and observed asexual recruitment was limited to root suckers produced only within the season. Presence of suckers was unrelated to browsing but strongly affected by a shifting ground surface. Viable theories for the absence of sexual recruitment include seedling destruction by herbivores, a lack of safe sites for seedling growth and establishment, and that sexual recruitment events might naturally occur rarely, but produce many recruits in the rare successful seasons. Significant recruitment of new individuals is likely to occur only with protection from current herbivore systems.

Controlling the Invasion of *Prosopis juliflora*. The efforts to control the *Prosopis juliflora* problem have been undertaken at the Ministry of Municipality and Environment level. In 2015 we got the ministry permit to protect the AlGhaf, Ghafat Makin, Umm Al Hefain in north of Qatar, we start fencing location with donation local company in early 2016, data in Figure 7 showed improvement *Prosopis juliflora from 2011*we removed 1008 *Prosopis juliflora* trees, shrubs and small seedling from this site. Monthly we resurvey site and remove any new vegetative reproductive. Now we didn’t have any single seedling in this site Figure 5. On the other hand, we get the highest benefits value from fencing we recorded height covering and density of associated native plant.

Effect of Distribution Locations on 100 Seed Weght and Viability and Germination Percentage of *P. cineraria*. Figure 6 reported that the seed of *P. cineraria* from Rawdat Rashed gave the lower 100 seeds wt. (2.73gm), and seeds from Al Ghaf, Ghafat Makin gave the highest 100 seed wt. (4.406gm).

This result might be effect on seed viability and germination in Figure 8. Figure 8 shows the effect of the locations on *P. cineraria* seeds viability and seed germination percentage seeds from Al Ghuwariyah resulted in the highest germination 97% followed by Al Ghaf, Ghafat Makin 87% the lowest germination resulted by seed from Rawdat Rashed 76%. viability percentage take the same trend. if the seed quality has relationship with age that will be clear the trees from Al Ghuwariyah its youngest and Rawdat Rashed and Al Ghaf, Ghafat Makin its oldest trees in Qatar.

Figure 4 *P. juliflora* in AlGhaf, Ghafat Makin, Umm Al Hefain.

Figure 5 AlGhaf, Ghafat Makin, Umm Al Hefain after Removal of *P. juliflora* and protect site 2017.

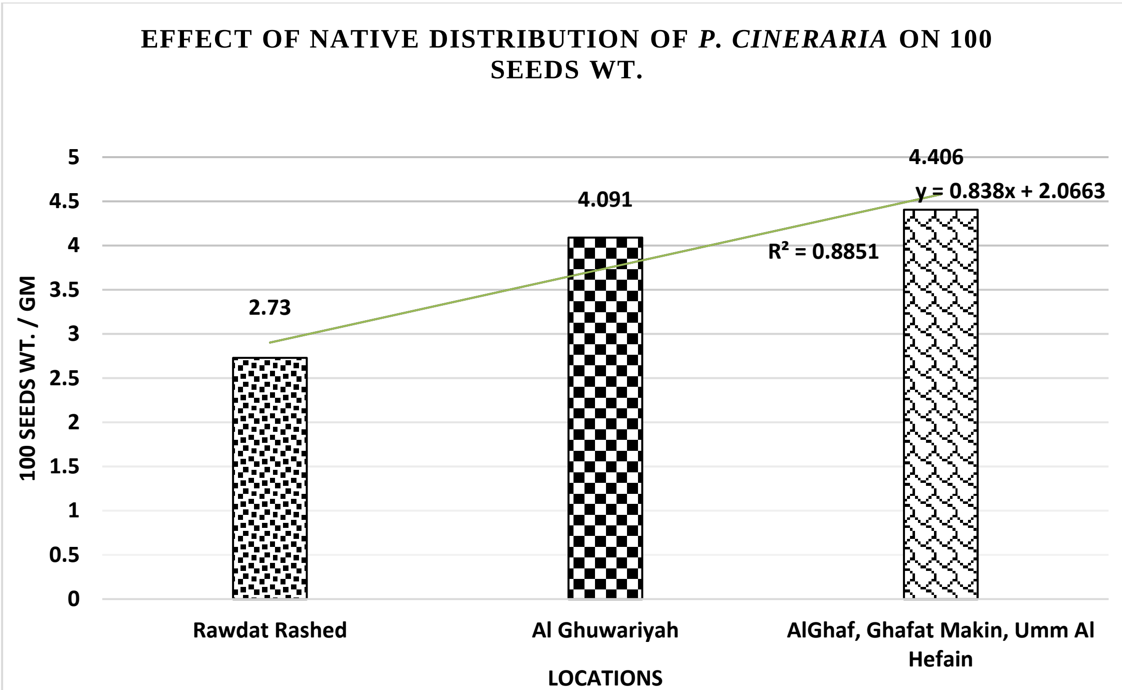


Figure 6. Effect of distribution site in 100 seed weight of *P. cineraria*.

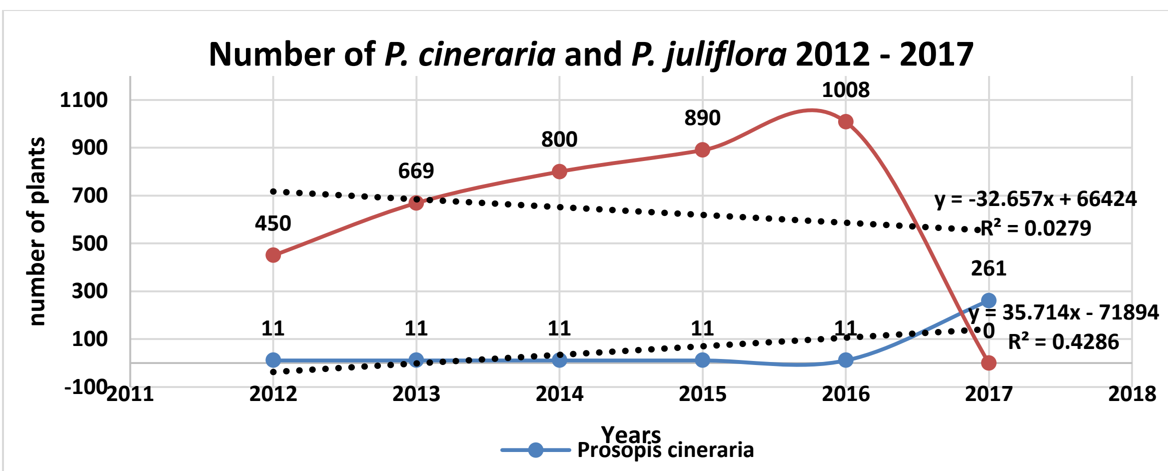


Figure 7. Number of *P .cineraria and P. juliflora* in AlGhaf, Ghafat Makin, Umm Al Hefain.

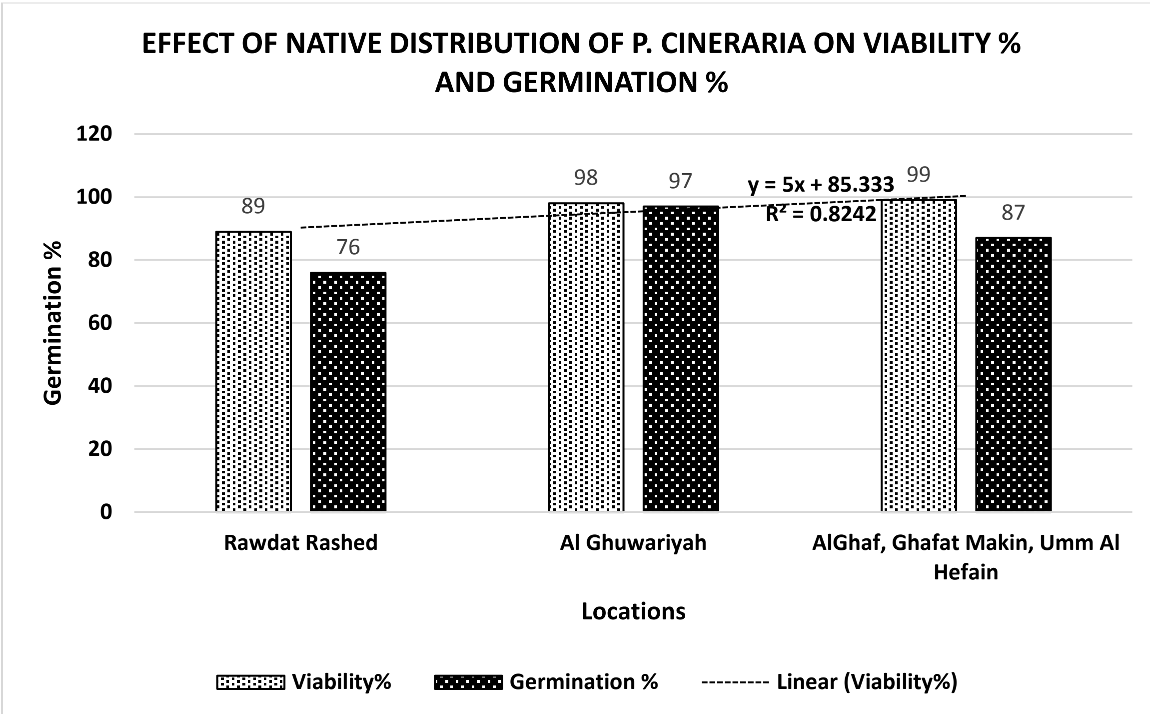


Figure 8. Effect of distribution of *P. cineraria* on Viability and Germination percentage.

Figure 9. Digital profiling of *P. cineraria.*

Conclusions

Conservation status of genetic resources of *P. cineraria* in the status of Qatar it's very endangered by many threats, we got very good results after protection site according to ministry permit 2015 to fence the most important Ghaf trees site in Al Ghaf, Ghafat Makin, Umm Al Hefain, North Qatar. We recorded during the current assessment of Ghaf trees in Qatar, the third site on the road from the north road to Al Zubarah historical site after 5.4 Km from Al-Ghwoyriyah, we recorded 8 trees on this site with high threatened by *P. juliflora* community. Seeds of *Prosopis cineraria* have highest viability % and 100 seed wt. from seed collected from Al Ghaf, Ghafat Makin, Umm Al Hefain. The oldest Ghaf trees that were recorded in North and Center of Qatar. Mechanical scarification improves Ghaf seed germination. Finally, when we looking in map Almost distributed on the same longitude.

References

Abdel-Bari, E. M. 2012. The Flora of Qatar. Vol 1. Qatar University.

Braun, B. J. 1932. Plant sociology, translated by G. D. Fuller and H.S. Conard McGraw-Hill Book Company, Inc., New York.

Gallacher, D. J. and A. El-Keblawy 2016. Macromorphology and recruitment of *Prosopis cineraria* in the United Arab Emirates. Eco. Env. & Cons. 22 (2): 499-504.

E. Abdel Bari, G. Fahmy, N. Al Thani, R. Al Thani, and M. Abdel-Dayem 2007. The Ghaf Tree *Prosopis cineraria* in Qatar. Qatar University Environmental Studies Centre, Doha.

El-Keblawy, A.; T. Ksiksi.; and H. El Alqamy. 2009. Camel grazing affects species diversity and community structure in the deserts of the UAE. *J. Arid Environ.* 73 : 347–354.

Norton J., S.A. Majid; D. Allan; M. Al Safran; B. Böer. and R. Richer. 2009. An illustrated checklist of the flora of Qatar. Browndown Publications, Gosport, UK.

Pasiecznik, N.; F. Peter.; P.J.C.; L.N. Harris; G. Harsh; J.C. Cruz; K. Tewari; Cadoret and L.J. Maldonado. 2001. The *Prosopis juliflora-Prosopis pallida* complex: A monograph. HDRA, Coventy, UK.

Leakey, R. B. and F. T. Last. 1980. Biology and Potential of *Prosopis* Species in Arid Environment with Particular Reference to *P. cineraria*. *Journal of Arid Environments*, Vol. 3, 1980, pp. 9-24.

Rao N. K.; Hanson, J.; M. E. Dulloo,; K. Ghosh,; D. Nowell; and Larinde M. 2006. Manual of seed handling in genebanks. Handbooks for Genebanks No. 8. Bioversity International, Rome, Italy.

Rastogi, A.1999. Methods in applied Ethnobotany: lesson from the field. Kathmandu, Nepal: International Center for Integrated Mountain Development (ICIMOD).

Sharma, S. K.; D. Rawat; S. Kumar; A. Kumar; S. Ku- maria; and S. R. Rao. 2010. Single Primer Amplification Reac- tion (SPAR) Reveals Intra-Specific Natural Variation in *Prosopis cineraria* (L.) Druce," *Trees-Structure and Function*, Vol. 24, pp. 855-864.

Secretariat, C. B. D. 1992. Convention on biological diversity. In *Convention on Biological Diversity*.

The Lonely Tree of Kuwait

Majda K. Suleiman, Narayana R. Bhat, Sheena Jacob and Rini R. Thomas
Kuwait Institute for Scientific Research, Kuwait
e-mail: mkhalil@kisr.edu.kw

Abstract

Kuwait is an arid country with a total area of 17,818 km^2. Its boundaries include the Arabian Gulf in the east, the Kingdom of Saudi Arabia in the north and the Republic of Iraq in the north and northwest. Due to the lack of rainfall and harsh environmental conditions, the vegetation cover in Kuwait is sparse. In spite of these conditions and limited water sources, Kuwait's desert ecosystem includes eight vegetation communities with a total of 374 species, which includes annuals, perennial woody shrubs, herbs and spring ephemerals and only one native tree, *Acacia pachyceras*, also termed the Lonely Tree. Presently, only one specimen of this species exists in the country in the Sabah Al-Ahmed Nature Reserve. Kuwait's native vegetation is highly valuable as they contain genes that are responsible for salt-, heat- and drought-tolerance. However, most of the native plant species are at the verge of possible extinction due to various natural and anthropogenic activities. For a sustainable rehabilitation, re-vegetation and/or restoration program to be implemented in degraded desert ecosystems, native plant genotypes must be preserved and propagated on a large-scale. The lack of literature on *Acacia pachyceras* hinder the restoration progress in this species in the country and hence, must be extensively studied. This paper discusses the importance of restoration of *Acacia pachyceras* and highlights studies needed for the implementing an efficient restoration process.

Keywords: Ecological restoration, desert ecosystems, *Acacia pachyceras*, stress tolerance, rehabilitation.

Background

Kuwait Environment. Kuwait is a small, flat to gently undulating desert country in the northeastern part of the Arabian Peninsula extending between latitudes 28º 33' and 30º 05' N and longitudes 46º 33' and 48º 30' E. The mainland and a number of offshore islands cover an area of 17,818 km^2 (KISR 1999). The climate is characterized by extremely hot dry summers with long, intense sunshine hours and moderately cool short winters with occasional rain. The average daily maximum temperatures vary from 18.9 ºC (based on a ten-year average from 1996 to 2004) during January to 46.8 ºC in July (Salam and Al-Mazrooei 2007). Rainfall, occuring between mid-October and late April, is minimal, averaging about 115 mm/y (ranging from 25 to 250 mm); however, evaporation is very high, in the 3.1−21.6 mm/d range. Low relative humidity and strong, dry and hot northwesterly winds prevail during summer, particularly in June and July. A recent analysis showed that the average maximum and minimum temperatures were 1.29 ºC and 1.14 ºC higher during the 1999−2004 period compared to those in 1962–1998. The annual rainfall during the 1999−2004 period increased by 18.67 mm, whereas pan-evaporation increased by 0.97 mm/d. Therefore, in Kuwait and most Gulf Cooperation Council (GCC) countries, the climate can be classified as hyper-arid (Middleton and Thomas 1997).

Soils and Land Use. Soils of Kuwait are poorly developed and predominantly sandy (> 85% sand) with negligible organic matter content (≈ 0.1%) and poor water retention capacity (KISR 1999).

In Kuwait, land resources are used for livestock grazing, water production, oil production, sand and gravel quarrying, agricultural production, and camping/ bird hunting during the winter season (Omar et al. 2001b). Rangeland (75.12% of the total surface area), which is mainly used for grazing and recreational (camping) activities, is the dominant land use (Omar et al. 2001a). Oilfields and military activities occupy 7 and 4% of the total land areas, respectively.

Terrestrial Biodiversity of Kuwait. Kuwait's biodiversity includes 374 plants, 28 mammalians, 40 reptilians and 300 bird species (Omar 1982; Omar et al. 2008). As in other arid and semi-arid countries, species composition is dominated by annuals (256 out of 374 plant species) followed by herbaceous perennials (83 species), shrubs and under-shrubs (34 species), and trees (one species). Kuwait's native vegetation reflects a transition between semi-desert and desert vegetation and a good indicator of human-induced changes (Omar and Bhat 2008). It offers a valuable gene pool and plant material for drought and salt-tolerance research (Omar and Bhat 2008).

Kuwait Vegetation. Low-fertility saline soils, prolonged drought, erratic and untimely precipitation, high evaporation rate and in the case of Kuwait, disruptive human interference have necessitated concerted efforts to restore fragile arid ecosystems. Re-establishing native vegetation can be very challenging, and strategies used for re-vegetation must be ecologically balanced, as well as technologically and economically feasible. In such situations, identifying and targeting functionally important species should be the primary focus of ecosystem restoration programs.

The native vegetation of Kuwait consists of scant perennial woody shrubs, herbs, and spring ephemerals. The perennial vegetation cover is sparse (Sudhersan et al. 2003; Brown 2001). In spite of the harsh climatic conditions and limited water sources, Kuwait has a unique desert ecosystem comprising of eight vegetation communities (Table 1)

Table 1.1. Vegetative Communities in Kuwait (source: Omar et al. 2008).

Map Unit	Area	
	($km^{2)}$)	(%)
Haloxyletum	3,696	23.0
Rhanterietum	338	2.0
Cyperetum	4,381	27.0
Stipagrostietum	6,407	39.0
Zygophylletum	41	0.5
Centropodietum	160	1.0
Panicetum	114	0.5
Halophyletum	303	2.0
Agricultural area	164	1.0
Urban area	686	4.0
Total	16,290	100.0

Kuwait's Lonely Tree. *Acacia pachyceras* Sw. (*Fabaceae;* synonyms - *Acacia gerrardii* Benth. *Acacia iraquensis* Reu.), commonly known as Talh in the middle east, is distributed in Saudi Arabia, Africa, Palestine, Kuwait and Iraq (Chaudhary, 1999; Al-Khulaidi, 2000; Le Houerou, 2003). Only one mature specimen of *Acacia pachyceras* which is more than 80 years old is still alive in Talha area of Sabah Al-Ahmed Nature Reserve (SANR). This species which is the only native tree species of Kuwait has become nearly extinct (Boulos and Al- Dosari, 1994). Thus, its preservation is of national importance (Boulos and Al-Dosari 1994; Omar et al. 2008). In other areas, such as the northern mountains of Oman, populations are reported to be well established and not threatened (World Conservation Monitoring Center, 1998).

Sabah Al-Ahmed Nature Reserve (previously known as Jal Az-Zor National Park) is situated in the northwest of Kuwait and covering an area of 330 km^2. It was established in 1986 to protect native fauna and flora from anthropogenic-related activities (El Sheikh and Abbadi 2004). The geomorphic features of the Sabah Al-Ahmed Nature Reserve include salt marches (Sabkhas), coastal desert plain, the Jal Az-Zor ridge, and the non-saline depressions representing the important habitats of the park. It protects 37% of Kuwait flora (Boulos and Al-Dosari 1994) and is habitat to the country's only native tree species, referred to as the "Lonely Tree" which accentuates the critical importance of its ecological preservation.

The Public Authority for Agriculture and Fish Resources (PAAFR) of Kuwait tried to protect the tree by erecting a fence in 1975. However, the fence attracted many types of desert users (hunters, campers, travelers and others) who caused serious damage to the trunk. After Kuwait's liberation in 1991, the tree collapsed and the trunk split into two parts. However, it started to sprout in 1992 and is now fully protected in the reserve (Omar et al., 2008). There are no other trees that grow naturally near the vicinity of this lonely tree raising serious questions regarding its reproductive capacity. As this native tree is at the verge of extinction, its restoration is urgently needed. Harsh climatic conditions and the physical dormancy of Acacia seeds make it necessary to initiate the conservation practices. Distribution of Acacia species in middle-east countries is shown in Fig. 1.

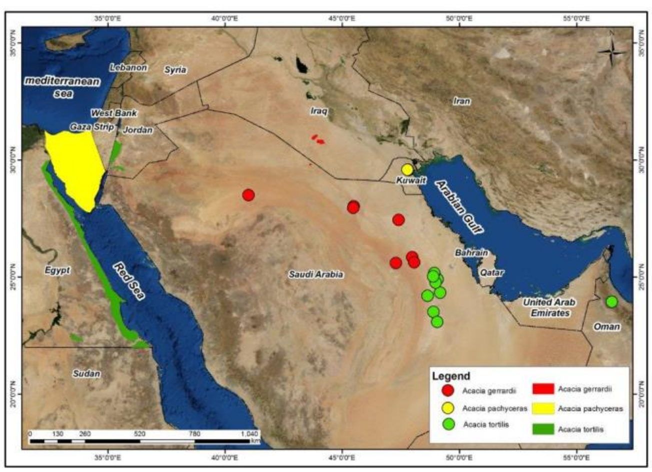


Figure 2. Distribution Map of *Acacia* Species in Middle East countries.

Restoration and rehabilitation can regenerate degraded ecosystems. Restoration involves supporting ecosystem recovery to its original condition through re-vegetation, improving soil properties, and retaining existing native plant species. On the other hand, rehabilitation is also aimed at improving degraded ecosystems. Empirical evidence shows that, in time, rehabilitation may result in an ecosystem returning to its original condition (Gilmour et al. 2000). In order to accomplish substantial rehabilitation required for restoring degraded desert ecosystems, native plant genotypes must be preserved and propagated on a large scale (Sudhersan et al. 2003).

Revegetation is currently of high priority in the country as Kuwait has embarked on the establishment of five new protected areas in Humailiya, Wadi Al-Batin, Um Gudair, Al-Kuwaiysat, and Um Nigga under the Kuwait Environmental Remediation Program (KERP), covering a total of 1680 km^2. Of the total area, 80 km^2 is currently being planned to be actively revegetated using native plants, which will require a substantial amount of seeds and seedlings including 33 million seedlings, 23 tons of seeds, three to five million seedlings and two to five tons of native seeds (Kuwait Environmental Remediation Program, 2013). Besides this, over 270 km^2 of tarcrete affected area has been included for less intensive revegetation (direct seeding). Additional quantities of seeds and nursery grown plants of keystone species will also be required for other restoration programs and ongoing / planned research and development activities.

The Lonely Tree, being the only native tree species of Kuwait, should be conserved through proper re-vegetation programs. Large-scale production of native plants is presently needed in Kuwait for national scale restoration projects. As seed production of the Lonely Tree is presently minimal in its natural habitat, it was important to conduct research on the restoration of this iconic native and last living individual tree. Absence of natural regeneration of this species should be investigated for its restoration into the desert ecosystem. To this end, information on the seed biology and ecology of the Lonely Tree should be studied in detail. Initial research should begin by developing its propagation protocols and providing a naturally healthy environment for its growth and performance.

Additionally, some projects which can be referred in relation to this area of research focused on the standardization of propagation techniques of selected indigenous plants and evaluation of their performance under urban conditions, mass propagation of native plants and establishment of field plantations, establishment of national seed bank for the native plants of Kuwait, enhancement of seed bank unit, and restoration potential of selected native plant species in Kuwait. One of the on-going projects has initiated research on *Acacia pachyceras*, suitable planting techniques and its salinity tolerance levels. Realizing the gap in the knowledge of this special species and the available information, another project focuses on the seed enhancement techniques for better germination.

Most importantly, researchers have to focus on (1) understanding seed biology to optimize dormancy break and germination conditions (2) determine seed storage behavior and longevity (3) agronomic cultural practices for efficient seedling growth and maintenance.

Recommendations

The main problem encountered in utilizing *Acacia pachyceras* (or *Acacia gerrardii* var *najvensis)* in restoration programs is the poor germination of its seeds. This could be due to impermeability of seed coat to water (Holmes et al., 1987), thickness of seed coat (Halavey, 1974; Janzen, 1975; Karschon, 1975; Sabiiti et al., 1991), susceptibility to insect damage (Donahaye et al., 1966; Halavey 1974; Janzen 1975), burial depth (Auld 1986; Fenner 1985; and Sabiiti et al., 1991) and/ or nonavailability of sufficient moisture in the soil (Mahgoub & Bebawi 1983) to initiate germination and sustain normal growth. Seeds that will not germinate promptly when placed under condition, which are normally regarded as suitable for germination are considered dormant. To overcome seed dormancy and obtain rapid and synchronous germination, these seeds must be subjected to some physical or chemical treatment (s) before sowing. Such pre-germination treatments will break the integrity of impermeable seed coat thereby allowing imbibition of water into the embryo and initiate germination process.

Overcoming Physical Dormancy. Generally, physical dormancy is reported in *Acacia* spp due to hard seed coat. The impermeable coat of seeds characterized by physical dormancy prevents water imbibition and thus restricts germination. These seeds generally have large embryos (bent, folded, investing or spatulate) and usually have a palisade layer of lignified cells in the seed coat, which makes them impermeable (Baskin and Baskin 2001; Vazquez-Yanes and Perez-Garcia 1976; Corner 1976).

Notwithstanding, physical dormancy is sometimes confused with physiological dormancy (due to physiological inhibiting factors) as some seeds with deep physiological dormancy respond to mechanical or chemical scarification. To eliminate this confusion, checking the impermeability of the seed coat by evaluating the imbibition of the scarified and control seeds is required (Baskin and Baskin 2003). Seeds that exhibit physical dormancy do not imbibe water, thus indicating impermeability of the seed coat. In contrast, seeds with physiological dormancy might imbibe water but will not germinate. Therefore, the presence of physiological mechanism can prevent germination.

In nature, physical dormancy is broken in many *Acacia* species by the exposure of seeds to wet and dry cycles (Bowen and Eusibio 1981), flooding (Badi et al. 1989), frequent fires (Sabiiti and Wein 1987; Kulkarni et al. 2007), or passage through the digestive tract of animals (Miller 1995; Cox et al. 1993).

Acid scarification, hot water treatment (wet heat), mechanical scarification, and dry heat treatment can break the physical dormancy in seeds. Treatment duration may vary with species, depending on seed coat thickness and other inherent characteristics. Immersion of seeds in hot water (wet heat treatment) also produced good results in several species. However, duration of exposure and treatment temperature differed with each species. For example, when the treatment temperature was lower, seeds could withstand longer treatment duration and vice versa (Baskin and Baskin 2001).

Exposing seeds to dry heat was also successful in breaking dormancy. Similar to wet heat, the temperature duration of dry heat varied with species. Although exposure to dry heat developed cracks on the seed coat and in the palisade layer of the lens/ strophiole area of the seeds of *Acacia* spp., the cracks on the seed coat were typically not deep enough to allow water to enter the embryo (Brown and Booysen 1969; Cavanagh 1987).

Usually, the impermeable coat of seeds characterized by physical dormancy has a weak point in the testa, which cracks upon treatment or under certain natural conditions to allow entry of water (water gap) into the seeds, thus facilitating imbibition and germination (Baskin and Baskin 2001). The point of water entry differs with each family (Baskin and Baskin 2000). Considering the variations in specialized water gap structures among Fabaceae sub-families, it is important to understand their morphology in *Acacia gerrardii* in order to gain a deeper knowledge and understanding of its seed biology.

Seed Storage Behavior and Longevity. Storing seeds in a seed bank is an effective method of *ex- situ* conservation and these seeds can be used in re-vegetation programs. Although a seed is viable at a particular point of time, its viability might be lost during prolonged storage. The period during which a seed can be stored without losing its viability under given storage conditions is referred to as longevity. Longevity can be determined by testing the viability of a seed lot at regular intervals. Since this method is time consuming, Millennium Seed Bank Project, KEW, developed a technique based on the use of accelerated aging of seeds. In this method, seeds are rehydrated and aged under high temperature and seed moisture conditions. Following the aging process, germination tests are conducted at regular intervals, and the total germination percentage is plotted against the storage period. Thus, the time taken for 50% of seeds to lose viability (p_{50} values) obtained from the probit analysis of the survival curve could be used to compare seed longevity in different species. These longevity studies help researchers and seed bank managers gain insight into the threshold storage period for each species, develop priority list for immediate storage, and determine the need for innovative storage techniques for preservation of desired seed quality (Merritt et al. 2014). The data on storage behavior and longevity of seeds are important in making decisions on storage and subsequent use of native plants in large-scale re-vegetation programs.

Seed traits, taxonomic relatedness, climate, environmental factors, seed mass, and oil content might influence longevity directly or indirectly at varying degrees (Walters et al. 2005; Probert et al. 2009; Merritt et al. 2014). Since seed longevity can be influenced by several factors, it is important to study the longevity behavior of *Acacia* spp. from Middle Eastern countries.

Seed persistence is the capacity of a seed to persist or survive in a soil seed bank, plants (in case of serotiny) and *ex-situ* seed banks (Long et al. 2015). Seed persistence varies with species, seed characteristics (seed size and shape), genetic factors and a number of abiotic factors (soil temperature, soil moisture, and pre- and post-dispersal environmental factors) (Long et al. 2015). Knowledge of seed persistence is particularly important for threatened species as it helps effectively manage restoration programs by planning and adopting appropriate methods to utilize seeds in their natural seed bank (CALM 2006). Seed persistence in the field can be predicted based on the results of controlled aging tests (CAT) conducted in the laboratory (Long et al. 2008). Seeds with p_{50} < 20 d were found to have transient persistence (< 1 year); those with p_{50} between 20 and 50 are deemed short-lived (1-3 years) and those with p_{50} >50 are considered long-lived (> 3 years) under field conditions. However, Walter et al. (2005) suggested correlation between *in-situ* persistence and *ex-situ* seed longevity. While it is not possible to predict accurately seed persistence-based CAT, rough estimates of seed persistence of threatened species could be of considerable value to restoration managers.

Seed Priming. Seed priming is a physiological seed enhancement technique (Taylor et al., 1998; Matsushima and Sakagami, 2013). It is beneficial to increase the rate; uniformity of seed germination improves leaf emergence and elongation, and shoots and root growth under drought (Farooq et al., 2013). Seed priming for seed effectiveness is an ideal method for the betterment of seed germination (Finch-Savage, 2004; Halmer, 2004). The mechanism of seed priming involves seed soaking in a priming agent in solution form, which is followed by drying to initiate germination processes without radicle emergence (McDonald, 2000). Many studies were conducted on this subject with positive (Hardegree, 1994a, 1994b, 1996, Hardegree and Van Vactor, 2000; Hardegree et al., 2002) and negative results (Emmerich and Hardegree, 1996). Seeds were soaked in different solutions with high osmotic potential, preventing seeds from absorbing enough water for radical emergence (Taylor et al., 1998). Research studies in this area will generate scientific information on seed priming methods that contributes towards significant improvement and uniformity of seed germination for an efficient revegetation program.

Salinity and Drought Tolerance. Salinity and drought are the main limiting factors for plant growth in arid environments since stress from any one of these factors or a combination of both can reduce plant growth. Salinity is a serious problem in desert environments, especially where lands are irrigated with water containing salts. Due to freshwater shortages, plants are currently being irrigated with saline water, leading to irrigation water salinization becoming a major concern (Miyamoto and Chacon, 2005). Salinity adversely affects plant growth and development. Although the virgin soil of Kuwait is non-saline, the irrigation water is brackish and saline with 3000 to 8000 ppm of dissolved salts (Suleiman and Grina, 2003). Tolerance to salinity levels by a species is a complex phenomenon which involves various genetic, physiological, and morphological adjustments. Prolonged exposure to salinity in known to reduce plant growth parameters such as height, leaf area, and number of leaf area (Sun and Dickinson, 1995). Salinity also reduces photosynthesis in woody species (Van et al., 1989). However, salt tolerant plants often are able to maintain reasonable growth by accumulating salt ions in their roots and restricting their movement to the shoots. Hence studies on the salinity and drought tolerance of *Acacia pachyceras* will be breakthrough in the revegetation efforts in Kuwait.

Restoration Ecology. The SER International Primer on Ecological Restoration (2004) defined the ecosystem restoration as the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed by using the original vegetation type. Restoration attempts to return an ecosystem to its original structure. However, the restored ecosystem may not necessarily recover its earlier state because of the existing constraints and conditions that forces it to follow an altered path. The simplest restoration programs involve removal of perturbations and permitting the ecosystem to recover through natural ecological processes. However, more often multiple perturbations are involved in degrading the ecosystems beyond their ability to recover spontaneously; the restoration strategy also requires multidimensional approach. The ecological restoration provides opportunities to conduct large-scale experiments and test basic ecological theory and in turn, help build the science of restoration ecology (Palmer et al., 1997).

Based on the results of several studies carried out to investigate the regeneration, distribution and succession in *Acacia* communities in different regions of the world (Liang, 1987; Pieterse and Cairns, 1987; Skelton, 1987; Kushalapa, 1988; Ashton and Chappill, 1989; Johansson and Kaarakka, 1992 and Diouf and Grouzis 1996) the following strategy is recommended to restore *Acacia pachyceras* in Kuwait:

- Determining the causes of degradation.
- Protecting the natural habitats.
- Setting the restoration goals (Planning).
- Establishing efficient techniques for
    - Seed collection
    - Mass propagation and seed enablement
    - Choosing suitable locations for restoration
    - Seedling establishment techniques including irrigation
- Finalization of treatments, experimental designs and layout.
- Seedling maintenance and growth monitoring.
- Monitoring of ecosystem functioning.
- Documentation.

Conclusions

Re-establishing native vegetation can be very challenging and strategies used for revegetation must be ecologically balanced as well as technologically and economically feasible. To develop a sustainable re-vegetation plan specific to Kuwait region, in depth research on all the aspects of seed biology and ecology are necessary. A comprehensive study on *Acacia pachyceras* should focus on the observation of Lonely tree, morphological characters, flowering pattern, timing and season, possible reasons for minimal seed setting, seed germination requirements, seed storage, persistence, longevity, seed enabling techniques, best planting techniques, irrigation requirements, soil amendments, and protocol for seedling maintenance. Understanding these aspects of *Acacia pachyceras* will help Kuwait to have sustainable and durable re-vegetation projects.

Acknowledgement

The authors would like to thank the Kuwait Foundation for the Advancement of Sciences (KFAS) and Kuwait Institute for Scientific Research for providing funds and encouragement during the investigation.

References

A-Khulaidi, A.W.A., 2000. Flora of Yemen, P.139.

Ashton, D.H. and J.A. Chappill, 1989. The Secondary succession in post-fire scrub dominated by *Acacia verticillata* (L'Herit.) *Australian Journal of Botany.*, **37**:1-18.

Auld, T.D., 1986. Population dynamics of the shrub of *Acacia suaveolans* (Sm.). Wild: dispersal and the dynamics of the soil seed- bank. *Australian Journal of Ecology*., **11**: 235-254.

Badi, K. A; A. E. Ahmed; A. M. S. Bayoumi. 1989 The Forest of the Sudan Agricultural Research Council (NCR), Khartoum.

Baskin, J. M., Baskin, C. C. 2003. Classification, biogeography, and phylogenetic relationships of seed dormancy. Seed Conservation: Turning Science into Practice *(eds* R.D.Smith, J.B.Dickie, S.H.Linnington, H.W.Pritchard *&* R.J.Probert*), pp.* 518-544*. Kew, The Royal Botanic Gardens.*

Baskin, J. M; C. C. Baskin. 2001. Seeds, ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego.

Baskin, J.M; Baskin CC, Li X. 2000 Taxonomy, anatomy and evolution of physical dormancy. *Plant Species Biology* **15**, 139-152.

Boulos, L; M. Al-Dosari. 1994. Checklist of the flora of Kuwait. *Journal of the University of Kuwait (Science)* **21**, 203-218.

Bowen,M.R; T. V. Eusibio. 1981 *Acacia mangium Updated information on seed collection, handling, and germination testing*. Occational Technical and Scientific notes, Seed Series No. 5, Forest Research Center, Sepilok, Sabah.

Brown, G. 2001 Vegetation Ecology and Biodiversity of Degraded Desert Areas in North-Eastern Arabia.-Habilitation Thesis. Rostock University. pp.9-92.

Brown, N. A. C; V. P. de Booysen. 1969. Seed coat impermeability in several *Acacia* species. *Agroplantae* **1**, 51-60.

CALM. 2006. Corrigin Grevillea (*Grevillea scar*pigera) Interim Recovery Plan No. 224, 2006-2011. Department of Conservation and Land Management, Perth.

Cavanagh, A.K. 1987. Germination of hard seeded species (order Fabales). In ‘Germination of Australian native palnt seeds’ (Eds. P Langkamp). Inkata Press, Melbourne.

Chaudhary, S. A, 1999. Flora of the Kingdom of Saudi Arabia. Ministry of Agri. and Water, Riyadh.

Corner, E. J. H. 1976. The seeds of Dicotyledons, Vols I and II Cambridge University Press, Cambridge/London/New York/Melbourne.

Cox, J. R; A. de Alba-Avila; R.W. Rice; J. N. Cox. 1993. Biological and physical factors influencing *Acacia constricta* and Prosopis velutina established in the Sonoran Desert. *Journal of Range Management* **46**, 43-48.

Donahaye, E., S. Navarro and M. Calderon, 1966. Observations on the life cycle of *Caryedon gonagra* (F) on its natural host in Israel, *Acacia spirocarpa* and *A. tortilis*. *Tropical Science.*, **8**:85-89.

Diouf, M. and M. Grouzis, 1996. Study the Natural distribution of *Accaia tortilis* (Forssk.) hayne Subsp. Raddiana (Savi). *The International tree crops J.*, **9**: 69-75.

El- Sheikh, M. A. E.; G. A. Abbadi. 2004. Biodiversity of plant communities in the al-Az-Zor National Park, Kuwait. Kuwait Journal of Science and Engineering **31**(1),77-105.

Farouq, M; M. Irfan; T. Aziz; I. Ahmad; and S. A. Cheema. 2013. Seed priming with ascorbic acid improves drought resistance of wheat. *Journal of Agronomy and Crop Science* **199**: 12-22.

Fenner, M. 1985. Seed Ecology. 1st edition. Chapman & Hall, London, 151 pp. Halavey, C., 1974. Effect of gazzell's and seed beetles (Bruchidae) on germination and establishment of *Acacia* species. *Israel Journal of Botany*., 223:120-126.

Finch-Savage, W. E .2004. The use of population-based threshold models to describe and predict the effects of seeded environments on germination and seedling emergence of crops, pp. 51-84 . In: Benech-Arnold R. L. and Sanchez R. (Eds.). *Handbook of Seed Physiology*. Food Product Press, New York.

Gilmour, D.A.; N. V. San; X. Tsechalicha. 2000. Rehabilitation of degraded forest ecosystems in Combodia, Lao PDR, Thailand and Vietnam: An Overview. Conservation Issues in Asia. IUCN-Asia, Cambridge.

Halmer, P. 2004. Methods to improve seed performance in the field, pp.125-156. In: Benech-Arnold R. L. and Sanchez R. (Eds.). Handbook of Seed Physiology. Food Product Press, New York.

Halavey, C., 1974. Effect of gazzell's and seed beetles (Bruchidae) on germination and establishment of *Acacia* species. *Israel Journal of Botany*., 223:120-126.

Hardegree S. P. 1994 a. Drying and storage effects on germination of primed grass seeds. *Journal of Range Management* **47**:196-199.

Hardegree S. P. 1994 b. Matric priming increases germination arte of Greaqt Basin native perennial grasses. *Agronomy Journal* **86**:289-293.

Hardegree S. P. 1996. Optimization of seed priming treatments to increase low-temperature germination rate. *Journal of Range Management*. **49**: 87-92.

Haredegree S. P. and S. S. Van Vactor, 2000. Germination and emergence of primed grass seeds under filed and simulated-field temperature regimes. *Annals of Botany* **85**: 379-390.

Hardegree S. P., T. A. Jones, and S. S. Van Vactor. 2002. Variability in Thermal Response of Primed and Non-primed seeds of Squirreltail [*Elymus elymoides* (Raf.) Swezey and *Elymus multisetus* (J. G. Smith) M.E. Jones]. *Annals of Botany* **89**: 311-319.

Holmes, R. J., J. N. A. W. McDonald, and J. Juritz. 1987. "Effects of clearing treatment on seed bank of the Alinene invasive shrubs *Acacia saligna* and *Acacia cyclops* in the southern and south western Cape, South Africa". *Journal of Applied Ecology*., **24**: 1045-1051.

Janzen, D.H, 1975. Interactions of seed and their insect predators/parasitoids in a tropical deciduous forest. In. Price, P.W. (ed.) Evolutionary strategies of parasites insects and mites. Plenum, New York.

Johansson, S.G. and V.J. Kaarakka, 1992. Regeneration of cleared *Acacia* in Kenya. *J. Vegetation Sci.*, 3: 401-406.

Karschon, R., 1975. Seed germination of *Acacia raddiana* Savi and A. *tortilis Hayne* as related to infestation by bruchids. Agricultural Research organization Leafnet No. 52, Division of Science Publication, Israel.

Kushalapa, K.A., 1988. Comparative productivity of *Acacia ariculiformis* and *Casuarina equisetifolia* in high rainfall areas. Nitrogen fixing tree research reports. **6**: 12.

KISR. 1999. Soil survey for the State of Kuwait. Volume II. Reconnaissance survey. (Adelaide, Australia: AACM Int.) pp. 270.

Kulkarni, M. G; S. G. Sparg; J. Van Staden. 2007. Germination and post-germination response of *Acacia* seeds to smoke-water and butenolide and a smoke derived compound. *Journal of Arid Environments* **69**, 177-187.

Kuwait Environmental Remediation Program, 2013.Volume II: Project plans and Supporting documents. Biannual Report No. 9 submitted to the United Nations Compensation Commission, February (SP006C).

Liang, S.B., 1987. Research on *Acacia mangium* in Sabah ACIAR proceedings series. 16: 164-166.

Le Houérou, H.N., 2003. Bioclimatology and phytogeography of the Red Sea and Aden Gulf Basins: A monograph (with a particular reference to the highlandevergreen Sclerophylls and lowland Halophytes), Arid Land Research and management 17: 177-256.

Long, R. L; M. J. Gorecki; M. Renton; J. K. Scott; L. Colville; D.E. Goggin; L. E. Commander; D. A.Westcott; H. Cherry; and W. E. Finch-Savage. 2015. The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biological Reviews* **90**, 31-59.

Long, R. L; F. D..Panetta; K. J. Steadman; R. J. Probert, R.M. Bekker; S. Brooks;, S. W. Adkins. 2008. Seed persistence in the field may be predicted by laboratoty-controlled ageing. *Weed Science* **56**, 523-528.

Mahgroub, S. and F. Bebawi, 1983.Seed dormancy rhythm of Sudanese Acacias. *Sudan Silva*. 25 (v): 108 – 123.

Matsushima K. and J. Sakagami. 2013. Effects of Seed Hydropriming on Germination and Seedling Vigor during Emergence of Rice under Different Soil Moisture Conditions. American Journal of Plant Sciences. Vol.4 No.8, Article ID:35353,10 pages.

McDonald M.B. 2000. Seed priming. In ‘‘Seed Technology and Its Biological Basis’’ (M. Black and J. D. Bewley, Eds.), pp. 287–325. Sheffield Academic Press, Sheffield, UK.

Merritt D. J. A. J.Martyn; P. Ainsley; R. E.Young; L.U.Seed; M. Thorpe; F. R. Hay; L.E. Commander; N. Shackelford; C.A.Offord; K.W. Dixon; R.J. Probert. 2014. A continental-scale study of seed lifespan in experimental storage examining seed, plant, and environmental traits associated with longevity. *Biodiversity Conservation* **23**, 1081-1104.

Middleton, N; D. Thomas. 1997. World Atlas of Desertification. United Nations Environment Program/ Amold, pp, 182.

Miller, M. F. 1995. *Acacia* seed survival, seed germination and seedling growth following pod consumption by large herbivores and seed chewing rodents. *African Journal of Ecology* **33**, 194-210

Miyamoto S., and A. Chacon. 2005. Desert dreamscapes: Residential landscape preference and behavior. *Landscape and Urban Planning*. **78** (1-2): 85-100.

Omar, S. A. 1982. Baseline information on native plants of Kuwait. Kuwait Institute for Scientific Research Technical Report No. KISR 1790, Kuwait.

Omar S. A.; R. Misak; H. Minkarah; H. Minkarah; P. King; A. Kwarting; H. Abo-Rezq; W. Roy. 2001a. Land-use mapping for the State of Kuwait Using the Geographical Information System (GIS). *Arabian Gulf Journal of Scientific Research* **19**(2), 59-65.

Omar, S.A; R. Misak; S. A. Shahid; R. Malik; T. Madouh; H. Abo-Rezq. 2001b. Rehabilitation and management of Kuwait’s rangelands for sustainable yield. *Kuwait Institute for Scientific Research* Report No. KISR 6087 Kuwait.

Omar S. A. S; Y. Al-Mutawa; S. Zaman. 2008. Vegetation of Kuwait- A compressive illustrative guide to the flora and ecology of the desert of Kuwait. Kuwait Institute for Scientific Research, Kuwait. P.I.

Omar S. A. S; N. R. Bhat. 2008. Alteration of the *Rhanterium epapposum* plant community in Kuwait and restoration measures. *International Journal Environment Stududies* **65**, 139-155.

Palmer, M. A., R. F. Ambrose, and N. L. Poff, 1997. Ecological theory and community ecology. *Restoration Ecology*. **5** (4): 291–300.

Pieterse, P. J. and A.L.P. Cairns, 1987. The Factors affecting the reproductive success of *Acacia longifolia*, *South African j. botany*, **19**: 461-464.

Probert, R. J.; Daws, M.I.; F.R. Hay. 2009. Ecological correlates of *ex-situ* seed longevity: a comparative study on 195 species. *Annals of Botany* **104**, 57-69.

Sabiiti, E.N., W.R Wein, and E. Edroma, 1991. Fire browsing effects on the biology of east African *Acacia* species: a review. *African Wildlife research and Management*., **1**: 26-29.

Sabiiti, E.N.; R.W. Wein. 1987. Fire and *Acacia* seeds:A hypothesis of colonization success. *Journal of Ecology* 74, 937-946.

Salam, A. M; S. Al-Mazrooei. 2007. Changing patterns of climate in Kuwait. *Asian Journal of Water Environment and Pollution* **4**, 119-124.

Skelton, D. J., 1987. Study the Distribution and ecology of Papua New Guinea *Acacia*s. ACIAR proceedings series. 16: 38-4 Diouf, M. and M. Grouzis, 1996. Study the Natural distribution of *Acacia tortilis* (Forssk.) Hayne subsp. *raddiana* (Savi). *The International tree crops J.,* **9**: 69-75.

Society for Ecological Restoration International Science & Policy Working Group., 2004. The SER International Primer on Ecological Restoration. www.ser.org & Tucson: Society for Ecological Restoration International.

Sudhersan, C.; M. AboEl-Nil; J. Hussain. 2003. Tissue culture technology for the conservation and propagation of certain native plants. *Journal of Arid Environments* **54**, 133-147.

Suleiman, M. K.; N. R. Bhat; 2003. performance of ornamental plants in bioremediated soil. *Arid Land Research and Management* **17**(2), 169-176.

Suleiman, M.K., and R.H. Grina. 2003 . Evaluation of new ornamental plants for use in Kuwait's landscape and demonstration gardens establishment. Proposal. Kuwait Institute for Scientific Research, Report No. KISR6342, Kuwait.

Sun, D., and G.R. Dickinson. 1995. Survival and growth responses of a number of Australian tree species planted on a saline site in tropical north Australia. *Journal of Applied Ecology* **32**: 817–826.

Taylor AG, Allen PS, Bennett MA, Bradford KJ, Burrisand JS, Misra MK.1998.Seed enhancements. *Seed Science Research.* **8**: 245-256

Van der Moezel, P.G., C.S. Walton, G.V. N. Pearce-Pinto, and D.T. Bell. 1989. Screening for salinity and waterlogging tolerance in five Casuarina species. *Landscape and Urban Planning* **17**(4): 331-337.

Vazquez-Yanes C., B. Perez-Garcia. 1976. Notas sobre la morfolgia y la anatomia de la testa de las semillas de *Ochroma lagopus* Sw. *Turrialba* **26,** 310-311.

Walters, C.; L. M. Wheeler,; J. M. Grotenhuis, 2005. Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research* **15**, 1-20.

World Conservation Monitoring Centre, 1998. Acacia pachyceras Var. Najdensis. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. < www.iucnredlist.org> . Down loaded on 22 August, 2012.

Species Restoration Through *Exsitu* Conservation: Role of Native Seeds in Reclaiming Lands and Environment

Sobhia M. Saifan
National Center for Agricultural Research and Extension (NCARE)
Baqa'1938, Jordan. Moteh University, Karak
e-mail: saifan.sabah@gmail.com

Abstract

Drylands play a vital part in resilient landscapes at multidisciplinary scales. Restoring degraded drylands ecosystems is a key not only for confronting climate change and biodiversity loss, but also for improving direct ecosystem services that support livelihoods. Achieving expected goals of restoration requires careful planning, scientific knowledge, and proper infrastructure. While the success of any ecological restoration efforts depends on several technical issues including assessment of vegetation cover and estimation of diversity index, production of phenology tables, identification of pollinators, collection and conservation of native seeds, tracking and monitoring of restored locations, and estimation of biomass production (kg/ha), integration of local communities in the restoration process is vital for the success of such activities. Local, regional, and international collaborations also play a key role in biodiversity conservation and sustainable management of restored areas. Ex situ conservation of native seeds is an important aspect of ecosystem restoration for supplying seeds for restoration activities. It also reflects the state of biodiversity and ecosystem in the designated restoration site. Ex situ conservation and seed bank in particular, provide important information relevant to native species that are valuable for rehabilitation process and to match local communities needs for improving their livelihood. For example, Jordan gene bank at National Center for Agricultural Research and Extension (NCARE) hosts wild species and Crop Wild Relatives(CWR) (2058 accessions) like wild wheat (*Triticum monococcum, T. turgidum*); barley (*Hordeumspontaneum*); oat (*Avenasp*); forages (*Medicago sativa*, *Trifoliumcampestri*, *Trigonella*spp, *Onobrychis* spp., *Lathyrus*spp, and several *Vicia* spp.); wild vegetables (*Brassica*, *Daucus*, *Eruca*, *Allium*, *Asparagus*, *Lactuca*,); trees (*Pyrus*, *Ficus*, *Amygdalus*, *Pistacia*); and medicinal plants like *Teucrium polium*that have good potential for utilization in restoration programs in the country.

Keywords: Seed Bank, drylands, native vegetation, diversity index, climate change, international collaboration.

Introduction

Dryland systems are ecosystems characterised by water scarecity, frequent drought, high climatic variability, high wind vilocity and various forms of land degradation including desertification and loss of biodiversity. Dry lands are diverse in terms of their climate, soils, flora, fauna, land use and people, thus no consistent characterization or practical definition of drylands can be made because of this diversity. According to FAO drylands are areas with a length of growing period (LGP) 1–179 days (FAO, 2000); this includes regions classified climatically as arid, semi-arid and dry subhumid. The UNCCD classification employs a ratio of annual precipitation to potential evapotranspiration (P/PET), it includes evaporation from the soil and transpiration from the vegetation from a specific region in a given time interval (UNCCD, 2000), thus drylands occurred where evaporation is greater than pricipitation and characterized by a P/PET of between 0.05 and 0.65. Accordingly, four categories of dry lands cover earth's surface: hyper arid (6.6%), arid (10.6%), semiarid (15.2%) and dry subhumid (8.7%) (UN 2010). Large areas of arid zones are located in north and south america, north africa, sahelian region, africa south, the near east and asia and Pacific region between latitude 15 and 30 in nothern and southern hemispheres. Thus, arid lands occupy 5.36 million Km^2 and cover 41% earth surface, it include cultivated lands, scrublands, shrublands, grasslands, savannas, semi-deserts and true deserts (Mortimore 2009). On the otherhand, drylands are a vital part of the earth's human and physical environments, their ecosystems play a major role in global biophysical processes by reflecting and absorbing solar radiation and maintaining the balance of atmospheric constituents (Ffolliott et al., 2002). Dry lands are home of 2.5 billion people who rely directly on arid land ecosystem services for their livelihoods (UN EMG 2011). Drylands support one-third of the global population, they provide much of the world's grain and livestock, forming the habitat that supports many vegetable species and fruit trees and micro-organisms. Mahesh and Victor 2017 reported that dry lands support up to 44% of all the world's cultivated systems and about 50% of the world's livestock. However human populations of drylands are suffering from hunger, malnutrition, insecure and poverty due to land degradation and desertification.

Dry areas are relatively vulnerable to soil erosion, salinization and land degradation, these features show the way to desertification which defined as degradation of resource (land, water, vegetation and biodiversity) and has been agreed to be the major environmental problem in dryland areas (UNCCD, 2000). The degradation of land resources in arid, semi-arid and dry sub-humid areas results from a process or a combination of processes primarily caused by: a) vegetation degradation, fragmentation, biodiversity loss and reduced cover due to factors such as excessive wood collection, encroachment, land conversion for inappropriate agricultural practices, unplanned grazing, and the invasion of exotic species; and b) soil degradation due to erosion, compaction, nutrient mining, the loss of soil biodiversity, salinization (especially associated with irrigated lands), sand encroachment and contamination. In general degradation decrease the capacity of arid or semiarid ecosystems to supply a range of services, including forage, fuel, timber, crops, fresh water, wild-harvested foods, biodiversity habitat and tourism opportunities (Scholes, 2009). The sustainable management of drylands is essential for achieving food security and conservation of biomass and biodiversity.

According to the desertification paradigm, which is based on the assumption that natural systems are in a state of equilibrium that can be irreversibly disrupted, desertification leads to loss in productivity and increasing poverty due to the losses of ecosystem services that typically occur when grasslands transition to systems dominated by bare (unvegetated) ground or by woody plants that are unpalatable to domestic livestock (Debra et al, 2015). However, there is increasing evidence of the recovery of areas that were previously thought to be irreversibly degraded. The present paper discuss the opportunity for successful restoration using seeds of native species conserved *Ex situ* in seed banks for reclaiming lands and environment.

Restoration goals

Restoration of degraded drylands is a key not only for confronting climate change and biodiversity loss, but also for improving direct ecosystem services that support livelihoods, land productivity, environmental services and the resilience of human and natural systems. Restoration is a practice of renewing and restoring degraded ecosystems and habitats in the environment by integrating human in restoration process. restoration covers a wide range of conservation and sustainable management that increase the quality and diversity of land resources, thus enhancing ecological integrity and human well-being. Forest and landscape restoration often encompassing several ecosystems and land uses, as a way of enabling users to achieve trade-offs among conflicting interests and balancing social, cultural, economic and environmental benefits. Ecological restoration is focussed on the recovery of functional plant communities as they have a controlling influence on energy flows, hydrology, soil stability, habitat quality and network dynamics (Pocock et al 2012), for example, restoration is used: as a tool for reducing the spread of invasive plant species and preserve community structure, in studying natural disturbance regime, in initiating and accelerating ecological successional processes, in reversing the effects of fragmentation and increasing habitat connectivity, and in ecosystem function that describes the foundational processes of natural systems including nutrient cycles and energy fluxes. Eecosystem restoration is the return of a damaged ecological system to a stable, healthy, and sustainable state that have been degraded, damaged, or destroyed, often together with associated ecosystem services. Thus, conceptual frameworks for dryland restoration are based on conceptual models of plant and human community development and response to disturbance.

Restoration Frame work

Restoration is globally acknowledged as a way of reversing degradation processes. The international policy framework for restoration has been agreed all through various conventions and meetings, amongs are: i) Aichi Biodiversity Targets of the Convention on Biological Biodiversity (CBD), target 15 (by 2020, ecosystem resilience and the contribution of biodiversity to carbon stocks has been enhanced, through conservation and restoration at least 15 per cent of degraded ecosystems); ii) The Bonn Challenge: a global effort launched at a ministerial conference in September 2011 to restore 150 million hectares of degraded and deforested land by 2020 as a contribution to Aichi Biodiversity Target 15; iii) United Nations Framework Convention on Climate Change (UNFCCC): the Intergovernmental Panel on Climate Change recommends forest restoration as an efficient means to considerably increase carbon stocks and reduce emissions; iv) The ten-year strategy (2008–2018) of the United Nations Convention to Combat Desertification (UNCCD) aims to take coordinated action nationally, regionally and internationally and to monitor, globally, land degradation and restore degraded lands in arid, semi-arid and dry subhumid areas"; v) In a joint statement issued at the Rio+20 conference, the executive secretaries of the three Rio conventions committed to tackling sustainable development challenges by focusing on prioritized cross-cutting themes, which include landscape and ecosystem-based approaches to adaptation (e.g. ecosystem restoration); vi) The Global Strategy for Plant Conservation (2011–2020): Target 4 "at least 15 percent of each ecological region or vegetation type secured through effective management and/or restoration"; vii) The FAO State of the World Forest Genetic Resources (FAO, 2014) and its Global plan of action for the conservation, sustainable use and development of forest genetic resources include priority area 3 and strategic priorities 12 and 13 on the use of appropriate genetic material in restoration, rehabilitation, and national plantation programmes.

Toward successful restoration (Guidelines)

Planning. Restoration initiatives implemented in separation and over short term are mostly unsustainable. Achieving expected goals of restoration requires careful planning to make initiatives strongly embedded in larger processes that enable them to interlink and complement other initiatives. Priority actions can be taken by policymakers at the national levels to enable successful restoration and deliver sustainable impacts. The Restoration Opportunities Assessment Methodology (ROAM) developed by the International Union for Conservation of Nature (IUCN) is a helpful reference becaause it provides a framework approach by which countries can identify and analyse the potential for forest and landscape restoration (FLR) and locate specific areas of opportunity at a national or subnational level (IUCN, 2014). Among assessments are: defining the potential goals and scope of restoration work; Identifying priority areas for restoration; developing a shortlist of feasible restoration interventions to work across the different kinds of degraded land; quantifying the costs and benefits of each intervention type; analyzing finance and investment options for restoration; diagnosing the presence of success factors to understand what social, legal, or political institutions must be strengthened or put in place for restoration to succeed. By implementing ROAM, decision-makers and stakeholders can expect the following: better information for improved land-use decision-making; high-level political support for FLR; fundamental inputs to national strategies on FLR, adaptation and biodiversity and reinforcing coherence among such strategies; a basis for the better allocation of resources in restoration programmes; the engagement and collaboration among key policymakers and decision-makers; and shared understanding of FLR opportunities and the value of multifunctional landscapes.

Knowledge and practice. The other signficant player in restoration process are practitioners who are the actors of restoration, so guiding them to actions they should consider in restoration initiatives is critical. Practitioners should meet the restoration goals and consider cost-effective strategies. On the ground, practitioners have to consider a range of restoration actions, from protection and management, to Assisted Natural Regeneration (ANR), to planting. Among guidlines is conducting baseline assessments to identify causes of degradation and designate proper restoration scenario (Newton and Tejedor, 2011). The assessment include: biophysical data(eg. land cover, geomorphology, soil properties, climate-change-related disturbances); ecological data (eg. species information, inventories, genetic diversity, distribution maps, ecological processes, environmental services); socioeconomic data(eg. livelihood , social environments, demography, gender relations) ; and capacities(eg. stakeholder analysis, legal context for land rights).

Restoration approaches and *Ex situ* conservation

Diverse restoration strategies should be promoted, the restoration type approach named planting is one of several possible activities that based on species selection (Table 1). Establishing planted forests or landscape in drylands is a commonly used approach for restoring degraded lands. Planted areas managed for the production of wood or non wood can help communities' livelihood if poorly designed and managed, however, they may show negative impacts on people, the environment and biodiversity, therefore, it is important to effectively plan any planting scheme. Successful restoration using planting approach should consider the following:

A) Selecting proper native species for the right site and consider criteria such as: i) Social preference because the well known species are easy to manage by communities. In Jordan the RBG set up the Community-Based Rangeland Rehabilitation project (CBRR) in 2007 where the first step was to hold meetings with community members, local livestock owners were offered forage replacement (barley) in exchange for not grazing on the site while the RBG conducted vegetation surveys, estimated the diversity index and biomass and developed sustainable stocking rates for restoration. In the hyper-arid coastal forests of southern Peru, both cultural identity and natural capital were enhanced by establishing a plant nursery for the production of seedlings of 30 native species from seeds and cuttings, among native species are keystone *Prosopis* spp. tree pods; ii) Soil improvement: In landscapes with specific environmental risks like erosion, salinization or pollution, it is strongly recommended to select species adapted to such limiting conditions, usually native species can withstand such circumstances; iv) Biodiversity conservation: landscapes with endangered species may require specific selection criteria for in situ or *ex situ* conservation, inventory of endangered flora (Red list) and assessment of their population status and reporoductive strategies are very helpful in the identification of species requiring restoration interventions. Assessments of endangered plant species may also consider the status of associated plant and wildlife species; v) Economic production criteria make the restoration actions more beneficiland bring support for local communities, species of economic value should assessed and discussed among stakeholders. Jordan gene bank at National Center for Agricultural Research and Extension (NCARE) is supporting a range of habitat restoration and conservation projects in arid areas in Jordan and countries of the region like Iraq, Egypt and Sudia Arabia. Seed bank database provide information on habitat, localities and status of species proposed for the type of approch planting, more than 25 habitats are represented as shown by database of NCARE genebank (Table 2).

Table 1. Restoration Approaches in Drylands (FAO, 2015)

Type of approach	Goal	Common measures
Protection and management	To protect against potential threats and prevent further degradation, and to remove barriers to natural forest regeneration.	Protection of soils against erosion. Grazing management Fire management
Assisted natural regeneration	Enhance the natural processes to regenerate tree and vegetation cover.	Enhancing seed dispersal Farmer-managed natural regeneration
Planting	Planting trees, shrubs and herbaceous species, and ensuring their survival and growth.	Species selection Production of planting material Site preparation Planting Silvicultural operations

Table 2. Habitat of Wild and Native Species Hosted by NCARE Genebank

Habitat	Number of Accessions	Glossary
Others	1233	
AC	424	AC - Agricultural crop
BY	13	BY - Backyard
CA	18	CM - Commercial market
DE	54	DE - Desert
DI	40	DI - Disturbed
ES	9	ES - Extreme arid saline
FA	19	FA - Fallow
FM	4	FM - Field margins and dividers
FO	169	FO - Forest
FS	7	FS - Farm store
GR	177	GR - Grassland

HS	49	HS - Hill side
IN	4	IN - Institution
IT	3	IT - Irano-turanian
LM	5	LM - Local market
MD	4	MD - Mediterranean
OR	15	OR - Orchard
OT	2	OT - Other (see notes)
PA	1	PA - Pasture
PE	187	PE - Protected or enclosed
RS	494	RS - Roadside
ST	14	ST - Sudano-turanian
TP	3	TP - Threshing place
WC	27	WC - Water catchment or riverside
WL	107	WL - Woodland or marginal forest
Total	3082	

B) Use Native Species and Avoid Exotic. Effeciency of using native species of trees, shrubs and grasses is high because they evolved naturally and are adapted to local ecological conditions, thus they will efficiently contribute to ecosystem resilience. Scientific and practical knowledge of indigenous species (for use, phenology, reproduction in nurseries, potential in field plantation, growth habit, nichies, dormancy, seed dispersal, pollination, productivity kg/h) in restoration is very important for successful re-establishment. Such knowledge could be obtained from local communities and from refereed institutes like ISTA and MSBP. In contrast using exotic (alien) species cause large-scale ecological disturbance by competing with and replacing native species and disrupting hydrological and other processes. *Prosopis juliflora* has been reported to inhibit the regeneration of other species in riverine forests in Kenya (Mukuria Muturi, 2012). In Jordan, NCARE genebank is contributing effectivly in restoration programs and supply high quality seeds for restoration practioners and stakeholders, it host wild species and Crop Wild Relatives (CWR) (2058 accessions) like wild wheat (*Triticum monococcum*, *T. turgidum*); barley (*Hordeumspontaneum*); oat (*Avenasp*); forages (*Medicago sativa*, *Trifoliumcampestri*, *Trigonella*spp, *Onobrychis* spp., *Lathyrus*spp, and several *Vicia* spp.); wild vegetables (*Brassica*, *Daucus*, *Eruca*, *Allium*, *Asparagus*, *Lactuca*,); trees (*Pyrus*, *Ficus*, *Amygdalus*, *Pistacia*); and medicinal plants like *Teucrium polium*, all have good potential for utilization in restoration programs. During 2003-2017, more than 1500 accessions representing various native species and with seeds amount not less than 106115 were distributed, those representing wild native species collected from Jordan and maintained by NCARE genebank (Table 3). It should be mentioned that native species supplied along with information and ecological data that expected to be very helpful in restoration process. *C) Using proper genetic material*: plant propagation material (e.g. seeds and cuttings) is a key for restoration using planting approach. Sourcing of the genetic material needs to accomplished before the proposed seeding or planting period in order to make available sufficient time for identifying and producing optimal material to meet restoration goals. On the other hand the propagated material should be matched to the environmental conditions and to the expected future conditions of the restoration site. In a domestication experiment in Jordan, NCARE seed bank established field genebank for sourcing the genetic material and in the meantime conducting a provenance tests to compare germplasm with that obtained from drier sites with the aim of improving drought resistance and commercially important traits in species such as *Gundelia tournifortii* and

Coridothymus capitatus. D) Maintaining diversity: effective restoration should consider fluctulation in climate limited knowledge of the performance of many species, so it's important to maximize genetic and species diversity from sources that are similar to existing site conditions. High genetic, species and habitat diversity is likely to provide a wide range of opportunities and options for coping with environmental change, thus increasing resilience. On the otherhand, connectivity refers to the extent to which species, resources and actors disperse, migrate and interact across patches, habitats or social domains in a socioecological system (Simonsen et al., 2014). Biodiversity conservation can be enhanced by increased landscape connectivity that allows the movement of species and genes between habitats within landscapes. An ecosystem is more resilient if more than one species or other ecosystem component can perform a given function (such as pollination) in the ecosystem (known as "functional redundancy"). Thus, the ecosystem will continue to function if an ecosystem component is lost; also, if the ecosystem is disturbed, functional redundancy should bring about a diversity of ecological responses that may help maintain functionality (Braatz, 2012). *E) Producing high-quality planting material*: The ex situe conservation of genebanks usually care to conserve high quality materials to withstand storage conditions, in this regards the applied efficient and cost-effective regeneration methods. *F) Identifying planting period, density and sites*: the best time for planting in dry areas is when the soil has sufficient water for germination and to satisfy the water needs of seedlings in the first few months. Using water-harvesting techniques and soil water storage potential can help good establishment.*G) Using water in dry* conditions: Only supplenet watering in benefit amount could be supplied if water stress occurred in the first two years after planting, using high quality seeds, seedlings and cuttings, sufficiently hardened to face field conditions.

Table 3. Distribution of Native Species Seeds by NCARE During 2003-2017

Taxons	Purpose
Pyrus syriaca	(OTH.NAT.)
Tetragonolobus sp., Sinapis sp., Tragopogon sp.	(OTH.NAT.)
Pistacia sp	Virginia University (FSH)
Medicago sp.	MSc at UOJ (OTH.NAT.)
Moringa peregrine	Medicinal plants project (FSH)
Thymus bovei, Thymus capitatus, Thymus vulgaris	Research at UOJ (OTH.NAT.)
Melissa officinalis	Medicinal plants project (FSH)
Ruta chalepensis, Sarcopoterium spinosum, Paronychia argentea, Retama raetam, Foeniculum vulgare, Teucrium polium, Urtica pilulifera, Thymus povei, Capparis spinosa, Silybum marianum, Arbutus andrachne	Royal Society for Conservation of Nature (RSCN) as plantations at the society conserves (NGOs)
Artemesia herba-alba, Teucrium polium	JUST (OTH.NAT.)
Artemesia herba-alba, Arbutus andrachne, Teucrium polium	JUST (OTH.NAT.)
Hyoscyamus desertorum, Hyoscyamus reticulatus	UOJ (OTH.NAT.)
Astragalus sp., Achilleea sp., Artemesia sp., Hirschfeldia sp., Phalaris sp., Silene sp., Sinapis sp., Malva sp., Diplotaxis sp.	UOJ (OTH.NAT.)
Artemesia herba-alba	JUST (OTH.NAT.)

Arbutus andrachne	JUST(OTH.NAT.)
Hordeum spontaneum, Torularia torulosa	(NARC)
Achillea fragrantissima	UOJ(OTH.NAT.)
Aegilops longissima, Aegilops ovata, Aegilops peregrine, Aegilops kotschyi, Triticum turgidum	UOJ (OTH.NAT.)
Salvia officinalis, Artemisia judica, Mentha longifolia, Achillea biebersteinii,Achillea falcta, Coridothymus capitatus,	MIF, KSA
Orchis anatolica, Orchis papilionacea, Orchis longifolia, Cephalanthera longifolia	(NARC)
NARC: National Agricultural Research Centers, OTH.NAT.: Others (National). FSH: Foreign stake holders.	

Conclusions

Restoring degraded drylands is essential for sustainable development of human livelihood and also for maintaining ecosystem component and servieses. Decision-making related to dryland restoration involves adequate planning and efficient technical process and both require pre assessment studies and surveys in order to identify better restoration type approach. On the other hand a complementary restoration approaches could be implemented. To meet restoration objectives, a key factor is to adopt participatory approaches with stakeholders and integrate local communities and consider soscioeconomic value of restored areas. *Ex situ* conservation of native seeds could be a significant rescue for restoration so proper collection and conservation for the native species is important. Gene banks could be an effective source of seeds for various native species as they are not only source of genetic material but also provid eco information relevant to conserved native species.

References

Bagchi, S., Briske, D.D., Wu, X.B., McClaran, M.P., Bestelmeyer, B.T. and Fernandez-Gimenez, M.E. 2012 Empirical assessment of state-andtransition models with a long-term vegetation record from the Sonoran Desert. Ecological Applications, 22, 400–411.

Braatz, S. 2012. Building resilience for adaptation to climate change through sustainable forest management. In: FAO/OECD. Building resilience for adaptation to climate change in the agriculture sector. Proceedings of a Joint FAO/OECD Workshop. Rome, FAO (available at: www.fao.org/ docrep/017/i3084e/i3084e09.pdf).

Debra PC Peters, Kris M Havstad, Steven R Archer, and Osvaldo E Sala. 2015. Beyond desertification: new paradigms for dryland landscapes. Front Ecol Environ. 13(1): 4–12.

FAO. 2000. World soil resources report No 90. Rome.

FAO. 2015. Global guidelines for the restoration of degraded forests and landscapesin drylands: building resilience and benefiting livelihoods. Forestry Paper No. 175. Rome, Food and Agriculture Organization of the United Nations.

IUCN, 2014. A guide to the Restoration Opportunities Assessment Methodology (ROAM) : Assessing forest landscape restoration opportunities at the national or sub-national level. https://portals.iucn.org. (accessed in Oct 2017).

Mahesh K. Gaur, Victor R. Squires. 2017. Climate Variability Impacts on Land Use and Livelihoods in Drylands. ISBN 978-3-3-56680-1. P 3-20

Martimore M. 2016. Dry lands opportunities, what is meant by drought. CIUCN/UNDP, Gland/London.

Newton, A.C. & Tejedor, N. 2011. Principles and practice of forest landscape restoration: case studies from the drylands of Latin America I. Gland, Switzerland, International Union for Conservation of Nature (IUCN). https://portals.iucn.org/library/efiles.

Pocock, M.J.O., Evans, D.M. & Memmott, J. (2012) The robustness and restoration of a network of ecological networks. Science, 335, 973–977.

Scholes R. J . 2009. Syndromes of dryland degradation in southern Africa. African Journal of range & forage Science.Vol 26 (3). P 113-125.

Simonsen, S.H., Biggs, R., Schlütter, M., Schoon, M., Bohensky, E., Cundill, G., Dakos, V., Daw, T., Kotschy, K., Leitsch, A., Quinlan, A., Peterson, G. & Moberg, F. 2014. Applying resilience thinking: seven principles for building resilience in social-ecological systems. Stockholm Resilience Center brochure.

UNCCD 2000. An introduction to the Unitaed Nations convention to combat desertification. http// www.unccd.int. (accessed in Oct 2017)

United Nations Environment Management Group. 2011. Global Drylands: A UN system wide. http://www.unccd.int/Global Drylands Full Report.pdf. (accessed in Oct 2017).

White, R. P., D. Tunstall, and N. Henninger. 2002. An ecosystem approach to drylands: Building support for new development policies. Information Policy Brief 1, W orld Resources Institute, Washington, DC, USA.

Conservation of native plant germplasm in Sharjah Seed Bank and Herbarium and future implications for seed-based restoration

Sanjay Gairola, Tamer Mahmoud and Hatem Shabana

Sharjah Seed Bank and Herbarium, Sharjah Research Academy
P.O. Box 60999, Sharjah, United Arab Emirates
E-mails: drsanjaygairola@gmail.com; tamer_mahmed@yahoo.com; hatem_shabana@yahoo.com

Abstract

In the United Arab Emirates (UAE), the Sharjah Seed Bank and Herbarium (SSBH) have been working to conserve the botanical heritage of the UAE in the form of seeds in long-term storage. The SSBH's collections currently represent over 1700 seed accessions from about 430 plant species. Besides, a comprehensive database is built containing all collected data related to seeds, plants or habitats and linked by photography and GIS systems. Seeds, herbarium collections and available information on these materials could be used for basic or applied research including habitat restoration purposes in the region. The availability of quality seed is considered as a foundation of a successful seed-based restoration program. To achieve quality seeds for such purposes, careful selection, collection, processing, and storage of seeds under optimal conditions are essential. Likewise, for potential use of native species in reintroduction or habitat restoration program, sound knowledge of their germination behavior is crucial. Because an efficient collection, processing, and storage of seed materials underpin the effective use of plants for habitat restoration. Seed germination tests are important for seed viability evaluation and contribute significantly to long-term seed storage in seed banks. The germinability of seeds of several plant species stored at low temperature (-18°C) in the SSBH was investigated. Seeds of tested species showed variations in germinability under seed bank conditions. Such information would be helpful in identifying species with rapid loss of germination capacity, so that old seed lots can be replaced with new collections to maintain high germination rate during storage. Constant monitoring of seed storage conditions along with periodic germination testing is an important process to ensure that the seeds being stored in the seed banks are able to produce plants for habitat restoration activities or other purposes related to the conservation biology of the species.

Keywords: Conservation, Germination, Restoration, Native seed, SSBH.

Introduction

World-wide it is now well recognized that the wild plants and their habitats are under increasing threat and many are in decline. Thus, maintaining a stock of germplasm under controlled conditions as an insurance against loss in the wild is essential. Seed banks are considered valuable for preservation of genetic diversity of wild plants threatened by land use changes, overexploitation and climate change (Millennium Ecosystem Assessment, 2005). Tus, seed banking is a viable and efficient plant conservation strategy; especially considering the impacts of anthropogenic disturbances and global climate change poses to plant diversity and for restoration purposes. Studies confirms that the desert plant seeds can remain viable for a long period of time with proper processing and storage (orthodox seed storage behaviour) (Baskin and Baskin, 2014), thus, seed banks provide an ex-situ option for conserving native plant diversity. The United Arab Emirates (UAE) is a country of contrasting landscapes, with a wide range of habitats including mountains, sand and gravel deserts, sabkhas (salt flats), and mangrove forests. The diverse flora and fauna exhibit a fascinating range of adaptations to survive in this harsh and forbidding landscape (Gardner and Howarth, 2009). Many of the natural habitats in this region are facing threats both naturally and anthropologically; affecting plant diversity in the region. In view of this, the diversity of genetic resources need to be preserved for long term utilization and research.

The global efforts in ex situ conservation through seed banking, increasing knowledge through seed biology research and sharing this knowledge ultimately contribute to global plant conservation efforts under the Convention on Biological Diversity's Global Strategy for Plant Conservation. The conservation of biological diversity is a crucial issue to the whole Arabian Gulf Region and in view of this, it is vital that the germplasm of species from UAE's arid desert ecosystems be conserved. The value of ex situ conservation is increasingly important in supporting UAE's biodiversity conservation efforts by providing resources and knowledge base to our mission to safeguard the country's flora. Seed banking is an important strategy for doing this and the Sharjah Seed Bank and Herbarium (SSBH) has pioneered seed banking of wild plant species in the UAE (Gairola et al., 2013). The technical support on seed banking process from selection and collection of species, seed processing, and storage of collections under controlled climatic conditions was provided by the RBG Kew following the established guidelines. Initially, the SSBH and the RBG Kew worked together for the seed conservation of wild rare and threatened plant species from the UAE. Consequently, some of our collections are duplicated in the Millennium Seed Bank (MSB) of the Royal Botanic Gardens, Kew (RBG Kew), as an effective means of ex situ conservation.

It has been reported that in several countries of the Arabian Peninsula there is a growing concern for the vegetation restoration of damaged landscapes, for which an in-depth knowledge of the species present there is necessary (Ghazanfar and Osborne, 2010). The restoration strategies in highly unpredictable environmental systems such as the arid and hyper-arid deserts, depend on the knowledge of limiting factors of biological processes for the different plant species. Some of these include aspects such as germination and seed dormancy of native species present in such ecosystems. Knowledge on seed germination of native species having potential use in restoration, and seed storage behavior of these species is crucial for success of restoration projects. Germination tests by SSBH are performed to evaluate the seed viability of the accessions before they are deposited and preserved under the seed bank conditions. In additions, the preserved seeds are occasionally tested for germination ability. For ecological restoration, native plants seeds are now widely advocated as they can deliver successful restoration outcomes. Because, seed material from native plants obviously will have strong adaptations to the local environmental conditions, low risks for diseases and pests and more importantly will have less water requirements for growth and establishment in dryland ecosystems. The SSBH play a significant role in securing the survival of native plant species by collection of seed and herbarium and maintaining them in safe place for long-term storage and future uses in various applications. This article provides an overview on the seed banking activities of SSBH for conservation of native germplasm in the UAE, and future implications for the seed-based restoration.

Native Seed Germplasm Conservation

Seed banking is the principal tool for the safe and efficient storage of wild plant genetic material and a sound understanding of seed maturation period, harvest, storage and germination requirements is crucial to efforts to conserve plant diversity. The native plants are locally adapted to the environment and often considered as a first choice in restoration projects. However, the seed material required for such purposes may not immediately available for use. In such cases, seed banks can provide the desired material for immediate use. Researchers typically monitor the flowering and seeding of plants in the field prior to collection. The seed banks follow advanced planning from monitoring of seeds when they are ripe and available for collections which follow a series to collect the maximum species and reflect the diversity of wild plant species. A first critical step of the seed bank process is collection of genetically representative sample of high quality seed from the wild populations in a sustainable way. The collection strategy attempts to incorporate the genetic diversity of the species throughout their geographic distribution. Within the target populations, fully mature seeds or fruits are collected. The strategy for collection of seeds from the target species followed the recommendations from the Millennium Seed Bank Project (Royal Botanic Gardens Kew, 2006) and collection storage conditions followed the international standard for long-term seed conservation (FAO/IPGRI, 1994). Further, seeds of the same species are collected from different populations to cover all genetic diversity that might be present in any single species. Species are identified carefully and detailed records of the collection source are documented. The identity of collected species from all populations sampled was verified by vouchering specimens at the Sharjah herbarium and consulting local flora books.

The SSBH is a strong conservation effort established by the Sharjah Research Academy to collect and bank the entire UAE flora. To date this effort has made considerable native seed collections across the country (Figure 1), banked them under proper storage conditions of seed bank, and also stored herbarium collections of plant species. The SSBH currently contains about 1710 seed collections (Figure 2) from all over the UAE, representing about 430 plant species, or a little over 50% of known UAE species. Approximately 15% of the collections are belonging to locally rare species. The top 10 families with a large number of seed accessions in SSBH are presented in Figure 3. More researches for determining the optimal conditions for seed storage need to be attempted to provide measures for establishing the longevity potential of seed banking for conservation. The stored collections in seed bank can serve as a safeguard opportunity for species lost in the wild to be reintroduced and also provide the necessary seeds for restoration purposes.

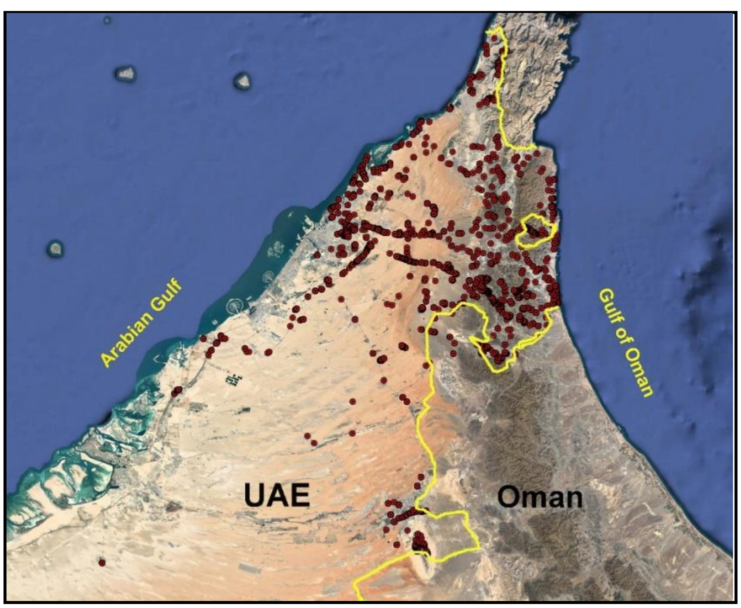


Figure 1. Distribution of collection points (ca. 275 field trips).

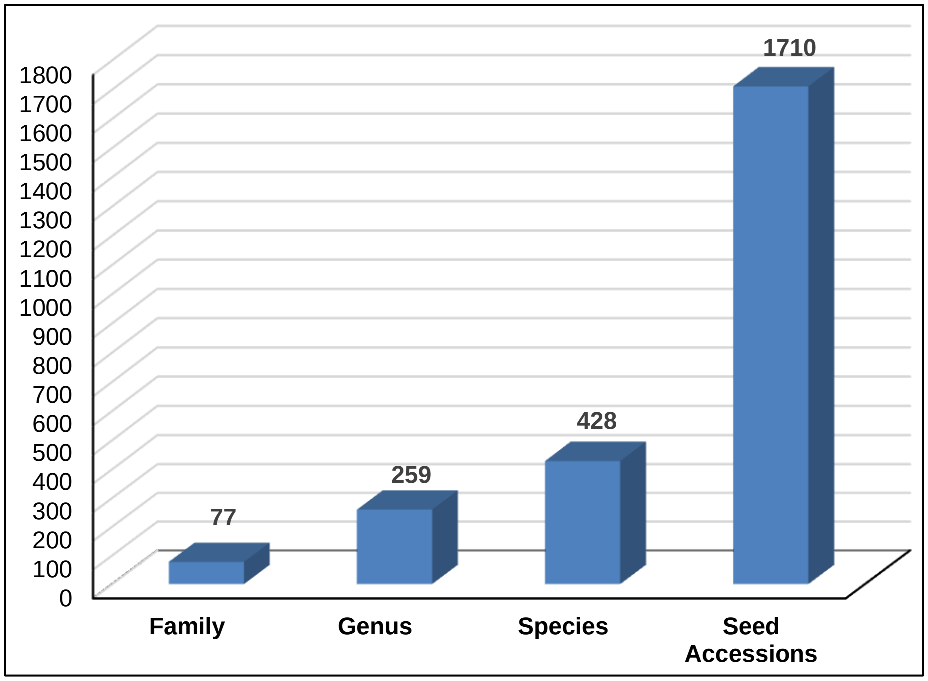


Figure 2. Representation of collections in SSBH.

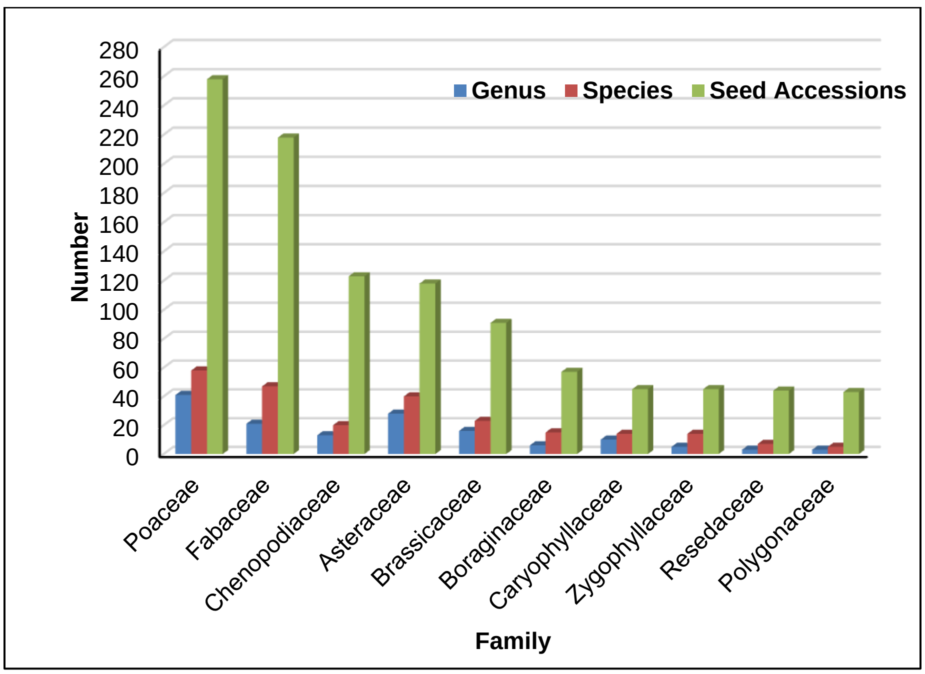


Figure 3. Top ten families with a large number of seed accessions in SSBH.

Relevance of Herbarium Collections in Restoration

Herbarium collections are a valuable adjuncts to all botanically oriented research and constitute permanent and often well-documented records of the distribution of a taxa. The knowledge of historical patterns of vegetation and species ranges in a given area offer a basis for rehabilitation of degraded lands and revegetation success. Records of the flora of the past become reference point to a more successful restoration and revegetation programs. The amassed database of herbarium specimen information for the region can be used to understand how the flora has changed over the time. This is especially critical in the desert ecosystems, where several native desert plants are being threatened and are facing danger of extinction due to anthropogenic causes (Sudhersan et al., 2003). Thus, to conserve the biodiversity of these important ecosystems, restoration and revegetation plans become crucial (Suileman et al., 2011).

Correctly identifying seed collections is essential if the seeds are to be utilized in restoration and revegetation programs. In this case, the herbarium specimen is the specimen that enables verifiable identification of a taxon. Consequently, it is essential to collect voucher materials such as herbarium specimens at the time of seed collection in order to accurately identify seeds to a particular species and to verify collection names. The details about collection location, vegetation information, date, collectors etc. are recorded and entered into the SSBH database. The herbarium collections in SSBH, are a unique library of ca. 4500 herbarium sheets belonging to 67 families, 254 genus and 390 species (Table 1). Number of species represented under different occurrence categories in SSBH herbarium are given in Figure 4, which shows that considerable species also falls under not - common and rare category. The herbarium collections cover most of the UAE habitats, where the highest number of collections are from sand plain and mountain areas while the lowest from the gravel plain. These collections are the best source of information on the flora of UAE and shows the potential of collections in Sharjah herbarium in the enrichment of biodiversity knowledge and their use in research applied particularly to vegetation restoration and conservation. Strict record keeping, modern technology and information sharing with other herbaria are essential to enable the SSBH herbarium to keep up to date with new ideas and utilization of collection's information in plant conservation.

Table 1. Main Families in SSBH Collection with Genus and Species Representation

Family	Genus	Species	Collections
Poaceae	35	50	148
Fabaceae	22	49	162
Asteraceae	31	45	121
Chenopodiaceae	13	19	67
Brassicaceae	15	18	55
Boraginaceae	9	16	53
Caryophyllaceae	7	11	33
Scrophulariaceae	7	11	32
Capparaceae	3	10	42
Convolvulaceae	3	10	33
Euphorbiaceae	4	10	39
Rest (56 families)	106	141	494

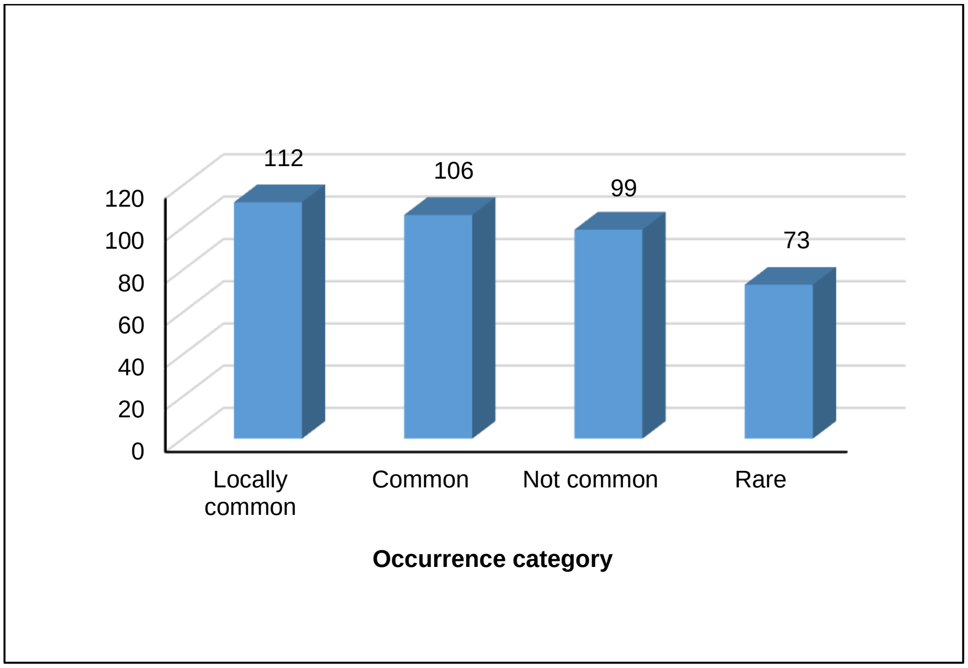


Figure 4. Occurrence category of species represented in SSBH herbarium.

Understanding Seed Germination Behaviour of Native Plant Species

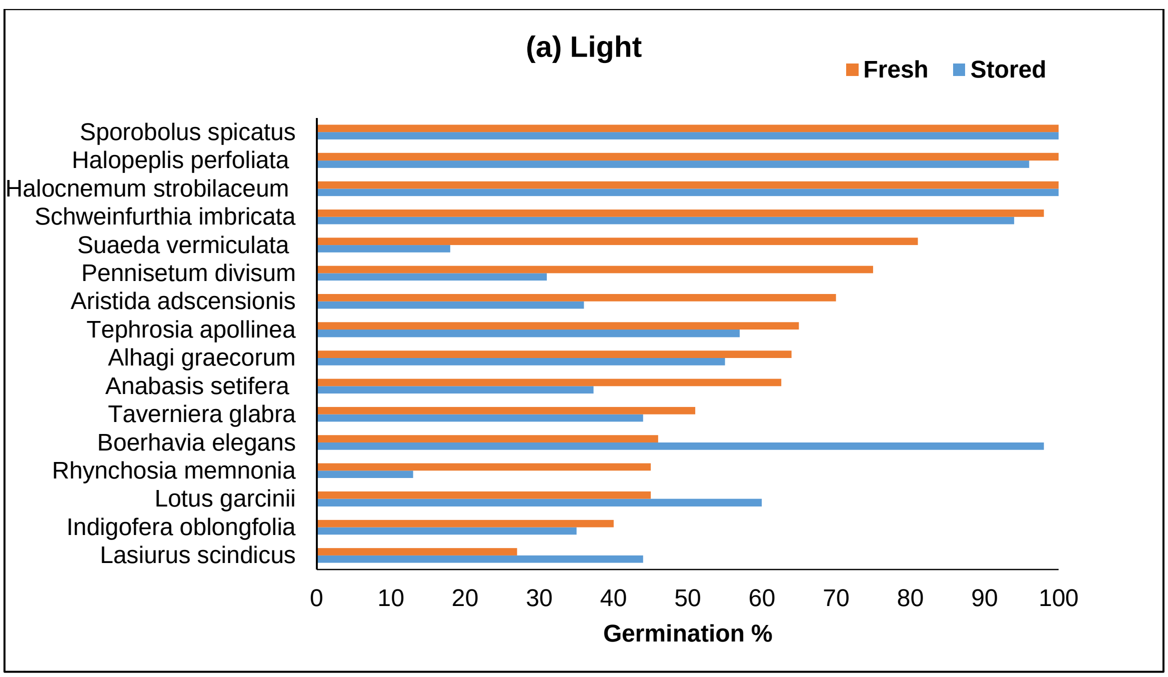
Understanding germination behaviour of wild species and knowledge of how to break their seed dormancy and enhance germination is fundamental to the potential use of these species germplasm for species reintroduction or habitat restoration (Hay and Probert, 2013). The germination results of a species are expected to increase the ecological knowledge that might promote success of establishment and growth in different areas. It is well known that the germination cues reflect the conditions under which a species is likely to succeed in recruitment. So, the seed germination experiments are performed at different conditions of photoperiod, temperatures and with different pretreatments for determining the optimum germination protocol of a species.

Seed germination tests of the native plant species are the important first step for long-term seeds storage in the SSBH. We do test seed collections by running germination tests. Through their testing, we keep track of the viability of our collections in SSBH. So far, the storage behavior and longevity of many native seeds in seed banks is not known, but with research and careful management, this knowledge is being acquired. The information on seed germination from Seed bank laboratories can be useful for the nurseries and landscaping sectors working with native plants suitable for restoration ecology and landscaping in the region. In this study, seed germination behaviour of 16 species belonging to five different families is presented. The details of tested species are listed in Table 2. Germination tests on freshly collected seed (within 1 month), and stored seeds (after 36 months in seed bank conditions: -18°C) were conducted. Seed germination was conducted using 9-cm Petri dishes containing two layers of filter paper (Whatman no.1) moistened with 10 ml of distilled water. For each species, three replicates of 25 seeds each were used. Petri dishes containing seeds were placed in an incubator set with an alternating temperature of 30/20°C, with a diurnal period of 12 hours light and 12 hours dark. Darkness was achieved by wrapping the Petri dishes with two layers of aluminium foil. Petri dishes were randomly distributed in the incubator and their position was changed daily. Germination was monitored until 1 month.

Table 2. Details of Plant Species Included in Germination Testing in this Study

Family	Species	Life habit	Habitat	Fruiting time
Chenopodiaceae	*Anabasis setifera* Moq.	Shrub	Coastal sand	Nov. to Feb.
Chenopodiaceae	*Halocnemum strobilaceum* (Pall.) M.Bieb.	Shrub	Saline sand	Nov. to Jan.
Chenopodiaceae	*Halopeplis perfoliata* (Forssk.) Bunge ex Asch. & Schweinf.	Shrublet	Saline sand	Aug. to Jan.
Chenopodiaceae	*Suaeda vermiculata* Forssk. ex J.F.Gmel.	Shrub	Saline sand	Nov. to Jan.
Fabaceae	*Alhagi graecorum* Boiss.	Shrub	Disturbed soil, saline areas	May to Aug.
Fabaceae	*Indigofera oblongifolia* Forssk.	Shrublet	Gravel	March to May
Fabaceae	*Lotus garcinii* DC.	Shrublet	Saline ground	March to May
Fabaceae	*Rhynchosia memnonia* (Delile) DC.	Subshrub	Sandy plain	March to May
Fabaceae	*Taverniera glabra* Boiss.	Shrub	Low mountain	March to May
Fabaceae	*Tephrosia apollinea (*Delile) DC.	Undershrub	Rocky and sandy places	March to May
Nyctaginaceae	*Boerhavia elegans* Choisy	Herb	Hillside, wadis	March to June
Poaceae	*Aristida adscensionis* L.	Grass	Sand	March to May
Poaceae	*Lasiurus scindicus* Henrard	Shrubby Grass	Sand	March to May
Poaceae	*Pennisetum divisum* (Forsk. ex F. Gmel.) Henrard	Shrubby Grass	Sand	April to July
Poaceae	*Sporobolus spicatus* (Vahl) Kunth	Grass	Saline sand	March to May
Scrophulariaceae	*Schweinfurthia imbricata* A.G.Mill., M.Short & D.A.Sutton	Herb	Gravel plain	March to May

Considering all species together, germination of fresh seeds reached on average 67% and 60% in light and dark conditions, and for stored seeds it was 58% in light and 63% in dark respectively (Figure 5 a and b). Out of 16 tested species, 10 showed a germination percentage of fresh seeds above 60% whereas, 6 had 50 % or less than germination in both light and dark conditions. For stored seeds, 6 species in light and 10 species in dark conditions showed a germination percentage of seeds above 60%. *Halocnemum strobilaceum*, *Halopeplis perfoliata* and *Sporobolus spicatus* kept their germination percentage after 3 years storage in dry-cold conditions. In dark condition, these species showed variable results (Figure 5b). Species like *Suaeda vermiculata*, *Rhynchosia memnonia*, *Pennisetum divisum* and *Aristida adscensionis* showed decrease in germination. Among these, *Suaeda vermiculata* had dramatic decrease in germination after storage. *Anabasis setifera* showed increase in germination under light conditions. *Schweinfurthia imbricata*, an endemic species, maintained its germination percentage after 3 years storage. However, in darkness the initial germination (fresh seeds) was low and stored seeds did not germinated. Conversely, two species namely *Lasiurus scindicus* and *Lotus garciniis* showed an increased germination after storage. In addition, *Boerhavia elegans* germination percentage increased considerably under light conditions. The variation in germination behaviour of tested species can be related to the taxa, to storage conditions or to other factors. In addition, germination percentage of some species might decline during storage because they are inherently short lived. For such species, a careful monitoring of storage behaviour and germination capacity is needed in order to maintain the viability of the accessions in seed banks. Studying germination behaviour of seeds collected from other representative sites of these species might be helpful in elaboration of their germination protocols that could be used in restoration ecology of degraded habitats. Moreover, it is important to routinely monitor different wild species for determining effective optimum seed storage conditions and investigate possible interactions with germination enhancement treatments.



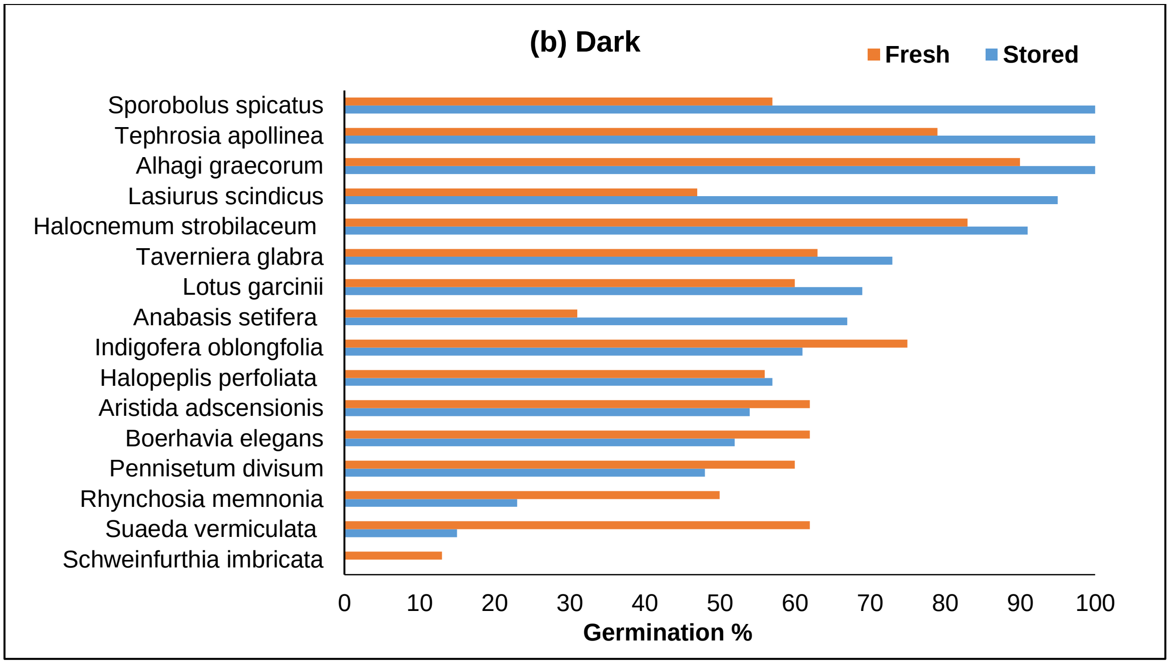


Figure 5. Germination percentage of tested species (fresh seeds vs. 3 years stored seeds in dry-cold conditions) under light (a) and dark conditions (b).

As far as germination percentage of accessions of top ten families ias concerned, Paoceae has the highest average germination percentage followed by Chenopodaceae and Fabaceae. However, the number of tested accessions varies among the families. Collections of family Resedaceae and Caryophyllaceae showed overall low germination and some accessions did not germinated without growth hormone pretreatments. Among the 10 families best represented in seed bank dataset, it was noticed that a few accessions have lost some viability with the storage. These are large families containing species with wide-ranging longevity, so the pattern we found based on our studies cannot be generalized and further studies are needed. Results of some germination experiments are published by the SSBH team, with particular emphasis on seed germination behavior, dormancy and factors influencing germination (mainly temperature, light and salinity) (e.g., El-Keblawy et al., 2011; El-Keblawy et al., 2015; El-Keblawy and Gairola, 2017; El-Keblawy et al., 2017b). Such studies of how different environmental factors influence the germination patterns and survival strategies of desert plants are of great relevance to our understanding of these interactions from the ecological, evolutionary, conservation and restoration perspectives.

Implications for Seed-based Restoration

Seed banking has been considered as a vital tool for the conservation of biological diversity, and play a significant role in protection of plants in their wild habitat by providing essential seed material for reintroduction and restoration. The conservation of biological diversity is important for the UAE; the conservation of germplasm of native species is an important contribution to this process. The availability of plant material and selection of native species is an important step in restoration planning and implementation. However, all restoration projects require careful and objective monitoring to measure their success so that the resulting data can enrich our understanding of restoration ecology

(Ghazanfar and Osborne, 2010). Seed bank collections have high potential for plant conservation, reintroduction and restoration efforts. According to Broadhurst et al. (2015), seed is fundamental to broad scale plant restoration when the goal is to re-establish species and ecosystems. Seed banks hold potential for storage of propagation material and its use for restoration purposes. The SSBH demonstrates potential for the conservation and use of native plant genetic resources for different purposes. Understanding the inter- and intra-specific variabilities in native seed physiology (germination, storage and stress tolerance characteristics) are critical for the selection of seed lots that are suitable regarding the specific environments for revegetation and restoration. The analyses of the main factors affecting seed germination processes in the investigated species provides relevant information about seed ecophysiology useful for the determination of appropriate propagation and conservation procedures and habitat restoration as well. The results of germination tests might be useful for the ex situ conservation of the tested species. In fact, for some species, especially those that showed high germination after the storage period, it would be relatively easy to establish plants from stored seeds for re-introduction into the wild and habitat restoration. On the contrary, species for which low germinability was identified after the three-year storage period, our results might be useful to better understand that further seed collection of those species are necessary, due to their fast declining germinability.

For restoration projects, it is generally advised to use local, site adapted native seeds, which are collected from healthy, local populations using a broad genetic base. Because plant seeds from more distant sources like mountainous habitat may not perform as well in the sandy plan or moist coastal habitats, as plants from nearer seed sources. Thus, the importance of using local seed sources needs great attention during selection of planting native material in restoration projects. In addition, the collected seeds can be banked for future uses though seed viability should be monitored over time (Bainbridge, 2007; Ghazanfar and Osborne, 2010). Sound knowledge about seed germination level and speed under different light regimes is important for restoration projects in sandy deserts as the interaction between storage and response to light would determine the change in seed response of light after storage. It has been well established that for many plants that inhabit sandy soils, light is one of the most important signals for germination (Gutterman, 1993). Seed collections that are stored at SSBH can be made available for propagation and plant material development to serve the current and future needs of restorationists, native plants landscaping, conservationists and researches.

Conclusions

The important role seed banks play in the conservation of wild plant genetic resources and supply of seed material for restoration projects is now globally recognized. Nevertheless, more efforts and research are still required to consolidate the achievements as well as to devise the ways of implementing effective seed-based restoration. As the restoration of degraded desert ecosystems is a long-term process; good knowledge of phenology of selected native species, including their germination behaviour and dormancy is essential. Moreover, careful planning would require for selection of potential species, seed collections from native populations, availability of quality seeds and proper storage of seed material for successful restoration.

Acknowledgements

We thank Director General of SRA, Dr. Amr Abdel-Hamid, for his encouragement and support. The authors are grateful for the help and expertise provided by the RBG Kew members, Shahina Ghazanfar, Thomas Heller and Joanna Osborne, and members of the SSBH field team for assistance. We are also grateful to KISR for invitation to participate in this international symposium.

References

Bainbridge, D. 2007. A Guide for Desert and Dryland Restoration. Island Press.

Baskin, C. C. and Baskin, J. M. 2014. Seeds: ecology, biogeography, and evolution of dormancy and germination. 2nd. edn. San Diego, Academic Press.

Broadhurst, L. M., Lowe, A., Coates, D. J., Cunningham, S. A., McDonald, M., Vesk, P. A. and Yates, C. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* 1: 587-597.

El-Keblawy, A. and Gairola, S. 2017. Dormancy regulating chemicals alleviate innate seed dormancy and promote germination of desert annuals. *Journal of Plant Growth Regulation* 36: 300-311.

El-Keblawy, A., Al-Ansari, F. and Al-Shamsi, N. 2011. Effects of temperature and light on salinity tolerance during germination in two desert glycophytic grasses, *Lasiurus scindicus* and *Panicum turgidum*. *Grass and Forage Science* 66: 173-182.

El-Keblawy, A., Bhatt, A. and Gairola, S. 2015. Storage on maternal plants affects light and temperature requirements during germination in two small seeded halophytes in the Arabian deserts. *Pakistan Journal of Botany* 47: 1701-1708.

El-Keblawy, A., Gairola, S., Bhatt, A. and Mahmoud, T. 2017. Effects of maternal salinity on salt tolerance during germination of *Suaeda aegyptiaca*, a facultative halophyte in the Arab Gulf desert. *Plant Species Biology* 32: 45-53.

FAO/IPGRI (1994) Genebanks standards. Food and Agriculture Organisation of the United Nations/International Plant Genetic Resources Institute, Rome.

Gairola, S., Mahmoud, T., Bhatt, A. and El-Keblawy, A. 2013. Importance of seed banking and herbarium collections in biodiversity conservation and research: a new initiative in the United Arab Emirates. *Current Science* 105: 1048-1050.

Gardner, A. S. and Howarth, B. 2009. Urbanisation in the United Arab Emirates: the challenges for ecological mitigation in a rapidly developing country. *BioRisk* 3: 27-38.

Ghazanfar, S. A. and Osborne, J. 2010. Conservation through restoration: study of a degraded gravel plain in South Eastern Arabia. *Pakistan Journal of Botany* 42: 193-204.

Hay FR, Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology* 1:1-11.

Millennium Ecosystem Assessment (2005). Ecosystems and Human Well Being: Synthesis. Island Press, Washington DC.

Royal Botanic Gardens Kew (2006). A field manual for seed collectors. Seed collecting for the millennium seed bank project. Kew, Royal Botanic Gardens.

Sudhersan, C., AboEl-Nil, M. and Hussain, J. 2003. Tissue culture technology for conservation and propagation of certain native plants of Kuwait. *Journal of Arid Environment* 54: 133-147.

Suleiman, M. K., Bhat, N. R., Jacob, S., and Thomas, R. 2011. Germination studies in *Ochradenus baccatus* Delile., *Peganum harmala* L. and *Gynandriris sisyrinchium* Par. *Research Journal of Seed Science* 4: 58-68.

Role of ISSR Markers for Conservation of *Rhanterium epapposum* Oliv. in Kuwait

Nazima Habibi and Fadila Al-Salameen
Kuwait Institute for Scientific Research, P. O. Box 24885 Safat 13109, Kuwait
e-mail: nhabibi@kisr.edu.kw

Abstract

Assessment of genetic diversity plays an important role in conservation management and desert rehabilitation. Molecular markers have proved to be a very powerful tool in characterizing, genotyping, and estimating genetic diversity in plants. Inter-simple sequence repeats (ISSR) are PCR based molecular markers with high degree of efficiency and precision to uncover differences among individual specimens. *Rhanterium epapposum* Oliv. (commonly known as Arfaj in Kuwait, and other Arab countries) is the national flower of Kuwait and is one of the most palatable forage plant grazed extensively by sheep and camels. It also has medicinal properties. The population of species is disappearing from the Kuwaiti desert at an alarming rate due to climatic change and human causes. Therefore, effective measures are needed to conserve this species to save it from extinction in the near future. In the current study, attempts were made to determine the genetic diversity using ISSR markers. Samples collected from six desert locations namely, Al Kabd, Sabah Al Ahmed Nature Reserve (SANR), Al Salmi, Mina Abdullah, Om Qaser, and Al Maqwa, were tested with 17 ISSR primers. This technique was found to be highly discriminatory. Genetic differences among the samples were estimated. The results were further explored to formulate conservation strategies of *R. epapposum*.

Keywords: Conservation management, genetic diversity, molecular markers, Arfaj, ISSR primers.

Introduction

The native plants of Kuwait demonstrate remarkable adaptation to the harsh desert climate and ecosystem. These species have enormous scientific value because they offer a valuable genetic pool for drought, heat and salt-tolerance research that can be utilized for the improvement of wide range of cultivated plants (Omar, 2007). Unfortunately, the native plant biodiversity of the Arabian Peninsula is rapidly depleting over the recent years (Halwagy & Halwagy, 1974; Halwagy et al., 1982). The main causes of this problem include global warming, overgrazing, increase in off-road driving activity, desert camping, expansion in urban areas, and damage caused during the Iraqi invasion (Omar and Bhat, 2008). Therefore, there is an urgent need to conserve the flora of Kuwait for future generations.

The understanding of genetic variation in the community is essential for the establishment of effective and efficient conservation practice for desert native plants (Zaman et al., 2006). Molecular techniques such as Sequence Related Amplified Polymorphism (SRAP), Inter-Simple Sequence Repeats (ISSR), and genotyping by sequencing (GBS) have been known for accurate assessment of variation within the population. ISSR is a popular technique in the detection of genetic variations due to its many advantages such as quick performance, cost effectiveness, low quantity of template and no prior knowledge about the target sequences (Ng and Tan, 2015). ISSR DNA markers have been widely applied as a method to establish conservation strategies for endangered plants such as *Galium catalinense* (Mcglaughlin et al., 2009) and *Breonadia salicina* (Gaafar et al., 2014).

Rhanterium epapposum (Arfaj), national plant of Kuwait (Halwagy et al., 1982), grows on deep, sandy soils and shallow substrate (Halwagy et al., 1982). It is more abundant in the south of Kuwait, but now, it is confined to protected areas (Halwagy & Halwagy, 1974). It is considered as one of the desert forage plants for camel and sheep grazing and it is used as fuel by Bedouins. The percentage distribution of the *R. epapposum* community was considerably reduced from 30.6% (Halwagy & Halwagy, 1974) to 2.1% (Omar et al., 2001). This is an indication that this community has considerably retreated from the rangelands of Kuwait and is more susceptible to grazing. Presently, it is confined to protected areas such as the Sulaybia Field Station, military air bases, military camps and some restricted oil fields (Omar et al., 2001). Literature survey indicates the lack of published research on the genetic diversity and population structure of *R. epapposum* communities in Kuwait and in Gulf Co-operation Council (GCC) GCC countries. Thus, an understanding of the extent and distribution of the genetic variation within these populations is necessary for devising sampling strategies that efficiently capture the genetic diversity for selection trials and subsequent use of the material in revegetation and rehabilitation projects that aimed at maintaining the biological diversity of native plants in Kuwait.

In view of the importance of *R. epapposum* to Kuwait environment and the utility of ISSR technique, the objective of this study was to use DNA-based markers to evaluate the genetic variation among the *R. epapposum* samples.

Materials and Methods

Sample Collection and Sampling Site. Samples of *R. epapposum* were collected from various locations in Kuwait during January to June, 2016. The locations were chosen based on the availability of plants in the region of Kuwait. Locations from the Central, Northern and Southern part of Kuwait such as Al Kabd, Sabah Al Ahmed Nature Reserve (SANR), Al Salmi, Mina Abdullah, Om Qaser, and Al Maqwa where these plants are found were chosen for collection. Several field visits were made in the designated areas and samples were collected from plants that were at least 30 meters apart.

DNA Extraction. DNA isolation from leaf tissues was carried out using GenElute™ Plant Genomic DNA Miniprep Kit (Sigma, St. Louis, MO), as per the manufacturer's instructions. One hundred mg of leaf tissue was weighed and ground to a fine powder in liquid nitrogen using a pre-chilled mortar and pestle. Cell lysis followed by protein precipitation and washing was carried out using the different reagents supplied in the kit. DNA purity (Absorbance ratio A260/A280) and quantity (Absorbance at 260 nm) were measured by the Nanodrop (Thermo Scientific, Carlsbad, CA). All the samples of *R. epapposum* were run on the 0.8% of agarose gel to check the intactness of the isolated DNA. All the DNA samples were also quantified through a Qubit fluorometer for further accurate measurement (Thermo Fisher Scientific, Carlsbad, CA). The samples were normalized at 10ng/µl for ISSR analysis following the Qubit concentrations.

PCR amplification. ISSR PCR was conducted with seventeen ISSR primers with 93 samples of *R. eppapsoum*. A total reaction volume of 20 µl was set by mixing 4 µl of master mix (5x HOT FIREPol® Blend Master Mix, Solis Biodyne, Estonia) containing 1X buffer, 200µM dNTPs, 1.5 mM $MgCl_2$ and HOT FIREPol® DNA polymerase. Primers (2µl) were added at a concentration of 10µM to yield a final concentration of 1.5µM in the final mix. Template concentration was 10 ng/µl and 1µl volume was added in the PCR reaction. Reaction was carried out in Veriti Thermal Cyclers (Applied Biosystems, Grand Island, NY). The PCR reaction was carried out by initial enzyme activation of 12 mins followed by 45 cycles of denaturation at 95°C for 45 sec, annealing at 45-60°C for 45 sec and extension at 72°C for 1.5 mins. Final extension was done for 7 mins at 72°C and bands were visualized on 1.8% agarose gels run at 5V/cm for 3-4 hrs. Gel images were documented using the BioRad gel documentation system. One Kb and 100 bp ladder were used as reference.

Data Scoring. The ISSR band profiles from each DNA sample were analyzed using the Bionumerics software (Applied Maths, Belgium), and scored for the presence (1) or absence (0) of bands for each primer. Weak and smeared bands were excluded from the study in order to get clear and reproducible data. A binary matrix (1/0) was generated, *ca.*200 multiple bands in total.

Statistical Analysis. The informativeness of markers was evaluated by estimating the polymorphic information content (PIC) using the following formula:

$$PIC = 1 - \sum (Pi)^2 \qquad (1)$$

Where, Pi depicts the proportion of samples carrying the ith allele

Genetic diversity within each population was estimated through Nei's gene diversity (*h'*), by using the GenAlEx 6.5 software. Pairwise genetic distance between each population was used to construct a dendrogram following the Unweighted Pair Group Method (UPGMA) by the POPGene v 1.32 software.

Results and Discussion

For *R. epapposum,* 17 primers produced 195 unambiguous and reproducible bands. A total of 182 bands were polymorphic (92.8%). The bands sizes ranged from 175-2000 bp. Number of bands per primer ranged from minimum 8 (ISSR 8 and 809) to maximum of 17 (ISSR 2) with an average of 10.7 bands per primer (Table 1). All the primers had a high discriminating power as observed by their PIC (mean-0.31) values. Highest PIC was obtained with ISSR 826 (0.42) and the lowest (0.12) with ISSR 8. ISSR markers are reproducible, cost-effective and capable of detecting high level of polymorphism. A good genetic marker is defined by its high rate of polymorphism and the ability to generate multi-locus data from the genome under study. The ISSR markers make use of microsatellite sequences that are inherently highly variable and ubiquitously distributed across the genome, at the same time achieving higher reproducibility (Ng and Tan, 2015) compared to other genetic markers such as RAPD. Wu et al., (2015) reported ISSR markers as highly efficient in detecting the polymorphism in the narrowly distributed *Lilium regale* in China. Similar results were reported in Chinese fir from Fujian province of China (Chen et al., 2017).

Generally genetic diversity is high within populations of cross-pollinated plants (Hamrick, 1989). Our results also indicate high values of *h'* compared to other plants with narrow distribution (Wu et al., 2015). In *R. epapposum,* the gentic diversity ranged from 0.360 (Kabd) to 0.235 (AL Maqwa) with an average value of 0.286. Th e percentage of polymorphic loci observed was also highest in Al Kabd followed by SANR >Al Salmi > Mina Abdullah > Om Qaser > Al Maqwa (Table 2).The high diversity and polymorphism in both the native plants could be attributed to the mixed mating system through cross-pollination.

Table 1- Polymorphic Information Content of ISSR Primers used for Assessment of Genetic Diversity of *R. epapposum* Community in Kuwait

Primer Code	NPB	BR	PIC
ISSR 1	7	300-1100	0.30
ISSR 2	17	240-1500	0.35
ISSR 3	13	250-1500	0.39
ISSR 5	6	300-1100	0.29
ISSR 7	12	200-2000	0.34
ISSR 8	8	800-1500	0.34
ISSR 11	5	400-1100	0.24
ISSR 12	9	300-1100	0.33
ISSR 13	9	500-1175	0.12
ISSR 17	14	175-1400	0.26
ISSR 23	12	265-1200	0.37
ISSR 809	8	500-1090	0.17
ISSR 810	16	200-1100	0.30
ISSR 811	10	285-1200	0.41
ISSR 820	15	310-1100	0.36
ISSR 826	12	300-1100	0.42
ISSR 827	9	250-1200	0.25
Total	**182**		
Average	**10.7**	**175-2000**	**0.31**

NPB-Number of polymorphic bands; BR-Band Range; PIC-Polymorphic information content.

Table 2. Measures of Genetic Diversity Among Six Populations of *R. epapposum* in Kuwait

Pop	*h'*	%P
Al Kabd	0.360	83.59%
SANR	0.308	74.36%
Om Qaser	0.240	54.36%
Al Maqwa	0.235	53.33%
Al Salmi	0.294	69.74%
Mina Abdullah	0.280	68.72%
Mean	**0.286**	**67.35%**

h'-Nie's gene diversity; %P- percentage of polymorphic loci

In *R. epapposum*, the pairwise Nei's genetic distances (D_S) among populations ranged from 0.061 between Kabd and SANR to 0.158 between the Mina Abdullah and Al Maqwa populations. Similarly lowest Ds of 0.50 existed between Kabd and SANR whereas, highest 0.227 showed between Mina Abdullah and Al Maqwa. Pairwise genetic distances based on Nei's analysis were observed to be moderate to low among the different populations in the present study. This could suggest a continuous distribution of both the plants throughout the desert habitat without any topographical barriers (Wu et al., 2015) and having a mixed ancestery.

Cluster analysis of *R. epapposum* populations of Kuwait revealed three distinct groups. First group consisted of Kabd, Salmi and SANR areas, the second group had the populations of Om Qaser and Maqwa with the third group consisting of a single population of Mina Abdulla (Figure 1).

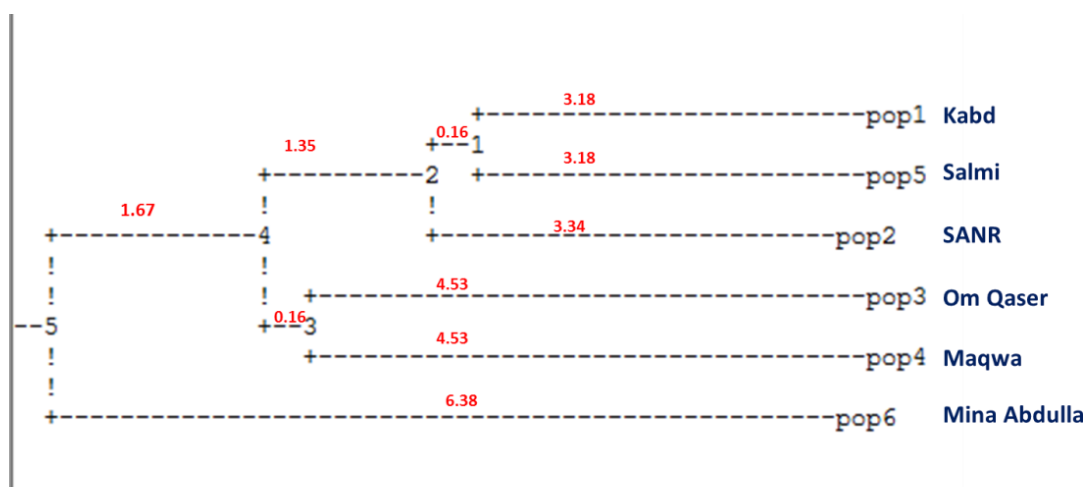


Figure1. UPGMA based cluster analysis of six populations of *R. epapposum* existing in Kuwait.

Conclusions

Results indicated that ISSR primers used had significant discriminatory power to assess the polymorphism in different populations of *R. epapposum*. High degree of polymorphism and overall genetic diversity was detected within the populations of *R. epapposum*. Pairwise gentic distances were low. Population structure of Kuwait native plants was in the form of 3 unique clusters

Acknowledgements

The authors are grateful to the Kuwait Foundation for Advancement of Sciences (KFAS) for funding the project (Project No. FB089C). The support lent by the management and other departments of KISR is gratefully acknowledged.

References

Chen, Y.; Z. Peng; C. Wu; Z. Ma; G. Ding; G. Cao; S. Ruan; and S. Lin. 2017. Genetic diversity and variation of Chinese fir from Fujian province and Taiwan, China, based on ISSR markers. *PLoS ONE* **12**(4): 1-14.

Gaafar, A.R.Z.; F. Al-Qurainy; and S. Khan. 2014. Assessment of genetic diversity in the endangered populations of *Breonadia salicina* (Rubiaceae) growing in The Kingdom of Saudi Arabia using inter-simple sequence repeat markers. *BMC Genetics* **201415**:109 https://doi.org/10.1186/s12863-014-0109-4.

Halwagy, R. and M. Halwagy. 1974. Ecological studies on the desert of Kuwait. II. The vegetation. *Journal of the University of Kuwait* **1**: 87–95.

Halwagy, R.; A. F. Moustafa; and S. M. Kamel. 1982. On the ecology of the desert vegetation in Kuwait. *Journal of Arid Environments* **5**: 95-107.

Hamrick, J.L. 1989. Isozymes and analyses of genetic structure of plant populations. In: Soltis, D.E. and P.S. Soltis (Eds.). Isozymes in Plant Biology. Dioscorides Press, Portland, OR, p. 87–105.

Mcglaughlin, M.E; L. Riley; and K. Helenurm. 2009. Isolation of microsatellite loci from the endangered plant *Galium talinense* subspecies *acrispum* (Rubiaceae) *Molecular Ecology Resources* **9**: 984–986.

Ng, W.L. and S.G. Tan. 2015. Inter-Simple Sequence Repeat (ISSR) Markers: Are We Doing It Right? *ASM Science Journal* **9**: 30–39.

Omar, S. 2007. Vegetation of Kuwait. Kuwait Institute for Scientific Research. *Kuwait*, *161*.

Omar, S. A. S. and N. R. Bhat. 2008. Alteration of the *Rhanterium epapposum* plant community in Kuwait and restoration measures. *International Journal of Environmental Studies* **65**: 139-155.

Omar, S.A.S.; R. Misak; P. King; S.A. Shahid; H. Abo-Rizq; G. Grealish; and W. Roy. 2001. Mapping the vegetation of Kuwait through reconnaissance soil survey. *Journal of Arid Environments* **48**(3): 341–355.

Wu Z.H., J. Shi; M.I. Xi; F.X. Jiang; M.W. Deng; and S. Dayanandan. 2015. Inter-simple sequence repeat data reveals high genetic diversity in wild populations of the narrowly distributed endemic *Lilium regale* in the Minjiang River Valley of China. *PLOS ONE* **10**(3): e0118831. https://doi.org/10.1371/journal.pone.011883.

Zaman, S.; S. Padmesh; N. R. Bhat; and H. Tawfiq. 2006. Germination of some Kuwait's native plants under saline conditions. *American-Eurasian Journal of Agricultural & Environmental Sciences* **1**(2): 146–148.

Remediation and Rehabilitation of Existing Pits and Contaminated Soil in the KOC Foields

Fahad Al Naqi
Kuwait Oil Company, Soil Remediation Group, Soil Remediation Office Building, Kuwait
Fnaqi@kockw.com

Abstract

SEED is the first remediation project of its kind to be undertaken by KOC carrying out oil recovery, remediation and rehabilitation of contaminated areas arising from historical exploration and production activities. The objectives of the project are to remediate contamination to acceptable limits (Primary 5,580mg/kg, Alternate 10,000mg/kg), restore Ecological Function in remediated soils, and identify lessons learned to allow continual improvement for future Remediation projects. The rehabilitation program was designed to measure the effectiveness of the remediation processes used by the consortia engaged to remediate effluent pits, sludge pits and Gatch pits in the Burgan Oil Field. Test areas were established in which planting blocks were constructed utilizing remediated soils to test a variety of scenarios with respect to the ecological function of the soils. In order to best evaluate the ecological function of the treated soils it was decided to use established nursery grown native plants as opposed to seeds as growth rates could be better monitored than the germination and survival of seedlings. The planting scenarios included a control area of native desert, within the remediated soils the planting varied depending on the irrigation levels (high and low) and blocks with and without soil amendments. The amendments added to remediated soil provided potential benefits to plants by adding nutrients and improving soil texture and water holding capacity. The Control areas comprised native desert soils, which are naturally low in organic matter content, friable, sandy and have low soil moisture content. The growth and survival rates of the plants was monitored over a number of growing seasons. Results from this study indicate that application of a soil amendment did not markedly improve plant survival in remediated soil and plant growth and survivorship in remediated areas was slightly lower than in the non-Contaminated soils in Control Areas and Gatch Pits.

Keywords: Ecological restoration, sludge pits, *gatch* pits, effluent pits, revegetation, restoration of native plants, colonization.

Introduction

The Soil Remediation Group of the Kuwait Oil Company (KOC) developed the Sustainable Environmental Economic Development Program (SEED) with a view to remediating contamination that has arisen as consequence of historical exploration and production activities. The project was developed in order to gain an understanding of the capability remediation technologies and the potential for restoration of ecological function of remediated soils and subsequent rehabilitation with native plant species.

The works comprised an assessment of available satellite imagery, ground verification and the use of a Geographical Information System (GIS) to develop a database of potential sites. A total of 25 sites were selected from the database for remediation and rehabilitation, these comprised Contaminated Soil Piles, Effluent Pits, Gatch Pits and Sludge Pits. For the purposes of the project these were split into 3 lots (Lot A, Lot B and Lot C) in order that a variety of remedial technology could be assessed. Remedial targets were established in order to mitigate potential risks posed by the contamination. The Contractors appointed to carry out the works provided a variety of remedial solutions for the treatment of contaminated soils including Bioremediation, Soil washing and Thermal Desorption. The contaminated soils were treated to achieve the remediation criteria specified (Table 1) for the feature and backfilled. Of the features included in the project a number were identified for rehabilitation including 3 control areas established in native desert soils, 3 Sludge Pits and 6 Effluent Pits. In each of these locations planting areas were established with a selection of native desert plants. A total 432 plants were placed in the Control areas and 12,960 plants in Sludge and Effluent Pits. A variety of scenarios were established in each of the blocks allowing for the comparison of the effects of differing irrigation levels and the impact of soil amendments in comparison to the Control areas. In addition, 3 Gatch Pits have undergone ecological restoration with the removal of waste and the implementation of slope stabilization and erosion control measures, including the planting of 216 trees and 11,080 plants.

Table 1. Remediation Standards (RS)

Parameter	Commercial/ Industrial RS (mg/kg)	Primary Ecotoxicity RS (mg/kg)	Alternate Ecotoxicity RS (mg/kg)
Petroleum Hydrocarbons			
PHC (total*) C6-C10	19,000	320	---
PHC (total*) C10-C16	10,000	260	---
PHC (total*) C16-C35	23,000 (>Csat)	1,700	---
PHC (total*) >C35	30,000 (>Csat)	3,300	---
Total PHC*,***	30,000 (>Csat)	5,580	10,000
Polycyclic Aromatic Hydrocarbons			
Acenaphthene	10,457	---	---
Anthracene	---	5.8	---
Benz[a]anthracene	20	2.5	---
Benzo[b]fluoranthene	20	20	---
Benzo[k]fluoranthene	196	38	---
Benzo[a]pyrene	11	4	---
Chrysene	1,955	35	---
Dibenz[ah]anthracene	2	2	---
Fluoranthene	21,501	---	---
Fluorene	10,887	---	---
Indeno[1 ,2,3-cd]pyrene	20	20	---
Naphthalene	62	11	---
Pyrene	15,909	10	---
Benzo(ghi)perylene	18,352	33	---
Phenanthrene	15,336	20.5	---
Acenaphthylene	1,001	1,001	---
Salinity			
Electrical Conductivity	---	4.5	4.5
Sodium Absorption Ratio	---	12	12

Notes: Parameter not regulated; >Csat calculated value is greater than residual saturation. For soil to meet this standard there can be no evidence of free phase petroleum hydrocarbon and the total PHC concentration must be less than 30,000 mg/kg; * total of aromatic and aliphatic compounds within fraction identified; ** alternate ecotoxicity RS to be applied at selected locations. Total PHC to include extractable PHC up to and including C90, at a minimum; *** Maximum tolerable total PHC concentration. Total PHC to include extractable PHC up to and including C90, at a minimum; # Salinity standards apply to effluent pits and sludge pits only.

The rehabilitation study was carried out over a 2 year period from November 2012 to April 2014, this constitutes 2 growing seasons. This paper discusses the materials, methodology and presents the results and conclusions for the Rehabilitation portion of the SEED project.

Materials and Methods

In order to evaluate the ecological function of the remediated soils planting areas were established in 3 Sludge Pits and 6 Effluent pits, of the features selected 3 of the Effluent pits were remediated to the Alternate standard (10,000mg/kg), the remaining features used soil remediated to the primary ecotoxicity standard (5,580mg/kg) (Table 1), with 3 control areas constructed in pristine desert soils. Within each of the planting areas 6 blocks were defined. The planting areas were split in half with soil amendments added to 3 of the planting blocks and the remaining areas had no soil amendments. Within each block irrigation systems were installed, these create suitable conditions to facilitate and support the initial growth of native plant species until they have been established. The specifications of the irrigation system include a low cost, low technology reusable, vertical deep pipe irrigation system which is hyper efficient in terms of water usage. This system delivers the minimal amount of water required for plant establishment directly to the root zone in a controlled measurable manner. In addition, it eliminates deposition of surface salts which is detrimental to ecological rehabilitation, which is typical of conventional drip irrigation systems.

The Planting blocks were further subdivided into areas of high and low irrigation representing two different water application rates. These are described as follows:

- High Level Irrigation pipes were constructed from poyvinyl chloride, 50 cms long and 10 cms in diameter, to give an internal volume of approximately 3.92 liters. The top of the pipe was covered with a galvanised wire or mesh screen (mesh size <3mm in diameter) and securely fixed in place to prevent ingress and trapping of wildlife and reduce infilling by sand.

- Low Level Irrigation pipes were constructed from poyvinyl chloride, 50 cm long and 7.5 cm in diameter, to give an internal volume of approximately 2.2 liters. The top of the pipe was covered with a galvanized wire or mesh screen (mesh size <3mm in diameter) and securely fixed in place to prevent ingress and trapping of wildlife and reduce infilling by sand.

Each Individual irrigation tube was installed 7.5cm away from its corresponding plant and was installed vertically to prevent any damage to the plants.

The following range of native Kuwait Desert plants were planted;

Panicum turgidum (4,110 plants)
Cyperus conglomeratus (4,138 plants)
Rhanterium epapposum (4,102 plants)

Nitraria retusa (4,130 plants)
Prosopis farcta (6,228 plants)
Astragalus spinosus (468 plants)
Ziziphus spina-christi (1,548 plants)
Acacia gerrardii (72 trees)
Prosopis farcta (72 trees)
Ziziphus spina Christi (72 trees)

During the course of the study the plants were monitored quarterly for key indicator parameters including survivorship and growth (height and width). Irrigation was carried out every 2 weeks during the growing season which was considered to be from November to May. In addition, physiochemical parameters were monitored during this period including nutrient levels, moisture content and soil pH.

Results and Discussion

The successful establishment of the Kuwait desert species installed across the various lot features differed markedly. A summary of survivorship is presented in Table 2. The reference areas, which presumably were similar to one another and characterized as undisturbed had very different rates of plant survival. Lot C had the greatest survival (79% and 90% under High / Low Irrigation rates, respectively) after two growing seasons compared to Lot A (55% and 82% respectively) and lastly, Lot B (44% and 33% respectively). High versus low irrigation rates were tested to determine if plant survival and growth were improved by a greater amount of water available during the growing season (i.e. high irrigation would result in better plant survival and growth). Results appear to indicate that although irrigation events improved soil moisture levels in all reference areas, the difference in soil moisture between high and low irrigation rates was overall, not significant (Table 3). This indicates that plants receiving high-level irrigation had similar moisture near their roots as plants receiving low-level irrigation. This could be the reason why high irrigation did not appear to provide a greater benefit to plant survival, nor did it provide a marked increase in plant growth in reference areas (Table 4).

The addition of a soil amendment to remediated soil was tested because of the potential to provide useful nutrients to plants and to improve soil texture and water holding capacity. After thermal treatment, soil would be considered inert, having no organic matter or living microbes and poor physical structure. All three contractors handled the remediated soil slightly differently, but all did complete soil testing to determine the appropriate rate of amendment application. Soil testing indicated that cleaned material was suitable for planting (i.e. not expected to be harmful to plants); therefore, only a biogenic fertilizer amendment was added. The amount of amendment added varied between all contractors (Lot A features applied the amendment at a rate of 10 kg/m3; Lot B at a rate of 20 kg/m3; and Lot C at a rate of 5 kg/m3). Although the addition of a soil amendment could have improved the physical and biological condition of the soil, this was not evident through a clear benefit to plant survival and/or growth within amended planting blocks, or at any of the rates applied.

In the sludge and effluent pits, high and low irrigation rates were tested, as well as amended and unamended soil treatments. However, prior to receiving the amendment, soil from the pits was treated to remove hydrocarbons and other contaminants to meet Primary or Alternate RS (see Table 1). A summary comparison of the average plant survival and growth in Primary RS and Alternate RS soils within each Lot respectively revealed some interesting trends (Table 5).

Although plants tended to survive better in Primary RS soil in Lots A and B, this trend was only significant in the Lot B features where plant survival was approximately 10% better in Primary RS soil. Plants also tended to grow larger in Primary RS soil in all Lots; however, the trend was only significant in Lot A and B features. Plants grew approximately 30% and 19% larger in Primary RS soil, respectively. Plant growth and survival was highly variable in Lot C and therefore, significant differences in plant response to soil treatments were not observed. This is likely due to the very different way the soil was treated during the remediation process, although in the end, the remediation standards were met just as in the other Lot features.

Table 2. Summary of Plant Survival Across All Lot Features (End of Monitoring Period)

Features	Reference Area		Gatch Pit	Sludge and Effluent Pits			
				Amended Soil		Unamended Soil	
	High	Low	High	High	Low	High	Low
Lot A							
Reference Area	55	82					
GPA1			66				
SPA1†				51	51	28	18
EPA2†				45	32	46	26
EPA3‡				40	38	42	32
Lot B							
Reference Area	44	33					
GPB1			96				
GPB2			82				
SPB1†				88	88	96	93
EPB2‡				83	73	84	77
EPB3†				85	86	89	87
Lot C							
Reference Area	79	90					
SPC1†				39	23	24	20
EPC3†				33	12	22	10
EPC4‡				18	6	29	18

Notes: † = Cleaned soil met Primary RS; ‡ = Cleaned soil met Alternate RS.

Table 3. Summary of the Average Percent Difference in Soil Moisture Following Irrigation

Features	Reference Area		Gatch Pit	Sludge and Effluent Pits			
				Amended Soil		Unamended Soil	
	High	Low	High	High	Low	High	Low
Lot A							
Reference	3.55	3.09					
GPA1			5.41				
SPA1†				1.21	3.47	2.47	1.92
EPA2†				9.5	9.8	10.0	9.4
EPA3‡				6.6	7.4	7.3	7.7
Lot B							
Reference	1.92	2.08					
GPB1			3.18				
GPB2			2.20				

SPB1[†]				0.4	0.3	-0.6	-0.4
EPB2[‡]				-0.41	1.72	1.13	0.24
EPB3[†]				1.11	0.27	0.89	-0.35
Lot C							
Reference	2.99	2.24					
SPC1[†]				1.01	0.88	1.03	0.87
EPC3[†]				1.05	1.07	1.17	1.03
EPC4[‡]				1.19	1.05	1.31	0.97

Notes: [†] = Cleaned soil met Primary RS; [‡] = Cleaned soil met Alternate RS.

Table 4. Average Plant Growth (cm) Across All Lot Features (End of Monitoring Period)

Features	Reference Area		Gatch Pit	Sludge and Effluent Pits			
				Amended Soil		Unamended Soil	
	High	Low	High	High	Low	High	Low
Lot A							
Reference Area	19.53	17.45					
GPA1			51.27				
SPA1†				50.69	50.60	50.05	50.96
EPA2†				45.66	40.50	31.72	25.25
EPA3‡				16.50	15.82	7.86	6.74
Lot B							
Reference Area	22.73	16.11					
GPB1			44.05				
GPB2			15.93				
SPB1†				37.56	32.85	20.92	23.80
EPB2‡				5.93	8.36	-6.36	-5.23
EPB3†				17.98	16.93	8.13	2.40
Lot C							
Reference Area	34.72	40.57					
SPC1†				48.06	46.14	51.03	51.91
EPC3†				22.85	12.03	25.85	17.92
EPC4‡				32.89	32.40	25.94	25.46

Notes: † = Cleaned soil met Primary RS; ‡ = Cleaned soil met Alternate RS.

Table 5. Summary of Average Plant Response to Primary RS and Alternative RS Soils

Feature	Plant Survival (%)		Plant Growth (Average Increase in cm)		Soil Moisture (% Difference After	
	Primary RS	Alternate	Primary RS	Alternate	Primary RS	Alternate
Lot A	37	38	43	12	6	7
Lot B	89	79	20	1	0	1
Lot C	23	18	34	29	1	1

Conclusions

On evaluating the data collected during the 18 month study period from each of the respective Lots (A, B and C) the following observations can be made:

- Plant survivorship in remediated soils at the end of the experiment was at 56%.
- Plant growth and survivorship in remediated areas was slightly lower than in the non-Contaminated soils in Control Areas and Gatch Pits.
- Soil amendments do not significantly improve plant growth and survivorship.

Acknowledgements

I acknowledge the support received from various individuals who are colleagues and have played an integral role in imparting their valuable knowledge and input at all times. My gratitude and thanks to;

Mr. Jamal Jaafer (CEO-KOC)
Mr.Hashim Hashim (CEO-KIPIC)
Mr. Yousef Ali (DCEO-KOC)
Mr. Ismail Abdullah (DCEO- WK KOC)
Mr. Mansour Al Khareji (Manager- SRG KOC)
Mr. Najeeb Al-Zaid (Snr.Eng. Project 1)

References

Adams, J.A., A.S. Endo, P.G. Rowlands and H.B. Johnson. 1982. Controlled experiments on soil compaction produced by off-road vehicles in the Mojave Desert, California. J. appl. Ecology. 19:167-175.

Al-Ateeqi, Sarah Salah. 2014. Phytoremediation of oil-polluted desert soil in Kuwait using native plants. Ph.D. thesis, University of Glasgow.

Al-Awadhi, J.M., S.A. Omar and R.F. Misak. 2005. Land degradation indicators in Kuwait. Land Degradation & development. 16: 163-176.

Al Bakri, D. 1994. Geomorphic framework for developing a sustainable greenery programme in arid environments. The environmentalist. 14:4,

Al-Fredan, M.A.A. 2010. Nitrogen fixing legumes in the plant communities. Amer. J. Environ. Sci. 7:166-172.

Bainbridge, D. 2001. Buried clay-pot irrigation: A little known but very efficient traditional method of irrigation. Agricultural Water Management. 48: 79-88.

Bainbridge, D. 2007. A Guide for Desert and Dryland Restoration. Island Press. Washington.391 pp.

Batanouny, K.H. 1987. Current knowledge of plant ecology in the Arab Gulf countries. Catena. 14:291-316.

Brown, G. 2002. Species richness, diversity and biomass production of desert annuals in an ungrazed *Rhanterium epapposum* community over three growth seasons in Kuwait. Plant Ecology 165:53-68.

Brown, G. 2002. Community composition and population dynamics in response to artificial rainfall in an undisturbed desert annual community in Kuwait. Basic Appl. Ecol. 3:145-156.

Brown, G. & N. Schoknecht. 2001. Off-road vehicles and vegetation patterning in a degraded desert ecosystem in Kuwait. J. Arid Environments. 49:413-427.

Brown, G. and S. Al-Mazrooei. 2003. Rapid vegetation regeneration in a seriously degraded *Rhanterium epapposum* community in northern Kuwait after 4 years of protection. J. Environ. Manage. 68:387-395.

Phytochemical Composition and Antifungal Potential of Ethanolic Seed Extract from *Citrullus colocynthis* Native to Kuwait

Sheikh Shreaz and Narayana R. Bhat
Environment and Life Sciences Research Centre
Kuwait Institute for Scientific Research, Kuwait
*** E-mail:** shreazresearch@gmail.com

Abstract

Plant extracts and their constituents have a long history as an important source of secondary metabolites and antifungal agents, but their use as fungicides has rarely been reported. The current antifungals have serious drawbacks regarding toxicity and emergence of drug-resistant strains. The aim of this study was to assess the phytochemical composition and *in vitro* antifungal activity of ethanolic extract from *Citrullus colocynthis* seeds (CCS). Qualitative determination of the different biologically active compounds from ethanolic extracts of CCS using gas chromatography-mass spectrometry (GC-MS) revealed different types of high and low molecular weight chemical entities with varying quantities. The major compounds found were simiarenol (45.79%), stigmasta-7, 25-dien-3beta-ol (12.28%), gamma tocopherol (10.90%), squalene (8.51%), and ethyl linolate (5.47%). Effect of ethanolic extract from CCS on growth and important virulence factors of different *Candida* isolates (three reference strains and 27 oral isolates) has been investigated. Minimum inhibitory concentrations (MIC) ranged from 256 to 2048 µg/ml for both reference and oral isolates. Only *C. albicans* (One standard and 10 oral isolates) were found to be proteinase and phospholipase secretors. Average drop in proteinase secretion caused by ¼ MIC and ½ MIC value of the test extract were 34.7, 59.5, respectively. The biofilm results obtained for different *Candida* strains (3 standard and 5 oral isolates), as assessed by a colorimetric assay, demonstrated that MBIC, ½ MBIC, and ¼ MBIC values of the test extract showed the average biofilm inhibition by 70.4, 39.9, and 17.1%, respectively. Confocal Scanning Laser Microscopy (CLSM) results indicated that the mechanism of the test extracts could involve a primary lesion on the cell membrane that ultimately could lead to cell death. The results obtained would provide enough evidence for further work on CCS so that better strategies could be employed to treat *Candida* infections.

Keywords: Secondary metabolites, *Candida*, minimum inhibitory concentrations (MIC), biofilm, confocal microscopy.

Introduction

Fungal infections have increased globally at an alarming rate, resulting in the cause of morbidity and mortality (Akansha et al., 2010). The advancement of antimicrobial resistance and populations of patients at some risk, in relation to the extremely limited number of commercially available antifungal drugs that still create many side effects, are the main cause for this problem. Of the two major classes of antifungals; polyenes guide to intense host toxicity (Cohen, 1998). While as azoles are fungistatic, and their utilization leads to the expansion of drug resistance. The need of an hour is to develop effective strategies for the treatment of candidiasis and other fungal infections.

Kuwait's native plants have endured harsh environmental conditions for hundreds of years by producing and accumulating some of the above secondary metabolites. Besides protecting them against harsh environmental conditions, these metabolites are known to possess significant biological properties (Ali et al., 2016). Therefore, either the whole plant or specific parts (leaf, bark, stem, roots, fruits, pulp or seeds) were used for centuries in several Arab and Asian countries for curing a number of ailments and physiological disorders in humans and livestock.

Citrullus colocynthis (L.) Schrad., also known as desert gourd, Handhal and Thumba (in Arabic), is a member of the Cucurbitaceae family, known for its high seed protein (35%) and oil (50%) content. It grows in the wild in many regions of the world such as Middle East, India, Mediterranean Europe, Cyprus, Turkey, Iran, Pakistan, Afghanistan, and North Africa (Hussain et al., 2014). This native plant grows in the arid conditions of the Kuwait, thrives in sandy loam and sub-desert soil areas. *C. colocynthis* is a perennial creeper that grows on the ground, it can propagate both by generative and vegetative means. The plant is said to be highly xerophytic and is the best example of good water management. Different parts of the plant including seeds, fruit, root, stem, and leaves, used as either aqueous or oil extracts, dried or fresh, are believed to have significant antidiabetic and antifungal properties (Abdel-Hassan et al., 2000; Marzouk et al., 2009).

To the best of our knowledge, this is the first report on the effect of ethanolic extract of native *Citrullus colocynthis* seeds (CCS) on the growth and important pathogenicity markers of *Candida*. In this study, we carried out GC-MS analysis of ethanolic extract from CCS and examined its antifungal effect against different *Candida* isolates. Primary screening for antifungal activity was carried out by evaluating MIC. To gain insight into the mechanism of action, we have made an attempt to assess the antifungal role of this test extract by studying its effect on hydrolytic enzyme secretions, biofilm formation and membrane integrity.

Materials and Methods

Collection and Extraction Through Accelerated Solvent Extractor (ASE). Mature seeds of *C. colocynthis* (Figure 1) were collected in June 2017 from Sulaibia, Kuwait (Coordinate - 29° 10' 23.65" N; 47° 44' 14.94" E). The seeds (200 g) were dried and then grinded into powder using a Polymix grinder (Kinematica, Switzerland). The finely powdered sample was then mixed and dispersed with diatomaceous earth (1:1) to remove moisture. The samples were placed in a 33 mL stainless steel extraction cell, to perform extractions with solvent (ethanol) on an ASE 350 (Dionex) system (Thermo Scientific, Sunnyvale CA). The dry seed sample (5-6 g) was extracted by one extraction cycles with all solvent (% 99 pure) at $100^{o}C$, 1500 psi with a static time of 5 min. Then, the cell was rinsed with fresh extraction solvent and purged with a flow of nitrogen (150 psi during 90 s). In the final of the experiment, the extraction solution was collected in 66 mL glass vials. This procedure was performed for all the plant material in different stainless steel extraction cells (Elif et al., 2014). The extract was then filtered, mixed and concentrated under reduced pressure using a rotary evaporator. For antifungal screening the extract was redissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich), to get the working solution of the extract.

GC-MS Analysis. Mass Spectrometry analysis of ethanolic extract from CCS was carried out using a Shimadzu 2010 gas chromatograph fitted with an AB-Wax column. The column temperature was 60°C for 1 min and then raised to 180°C for the total analysis time (50 min). Sample (0.1 mL) was injected in the splitless mode**.** Helium gas was used as a carrier. The chemical component from the extract was identified by comparing the retention time of the chromatographic peaks with those of authentic compound with the use of NIST05s.LIB & WILEY8.LIB (Table 1).

Yeast Strains, Culture Conditions and Species Identification. The yeast strains used in this study are listed in (Table 2). Reference strains (*C. albicans* ATCC 24433, *C. glabrata* ATCC 15126, and *C. parapsilosis* ATCC 90018), and clinical *Candida* isolates were obtained from Oral Microbiology Laboratory, Faculty of Dentistry, Kuwait University, Kuwait. Each sample was then labelled with a CDC (Comprehensive Dental Care) number provided by Kuwait University Dental Clinic (KUDC). The strains were cultured in yeast extract, peptone and dextrose (YPD) medium containing 1% yeast extract, 2% peptone, and 2% dextrose (Sigma-Aldrich, St. Louis, MO, USA). For experimental purposes, the culture was maintained on YPD agar plates at 4ºC and re-streaked after every 4-6 weeks. Species identification was done through CHROMagar Candida culture medium (CHROMagar Candida, France).

Figure 1. *C. colocynthis* collected from KISR research station, Sulaiba Kuwait (A) Plant (B) Seeds.

Table 1. Chemical Composition of Ethanolic Extract of *C. colocynthis* Seeds

Compound name	Mol. formulae	Mol. weight	Ret. time	% of presence
1-Hexadecene	$C_{16}H_{32}$	224	18.76	0.23
Benzyl benzoate	$C_{14}H_{12}O_2$	211	22.64	0.82
Heptadecene	$C_{17}H_{34}$	238	23.21	0.16
Benzyl salicylate	$C_{14}H_{12}O_3$	228	24.79	1.79
Palmitic acid	$C_{16}H_{32}O_2$	256	26.68	0.60
Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	27.22	1.66
Methyl linoleate	$C_{19}H_{34}O_2$	294	29.06	0.10
Linoleic acid	$C_{18}H_{32}O_2$	280	29.97	2.31
Ethyl linolate	$C_{20}H_{36}O_2$	308	30.31	5.47
Ethyl elaidate	$C_{20}H_{38}O_2$	310	30.43	1.48
Ethyl octadecanoate	$C_{20}H_{40}O_2$	312	30.91	0.84
Eicosane	$C_{20}H_{42}$	282	32.76	0.32
Fumaric acid, 2-pentyl tridec-2-yn-1-yl ester	$C_{22}H_{36}O_4$	364	34.23	0.19
Glycerol 1-monolinolate	$C_{21}H_{38}O_4$	354	34.68	0.14
Lineoleoyl chloride	$C_{18}H_{31}ClO$	298	35.45	0.44
(6Z,9Z)-pentadeca-6,9-dien-1-ol	$C_{15}H_{28}O$	224	35.66	0.47
Methyl palmitoleate	$C_{17}H_{32}O_2$	268	36.02	0.79
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	36.44	0.20
Squalene	$C_{30}H_{50}$	410	42.27	8.51
delta -Tocopherol	$C_{27}H_{46}O_2$	402	44.03	0.21
gamma –Tocopherol	$C_{28}H_{48}O_2$	416	45.74	10.90
Stigmasterol	$C_{29}H_{48}O$	412	49.57	0.56
Stellasterol	$C_{28}H_{46}O$	398	50.07	1.17
Simiarenol	$C_{30}H_{50}O$	426	51.27	45.79
Stigmastanol	$C_{29}H_{52}O$	416	51.48	0.64
Stigmasta-7,25-dien-3-ol, (3.beta.,5.alpha.)-	$C_{29}H_{48}O$	412	52.29	12.28
Lupeol	$C_{30}H_{50}O$	426	52.95	0.49
Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	$C_{30}H_{50}O$	426	43.91	0.79

Table 2. Minimum Inhibitory Concentration (MIC) of Ethanolic Extract of *C. colocynthis* Seeds against 3 ATCC Reference Strains and 27 Clinical *Candida* Isolates used in the Study

Strains	CDC* Number	Species Name	MIC (µg/ml)
Reference strains (n=3)		*C. glabrata* ATCC 15126	256
		C. albicans ATCC 24433	256
		C. parapsilosis ATCC 90018	256
Clinical strains (n=27)	27977	*C. albicans*	512
	27975	*C. tropicalis*	256
	27903	*C. tropicalis*	512
	27901	*C. tropicalis*	2048
	27923	*C. glabrata*	2048
	27942	*C. albicans*	512
	27935	*C. parapsilosis*	1024
	27845	*C. glabrata*	1024
	27903	*C. albicans*	256
	28304	*C. albicans*	512
	28305	*C. dubliniensis*	256
	28306	*C. dubliniensis*	512
	28326	*C. glabrata*	1024
	17584	*C. albicans*	512
	25910	*C. albicans*	256
	28390	*C. albicans*	256
	28396	*C. albicans*	512
	28398	*C. glabrata*	2048
	28416	*C. albicans*	512
	28444	*C. albicans*	256
	28498	*C. dubliniensis*	1024
	28552	*C. dubliniensis*	512
	28573	*C. dubliniensis*	512
	28574	*C. dubliniensis*	512
	28575	*C. glabrata*	256
	28620	*C. glabrata*	512
	28621	*C. dubliniensis*	2048

***** Comprehensive Dental Care

Determination of Minimum Inhibitory Concentrations (MIC). The MIC was performed by broth microdilution methods as described in the document M27-A3 from the Clinical Laboratory Standards Institute (CLSI) for yeasts (CLSI, 2008).

Growth Studies. Yeast culture was inoculated into YPD broth to obtain a cell count of (~1×106) (optical density A595 = 0.1). MIC and sub-MIC values of test extract were then added to the medium. Growth was recorded as done previously (Shreaz et al., 2010), using Eppendorf Biospectrophotometer (Hamburg, Germany).

Proteinase Assay. *Candida* strains (5 µl) identified as proteinase positive from primary culture were transferred to flasks containing 5 ml YPD media alone and along with sub-MIC values of ethanolic extract from CCS and incubated at 35ºC for 18 h. Following incubation, the yeast culture was centrifuged, and the pallets were washed. After standardizing the suspensions, volumes of 2 µl were plated, at equidistant points, on proteinase agar-BSA fraction V, 2 g; yeast nitrogen base w/o amino acids; ammonium sulfate, 1.45 g; glucose, 20 g; distilled water added to 1000 ml respectively. The plates containing *Candida* cells treated with different concentrations of test extract for the detection of proteinase were incubated at 37ºC for two days. The effect of test extract on proteinase synthesis was measured by dividing the diameter of the zone by the diameter of the colony alone (Shreaz et al., 2012).

Assay for Inhibition of *Candida* Biofilm. Biofilm was formed by incubating 100 ml of standardized cell suspensions into selected wells of the 96-well microtiter plates (Corning Inc., Corning, NY, USA) for 2 h at 37ºC. After 2 h medium was aspirated, and the wells were washed with sterile PBS. For mature biofilm formation, fresh medium was added, and the plates were incubated for 48 h. The medium was aspirated again, and the wells were washed with sterile PBS. Minimum Biofilm Inhibitory Concentration (MBIC) and sub-MBIC values of test extract were added to the adherent cells (200 µl/well), and the plates were incubated again for 24 h at 37^{o}C. The effect of the test extract on the preformed biofilm was estimated by using the MTT reduction assay. Briefly, MTT (Sigma-Aldrich) (1 mg/ml) was prepared in PBS and stored at - 80°C. Prior to use, menadione solution was prepared in acetone and added to the MTT to a final concentration of 1 µM. A 100 µl aliquot of the MTT-menadione solution was added to each well of the microtiter plate, and the plate was then incubated for 3 h at 37ºC. After this 100 ml of DMSO was added to solubilize the MTT-formazan product, which was measured at 490 nm by using a microtiter plate reader (Microplate Reader, BioTek Instruments, and Winooski, USA). The percentage inhibition of biofilm was calculated by the equation reported by (Srivastava et al., 2016).

Confocal Scanning Laser Microscopy (CSLM). The disruptive effect of ethanolic extract from CCS on yeast cell membranes was assessed by using CCS-mediated propidium iodide (PI) (Sigma-Aldrich) uptake (Shreaz et al., 2016). *Candida* cells (~1×10^{6}) were suspended in YPD medium and incubated with test extract at 37 °C with constant shaking (200 rpm) for 30 m. For confirming cell membrane permeabilization, one µg/ml of PI was added, and the cell suspensions were then incubated again at 37 °C for 4 h. The dye uptake was visualized by confocal microscopy (ZEISS LSM 500)

Results and Discussion

We used CHROMagar Candida culture medium for the identification of yeasts used in this study. This medium better differentiated the color of colonies, after 72 hours of incubation at 37º C. In this study, a total of 30 yeast strains have been investigated for the presumptive identification. Stereo microscopic images (Stereomicroscope, Leica-M165) were taken for all the thirty isolates grown on CHROMagar culture medium. The results showed that different shades of green color were observed for *C. albicans* (Figure 2A), metallic blue for *C. tropicalis* surrounded by a slight creamy halo (Fure 2B), small smooth light purple to mauve colonies for *C. glabrata* (Fig. 2C), *C. dubliniensis* colonies gave dark green color, accompanied by cream colored ring on the outer side which diffuses into surrounding agar (Fig. 2D), and *C. parapsilosis* appeared off-white (Fig. 2E). To conclude out of the thirty isolates, identified on CHOMagar Candida, 11 isolates were identified as *C. albicans*, 7 as *C. dubliniensis*, 3 as *C. tropicalis*, 7 as *C. glabrata*, and 2 as *C. parapsilosis*.

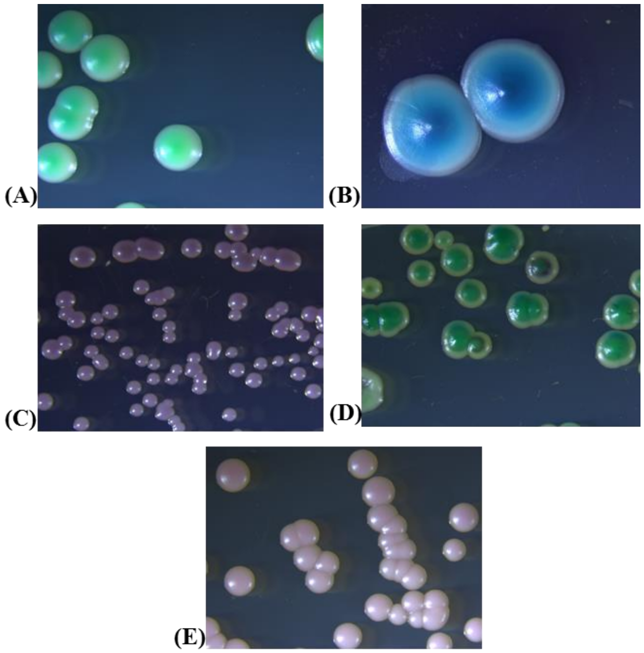


Figure 2. Stereo images showing typical colony colour of *Candida* colonies grown for 72 hours on CHROMagar Candida at 37º C. (A) *C. albicans* (B) *C. tropicalis* (C) *C. glabrata* (D) *C. dubliniensis* (E) *C. parapsilosis.*

The results pertaining to GC-MS analysis of ethanolic extract of CCS lead to the identification of a number of compounds. As indicated in (Table 1) the identified compounds were twenty eight, out of which five were found in abundance and rest twenty three compounds were in traces. The identification of phytochemicals is based on the molecular weight, molecular formulae, and peak area. The major compounds of the extract were simiarenol (45.79%), Stigmasta-7, 25-dien-3-ol, (3.beta, 5.alpha.)- (12.28%), gamma tocopherol (10.90%), squalene (8.51%), and ethyl linolate (5.47%), respectively.

The MIC of selected plant extract was defined as the lowest concentration of test agent that resulted in complete inhibition of visible fungal growth after 24 h. The growth inhibition results obtained on 96 well plates showed that ethanolic extract of CCS was found to be active against both the standard and clinical *Candida* isolates used in this study. Table 2 gives the MIC values of ethanolic extract of CCS against 3 ATCC reference strains and 27 clinical *Candida* isolates of different *Candida* species. The results obtained showed that ethanolic extract of CCS showed the MIC ranging from 256-2048 µg/ml (Table 2).

Growth curve has been plotted by recording in time vs absorbance at 595 nm. Figure 3 A-E show the growth curves of *C. albicans* CDC 28390, *C. glabrata* CDC 28575, *C. dubliniensis* CDC 28305, *C. tropicalis* CDC 27975, and *C. parapsilosis* CDC 27935 in presence of MIC and sub-MIC values of ethanolic extract of CCS. In these growth curves, the top dark blue curve depicts the normal or control cells, while as the red curve, green curve, and purple curve shows growth patterns at MIC/4, MIC/2 and MIC values of the test extract. The results obtained showed that control cells showed a normal pattern of growth with lag phase of 0-2 h, an active exponential phase of 8-14 h before attaining stationary phase. Log phase of control cells could be divided into initial (6-8 hours), active (8-10 hours) and late log phase (10-14 hours). The results showed that an increase in the concentration of the test extract leads to a significant decrease in growth with suppressed and delayed exponential phases. It was observed that at MIC/2 and MIC/4 concentrations of the test extract the lag phase was further extended by 2-4 hours with respect to control. The test extract in a concentration dependent manner suppressed growth and delayed exponential phases. At MIC values of the extract, the normal sigmoid growth curve was not seen and almost a flat line was observed indicating negligible growth. Growth inhibition was measured by comparing optical densities of control and treated samples at 24 hours. O.D value at 24 hours in case of the control sample is taken as 100% for growth comparison at different concentrations. The average decrease in growth of *C. albicans* CDC 28390, *C. glabrata* CDC 28575, *C. dubliniensis* CDC 28305, *C. tropicalis* CDC 27975, and *C. parapsilosis* CDC 27935 (after 24 hours) at MIC/4, MIC/2, and MIC values of ethanolic extract of CCS measured as a change in optical density with respect to control was 48.43%, 67.47%, and 95.22%, respectively.

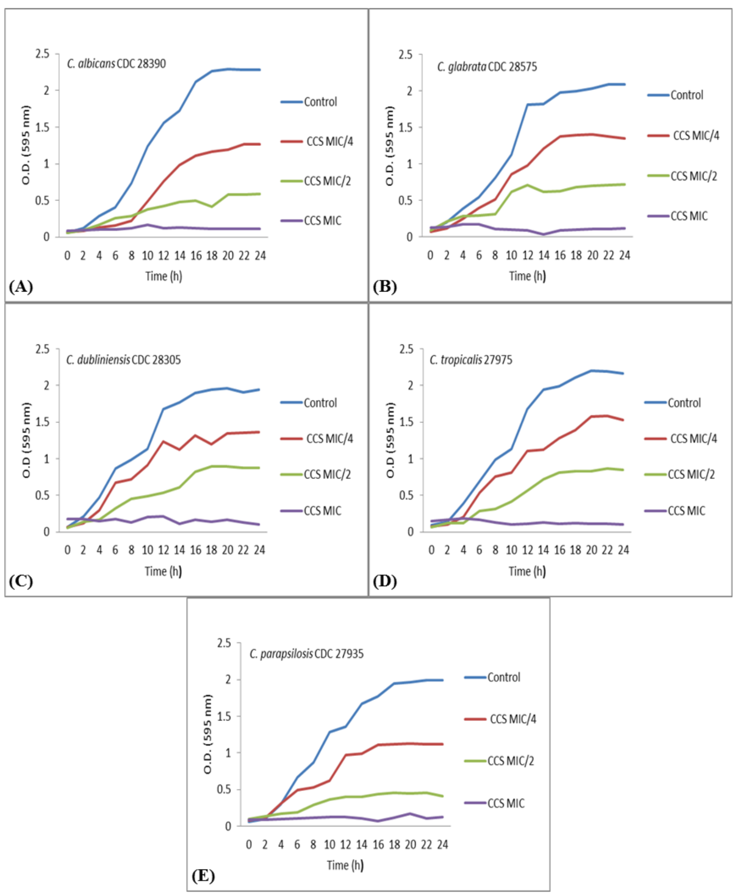


Figure 3. Effect of ethanolic extract of CCS on the growth of different *Candida* species. Growth curves of *C. albicans* CDC 28390 (A), *C. glabrata* CDC 28575 (B), *C. dubliniensis* CDC 28305 (C), *C. tropicalis* CDC 27975 (D), and *C. parapsilosis* CDC 27935 (E), in presence of MIC and sub-MIC values of ethanolic extract of CCS. Growth curve plotted against absorbance at 595 nm and time (h) shows almost complete inhibition of growth at MIC values.

In proteinase assay experiments were performed on one standard and ten different clinical strains of *C. albicans*. Figure 4 A-I shows typical randomly chosen plates of proteinase secretion following the exposure to sub-MIC values of ethanolic extract of CCS. A significant difference in precipitation zone was observed with respect to control. As the concentration increases the diameter of zone decreases indicating the proportional effect of test extract on proteinase secretion by various *C. albicans* strains. Precipitation Zone (PZ) = (Diameter of colony/ Total diameter) by different isolates, when grown with and without the test extract concentrations, was calculated separately. Average PZ (of eleven *C. albicans* isolates) for control was found to be 0.32 ± 0.018, ethanolic extract of CCS at MIC/4 and MIC/2 concentration showed average PZ of 0.49 ± 0.125 and 0.79 ± 0.108 (Figure 4). Average inhibition of proteinase secretion by MIC/4 and MIC/2 of ethanolic extract of CCS was 34.7% and 59.5%, respectively. The present work demonstrates that ethanolic extract of CCS interfere by inhibiting one of the initial stages of the infection process (hydrolytic enzyme secretion). The lowered expression of proteinase enzymes may indicate the less virulent nature of *Candida* isolates, in the presence of this extract. Future studies will explore this extract for the discovery of novel inhibitors to target hydrolytic enzyme secretion in *Candida*.

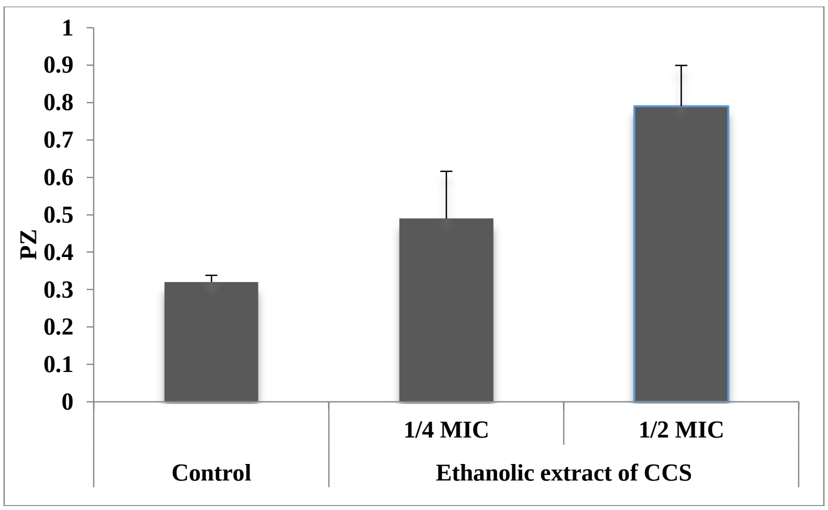


Figure 4. Effect on proteinase secretion by 11 strains (1 standard and 10 clinical) of *C. albicans* when 2 µl of culture was grown on proteinase agar-BSA plates in absence (control) and presence of sub-MIC concentrations of ethanolic extract of CCS. The effect on proteinase synthesis was determined by the formation of zone of degradation around the yeast colonies.

Biofilm is often associated with higher degree of resistance among *Candida* pathogens. A plant extract with potent anti-biofilm activity may offer great hope for treatment of candidiasis involving biofilm producing of oral *Candida* isolates. Therefore, it had been examined if ethanolic extract of CCS inhibited the biofilms produced by different *Candida* isolates. Activity of ethanolic extract of CCS was tested on biofilm formation of *C. albicans* ATCC 24433, *C. albicans* CDC 25910, *C. glabrata* ATCC 15126, *C. glabrata* CDC 28575, *C. parapsilosis* ATCC 90018, *C. parapsilosis* CDC 27935, *C. tropicalis* CDC 27975, and *C. dubliniensis* CDC 28306 (Table 3). Biofilm formation was initiated in 96-well microtiter plates. *Candida* cells were first incubated for 48 h for mature biofilm formation, after this different concentrations of the test extract were added and the cells were further incubated at 37 °C for 24 h. The effect of the extract on the preformed biofilm was then estimated by using the MTT reduction assay. The results of this experiment, as assessed by a colorimetric assay, demonstrated that MBIC, ½ MBIC and ¼ MBIC values of the test extract showed that the average biofilm inhibition (for eight *Candida* species) by 70.4%, 39.9% and 17.1% (Table 3). These results were obtained by visual observations as well as by MTT reduction assays which showed lowest MTT readings towards the highest concentration of the test extract and progressively higher readings as the concentration of the extract decreased. Our results indicate that ethanolic extract of CCS acts as naturally occurring seed extract that significantly inhibits biofilm formation by the different standard and clinical *Candida* isolates.

CLSM was performed to localize and confirm cell membrane damage in the presence of Propidium iodide (PI). Membrane damage due to the ethanolic extract of CCS treatment was monitored by using confocal microscopy. PI is a nucleic acid binding fluorescent probe commonly employed to evaluate the effect of drugs on cell membranes. Cells with severe membrane lesions leading to inherent loss of viability will internalize PI, resulting in an increase in red fluorescence. Yeast cells without the test extract treatment were taken as control and compared with the *Candida* cells treated with the MIC values of the test extract. As expected, the test extract treated cells exhibited an increased red fluorescence light. The laser confocal images of stained *Candida* cells exposed to MIC value of test extract are shown in lower panels of Figures 5, 6 and 7. In all the three figures the upper panels show the laser confocal images of stained *C. albicans* CDC 28390 (Figure 5), *C. glabrata* CDC 28575 (Figure 6), and *C. tropicalis* CDC 27975 cells (Figure 7), without any treatment while as the lower panel shows images of stained cells exposed to MIC value of the test extract. Our results confirm that PI penetrates in test extract treated cells implying that the structure of cell membrane was disrupted by the ethanolic extract of CCS. Penetration of the red dye into the *Candida* cells with damaged cell wall and cell membrane showed a staining fluorescence light with a strong red light under the confocal microscope as displayed in lower panels in Figures 5 - 7. Hence, it can be concluded that the observed dose-dependent fungicidal activity of ethanolic extract from CCS on yeasts, is due to a severe lesion in the membrane, leading to direct damage to the cell membrane. Thus, the excellence of this extract demands more insight studies into all possible mechanisms of action, compounds isolation and then examining the antifungal potential of those individual compounds to make them suitable candidates for fungal specific drug development.

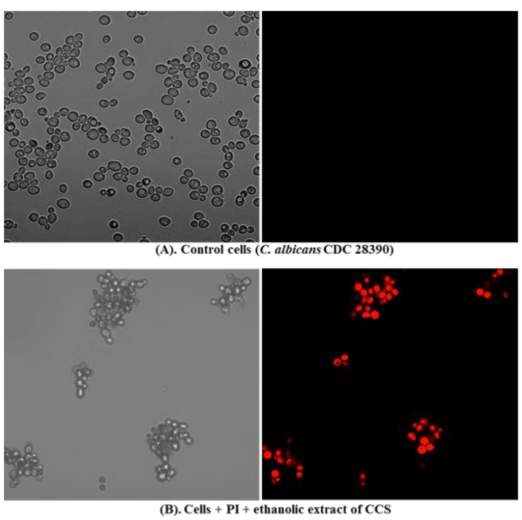


Figure 5. Laser confocal images of *C. albicans* CDC 28390. To confirm membrane damage cells were stained with PI (red signals). (A) Control cells (B) Cells treated with ethanolic extract of CCS (MIC).

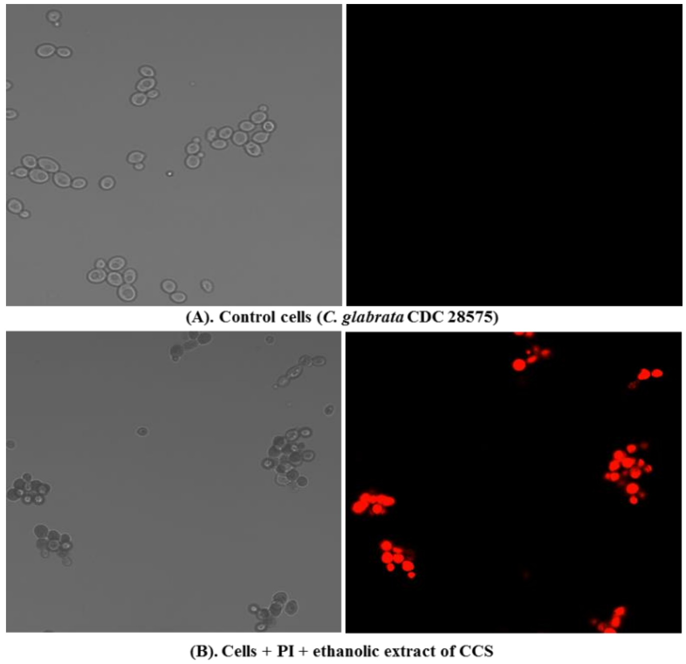


Figure 6. Laser confocal images of *C. glabrata* CDC 28575. To confirm membrane damage cells were stained with PI (red signals). (A) Control cells (B) Cells treated with ethanolic extract of CCS (MIC).

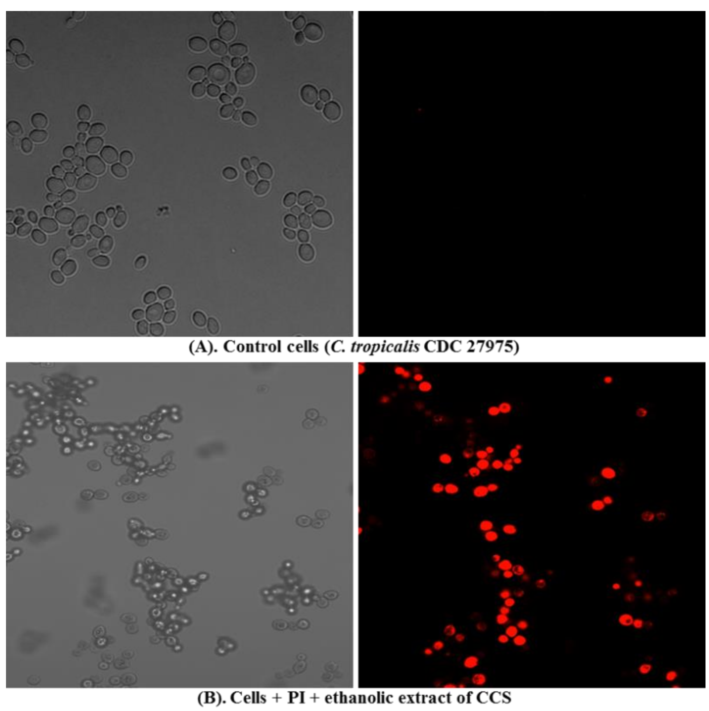


Figure 7. Laser confocal images of *C. tropicalis* CDC 27975. To confirm membrane damage cells were stained with PI (red signals). (A) Control cells (B) Cells treated with ethanolic extract of CCS (MIC).

Conclusions

Ethanolic extract from CCS proved to be a very active anticandidal agent. This test extract severely affected *Candida* growth, biofilm formation, and proteinase enzyme secretions. It leads to direct damage of the cell membrane as visible in our microscopy results. These results taken together make this test extract eligible for further development as antifungal agent.

Acknowledgements

Authors thank the Management of Kuwait Institute for Scientific Research for supporting this research. They also thank laboratory staff for their assistance in carrying out some of the analysis.

References

Abdel Hassan, I. A.; J. A. Abdel Barry; and S. T. Mohammeda. 2000. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. *Journal of Ethnopharmacology* **71**: 325-330.

Akansha, J.; J. Shubham; and R. Swati. 2010. Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention. *Journal of Pharmacy and Bioallied Sciences* **2***:* 314-320.

Ali, M. A.; M. A. Farah; F. M. Al-Hemaid; F. M. Abou-Tarboush; K. M. Al-Anazi; S.M. Wabaidur; Z. A. Alothman; and J. Lee. 2016. Assessment of biological activity and UPLC–MS based chromatographic profiling of ethanolic extract of *Ochradenus arabicus*. *Saudi Journal of Biological Sciences* **23**: 223-236.

Clinical and Laboratory Standards Institute (CLSI). 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Third Edition. CLSI Document M27-A3. Wayne, Penn, USA: Clinical and Laboratory Standards Institute.

Cohen, B. E. 1998. Amphotericin B toxicity, and lethality: a tale of two channels. *International Journal of Pharmaceutics* **162**: 95-106.

Elif, A. E.; G. Gulden; and E. Ayse. 2014. Anti-candidal activities of some *Myrtus Communis* L. extracts obtained using accelerated solvent extraction (ASE). *Journal of Applied Biology & Biotechnology* **2**: 012-014.

Hussain, A. I.; H. A. Rathore; M. Z. Sattar; S. A. Chatha; S. D. Sarker; and A. H. Gilani. 2014. *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): a review of its phytochemistry, pharmacology, traditional uses and nutritional potential. *Journal of Ethnopharmacology* **155**: 54-66.

Marzouk, B.; Z. Marzouk; and R. Décor. 2009. Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis* Schrad. from Medenine. *Journal of Ethnopharmacology* **125**: 344-349.

Shreaz, S.; R. A. Shiekh; B. Rimple; A. A. Hashmi; M. Nikhat; and L. A. Khan. 2010. Anticandidal activity of cinnamaldehyde, its ligand and Ni (II) complex: effect of increase in ring and side chain. *Microbial Pathogenesis* **49**: 75-82.

Shreaz, S.; R. A. Shiekh; V. Raja; W. A. Wani; and J. M. Behbehani. 2016. Impaired ergosterol biosynthesis mediated fungicidal activity of Co (II) complex with ligand derived from cinnamaldehyde. *Chemical and Biological Interactions* **247**: 64-74.

Shreaz, S.; R. Bhatia; N. Khan; I. K. Maurya; S. I. Ahmad; S. Muralidhar; M. Nikhat; and A. K. Luqman. 2012. Cinnamic aldehydes affect hydrolytic enzyme secretion and morphogenesis in oral *Candida* isolates. *Microbial Pathogenesis* **52**: 251-258.

Srivastava, V and A. K. Dubey. 2016. Anti-biofilm activity of the metabolites of *Streptomyces chrestomyceticus* strain ADP4 against *Candida albicans*. *Journal of Bioscience and Bioengineering* **122**: 434-440.

National Conservation Efforts of Socioeconomic Plant Genetic Resources in Oman – Issues of Climate Change Impact on Dryland Ecosystem and plant diversity Disruptions

Ali H. Al Lawati
Oman Animal and Plant Genetic Resources Center, The Research Council, Muscat 130, Sultanate of Oman.
email: ali.allawati@trc.gov.om

Abstract

Oman is a rich country in plant genetic resources (PGR) especially those that classified as socioeconomic important species. For about 1200-1400 plant species, more than 80% of these species have socioeconomic importance. These include for food and general agriculture purpose such as grains, legumes, vegetables, and fruits, which are mostly cultivated. While, medicinal and aromatic plants (MAP), forestry and ornamentals, and Crop Wild Relatives (CWR) are wildly grown. Conservation efforts in form of *ex situ* or *in situ* is taken by the country since decaes ago by different organizations in the country. However, full picture of PGR conservation status is not known at the national level. Therefore, the Oman Animal and Plant Genetic Resources Center was aimed to develop and plan a conservation strategy. This strategy provide an overview and analysis of Oman's PGR diversity, the challenges it faces and their causes. The strategy based on records of geographical distribution of the plant species either for wild species such as for MAPs and CWRs and landraces for cultivated. Information on conservation of each plant species were gathered from research publications or reports. Gap analysis of conservation for each socioeconomic important species were taken as well. In addition, Impact of climate change was projected for two species. The results presented in the strategy indicated that most of socioeconomic important plant species were in Dhofar region in the southern part of the country and Jabal Al Akhdar and Musandam in the northern part. Projected climate change showed that conservation and use of PGR is strongly impacted especially in the long term. The strategy concluded for activities for conservation and use.

Keywords: Oman, Socioeconomic, Plant Genetic Resources, Conservation

Introduction

Oman's plant genetic resources have actual and potential value that can directly and indirectly benefit current and future generations in Oman. This important natural resource is currently being threatened through unsustainable ecosystem management practices. While more sustainable ecosystem management practices will help secure Oman's food security, enhance ecosystem services and generate significant income for socioeconomic related industries and generally the economy of the country.

Several organizations in Oman involve in plant genetic resources conservation. Ministry of Agriculture and Fisheries mostly involve in conservation of PGR for food and agriculture. The conservation is usually in the form of field genebanks of different fruit tress including date palm, coconut, mango, citrus species, banana (MAF, 2008), and to some extent other type species such as medicinal plants (MAF, 2005) and small seeds. Oman Botanic Garden (OBG), which established in 2006 by a royal decree aims is to conserve wild plants of the country. OBG had several publication about their conservation efforts and status such as Patzelt (2014) and Patzelt et.al. (2008). Although, these organizations and other involved in conservation, information on status of conservation of socioeconomic PGR is not well known in national base.

Increasingly the techniques developed initially for PGR characterization and genetic conservation are being applied to the broader conservation of wild plant species that are only remotely related to any form of human utilization. The application of biotechnology and systematic bioprospecting has also meant that any plant species has potential to be of use to humankind in the future. Thus, the boundary between what may be and may not be regarded as a PGR is breaking down and becoming of limited semantic importance. In the future, a more appropriate definition might be the total genetic diversity found both between and within all plant species (Figure 1).

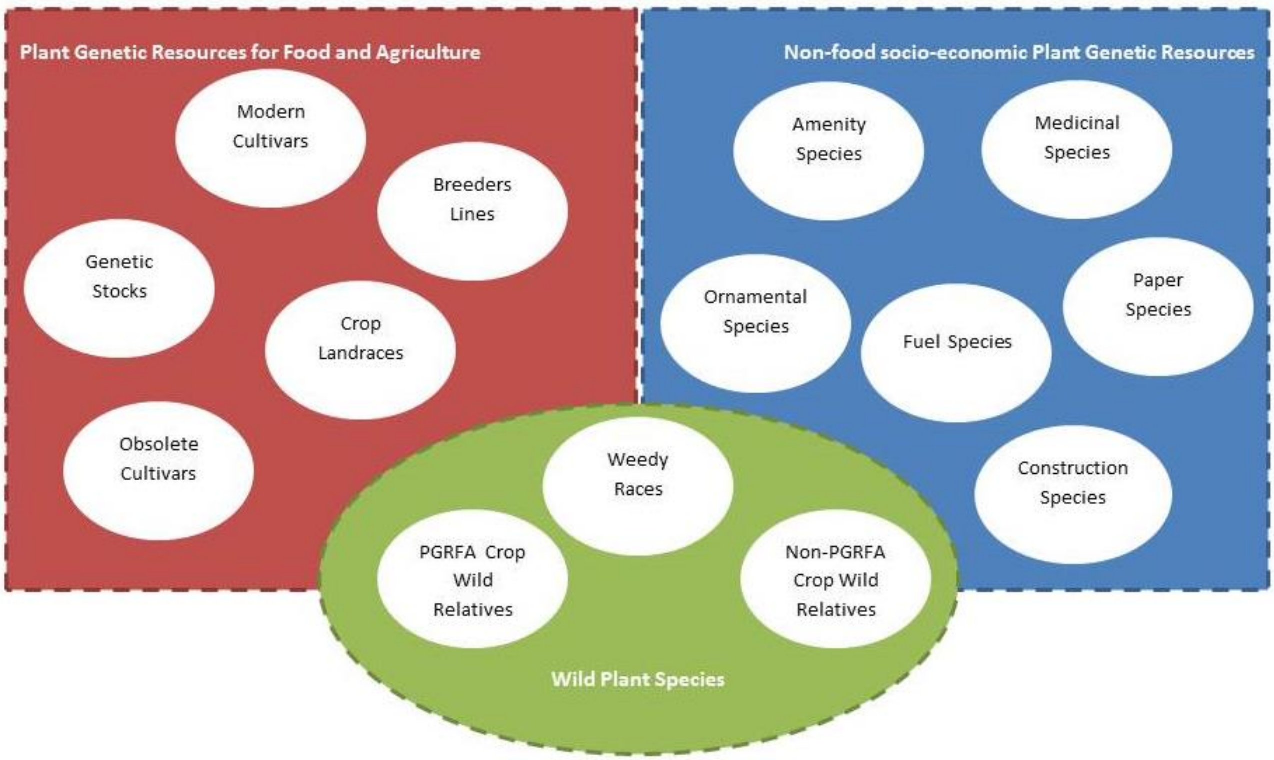


Figure 1. The diversity of plant genetic resources (Maxted *et al.*, 2008a).

While it is easy to see the link between crop cultivation and livelihoods increasingly; wild socioeconomic plants that are wild harvested and used by local communities are recognized as an important services provided by different ecosystems (Daily and Dasgupta, 2001; MEA, 2005; McNeely *et al.*, 2009). Such service supports, directly or indirectly, the survival and quality of life of local communities; some wild species are essential for life (e.g. food), others help improving quality of life (e.g. recreation). Internationally, 60% of the ecosystem services including wild plants are being degraded or used unsustainably, often resulting in significant harm to human well-being (MEA 2005), which reflects the urgent need for reversing the loss of environmental resources, particularly biodiversity. For many rural women, wild harvested plants represent a cornerstone of their livelihood, belief systems and basic survival. A substantial literature review (e.g. Tukan *et al.* 1998, Rivera *et al.* 2006, Aburjai *et al.* 2007, Takruri *et al.* 2008, Al-Assaf *et al.* 2014) affirm on the role of wild plants for income and subsistence for communities in marginal areas. There is clear evidential support that poorer communities have the highest dependency on wild biodiversity. However, the extensive use of such natural resource is limited as long as it remains relatively low-value and subsistence oriented.

Still, poor communities either loose access or are excluded from using other alternative valued resources. The collection and use of wild plants are part of the ecological services that biodiversity provides. The sustainable use of wild plant species thus form a fundamental link with the land and maintains individuals and group identity.

There is an important knowledge gap in how the wild plants are linked to the livelihoods of local communities. Therefore, systematic and robust studies that examine the linkages between wild plants, subsistence and the livelihood of local communities are required. Since the review of global ecosystems in the MEA (2005), there has been a lively discussion over the link between biodiversity conservation i.e. wild plants conservation and poverty reduction. The MEA (2005) highlighted the conceptual impact of biodiversity deterioration on poor communities. However, when the poverty and biodiversity are measured, a clear spatial link between biodiversity and poverty is often presented; thus, biodiversity conservation and poverty alleviation will often be pursued side by side in the same geographical places. Therefore, there is a clear opportunity for a much greater understanding of the link between biodiversity conservation and poverty alleviation.

Plant genetic resource conservation aims to maintain the taxonomic and genetic diversity of PGR, the habitats or ecosystems in which they live and the interrelationships between plants, other organisms and their environment. It aims to enhance or maintain diversity and halt habitat, species and genetic erosion by establishing and implementing conservation programmes. To achieve this goal the conservationist must clearly define and understand the processes involved, and then develop practical techniques to achieve taxonomic and genetic maintenance. It is important to stress that genetic conservation is not just about individual plant populations, but includes all levels of biodiversity from ecosystems (a community of organisms and its abiotic environment), through communities (collection of species found in a common environment or habitat), species and populations to genetic diversity within populations. To conserve the maximum range of diversity found in a species, populations of the species are likely to require protection in diverse location and in each of these; the habitat must be maintained that contains the target population.

The aim of genetic conservation of PGR is to maximize the maintenance of genetic diversity, but further it is explicitly utilitarian; in that, there is an intimate link between PGR conservation and utilization (Figure 2). The model includes a series of steps starting with the full range of genetic diversity for all plant species, through the prioritization of target taxa, the planning of conservation action, the implementation of the conservation action and leading through characterization and evaluation to utilization. In connection to this notion, the aim of this strategy is to cover the gap on information on required conservation efforts that need to be taken nationally.

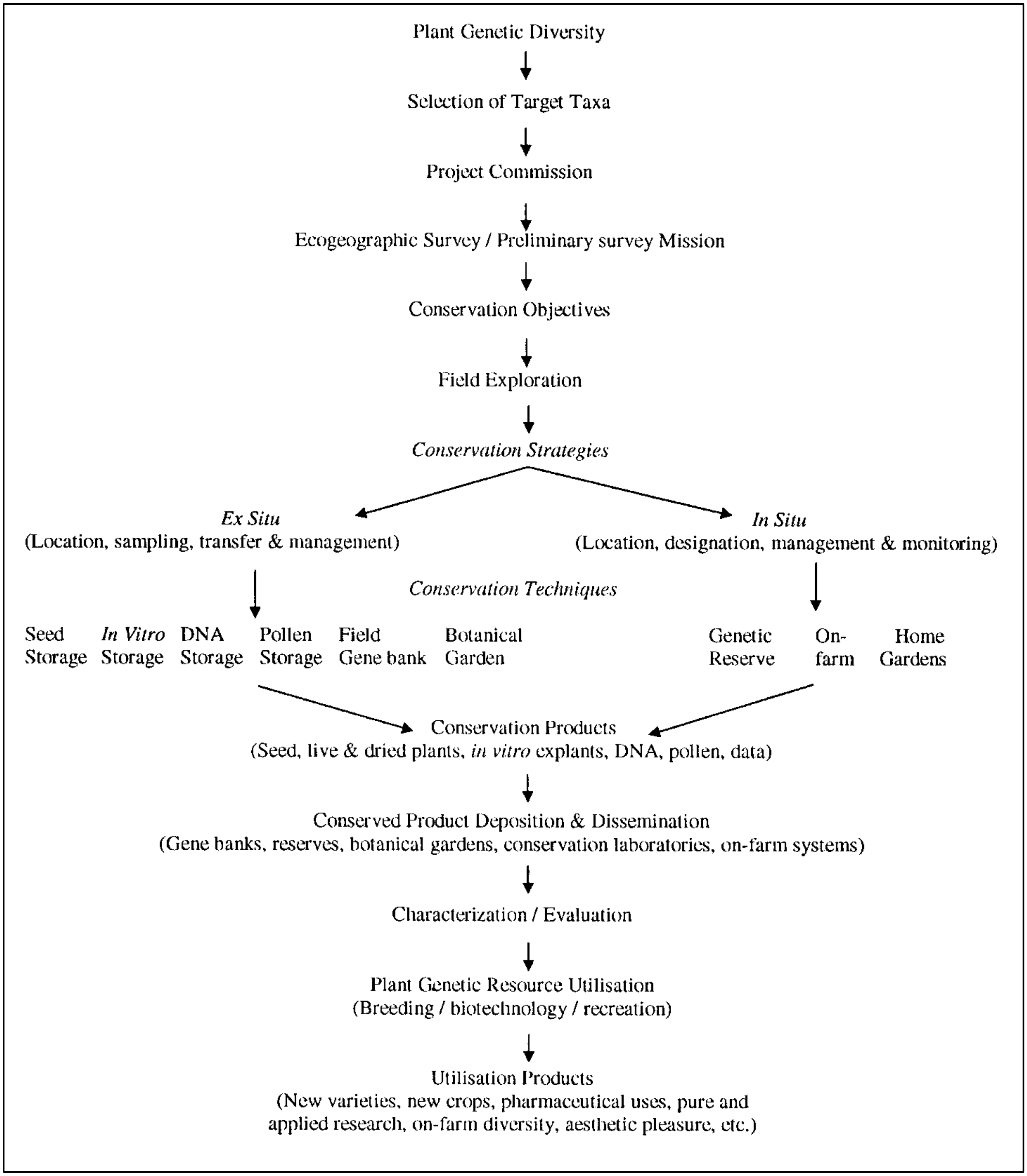


Figure 2. Model of plant genetic conservation (Maxted *et al.*, 1997).

Materials and Methods

Selection of Target Taxa, CWR, and MP Checklist. The main source of data were the Oman Natural History Museum and its herbarium (OM) within Oman. Data from the National Herbarium of

Oman (13,200 accessions) and data available from GBIF (2,132 accessions). These were then matched against the Crop Wild Relative (CWR) and Medicinal Plants (MP) checklists and those with latitude and longitude were used in the analysis. Crop Wild Relatives (2,721) and MP (1,481) dataset of georeferenced presence points is used to study species distribution. The minimum data required for presence point information includes: identification number, species name, longitude and latitude coordinates. Further passport information can include locality descriptions, institution, type of accession, date of collection, collector name etc. Presence point data (georeferenced presence points) has been collated into an appropriate database format (a CSV file, Microsoft Excel table or Microsoft Access database). The data were checked for reliability and precision before undertaking any analysis to limit model inaccuracy. DIVA GIS (Hijmans *et al.*, 2012), the CAPFITOGEN tool, and GEOQUAL (Parra-Quijano *et al.*, 2014) were used for such checking.

Species richness analysis and complementary reserve selection analysis (see Rebelo, 1994) performed as described in Scheldeman and von Zonneveld (2010). Gap analysis (Maxted *et al.*, 2012b) which compares the observed presence points with the predicted distribution of the species to produce a map illustrating where a species is predicted to be found but as yet has not been observed in this location.

Assessing Climate Change Impact. Climate change modelling was initiated for *Medicago sativa* and *Aerva javanica*, both species were selected as they had a relatively large number of presence points available for the analysis (the higher the number of points the greater the accuracy of the prediction) and *M. sativa* because it is cultivated and *A. javanica* because it is wild. Current climatic data was obtained from WorldClim (*Hijmans et al*., 2005) and future climate projections were from CCAFS (www.ccafs-climate.org). The SRES-A2 emission scenario was selected as it is considered the most realistic and due to its use in similar studies (Ramirez-Villegas et al., 2014). The General Circulation Model (GCM) used was the UKMO-HadCM3 (http://www.metoffice.gov.uk/research/modelling-systems/unified-model/climate-odels/hadcm3).

The variables used to create the potential species distribution maps can be seen in Table 1 and were selected using objective methods that included a test for collinearity and PCA that resulted in the removal of correlated variables. Maxent (*Phillips et al*., 2006) was used to create the predicted distribution at a resolution of 2.5 arc-minutes (~5km^2 cell size at the equator). Parameters were kept at default settings apart from the following: Random test percentage=30% and 'Equal training sensitivity and specificity' was selected.

Ex situ gap analysis was undertaken using the results from the observed species richness and the predicted distribution. In DIVA GIS the predicted distribution file was subtracted from the observed distribution grid file (in Grid>Overlay) to produce a new grid file showing those areas where there are gaps between observed and predicted.

In this paper, only results for Medicinal plants are presented. These information that is available can be used (i) to make initial genetic conservation plans and (ii) act a guide to the systematic surveying of plant occurrence in Oman more systematic wild plant surveying in Oman is an urgent priority if its conservation is to be efficient and effective.

Results and Discussion

There were 448 species of medicinal plants for Oman were used as a basis for ecogeographic data collection based on the checklist used in this study. Longitude and latitude for 1481 MP presence points were established. Medicinal plants was based on 316 taxa and 132 MP taxa without presence points.

Table 1. ***Environmental Variables Available for Predictive Specie Distribution Modelling***

Name	Description	Units		Source	
Altitude	Elevation above sea level	meters (m)	Geophysical	WorldClim but derived from SRTM	www.worldclim.org and www2.jpl.nasa.gov/srtm/
Aspect	Aspect (in degrees) of the land surface. 0 and 359 correspond to north.	o	Geophysical	Derived from SRTM DEM	
Slope	Slope (in degrees) of the land surface	o	Geophysical	Derived from SRTM DEM	
bio_10	Mean Temperature of Warmest Quarter	°C	Bioclimatic	Worldclim	http://worldclim.org
bio_11	Mean Temperature of Coldest Quarter	°C	Bioclimatic	Worldclim	http://worldclim.org
bio_2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	°C	Bioclimatic	Worldclim	http://worldclim.org
bio_9	Mean Temperature of Driest Quarter	°C	Bioclimatic	Worldclim	http://worldclim.org
prec_10	Average october precipitation	Mm	Bioclimatic	Worldclim	http://worldclim.org

prec_11	Average november precipitation	Mm	Bioclimatic	Worldclim	http://worldclim.org
t_caso4	Topsoil Gypsum. Calcium sulphate (gypsum) content in topsoil.	% weight	Edaphic	HWS Database	http://www.iiasa.ac.at/Research/LUC/External-World-soil-database/
t_cec_clay	Topsoil CEC (clay). This field gives the cation exchange capacity of the clay fraction.	cmol/kg	Edaphic	HWS Database	http://www.iiasa.ac.at/Research/LUC/External-World-soil-database/
t_esp	Topsoil Sodicity (ESP). This field gives the exchangeable sodium percentage in the topsoil.	%	Edaphic	HWS Database	http://www.iiasa.ac.at/Research/LUC/External-World-soil-database/
t_ph_h2o	Topsoil pH (H2O). pH, measured in a soil-water solution, is a measure for the acidity and alkalinity of the soil.	-log(H+)	Edaphic	HWS Database	http://www.iiasa.ac.at/Research/LUC/External-World-soil-database/

Sampling bias (Figure 3) and taxon richness (Figure 4) maps were assessed using the methodology proposed by Scheldeman and von Zonneveld (2010) and in the analysis each grid cell is equivalent to 2.5km^2 at the equator. Herbaria and gene bank sampling is often *ad hoc*, non-systematic sampling and uneven sampling efforts (Chapman, 2005). Therefore, although Figure 3 shows bias in the pattern of collections, around Musandam, northeast Al Dakhiliya and particularly coastal Dhofar, these areas also have of high MP richness (Figure 4), so the bias was in areas of diversity, which was expected. For medicinal taxa 83 presence points (of 1,349 totals) were found inside four different protected areas (PA), so that 42 MP taxa (of a total of 272 taxa) were being passively conserved.

The predictive modelling based on the available historic data for MP in Oman is shown in Figure 5 and clearly indicated four areas of high predicted MP presence, Musandam, the Northern range of Al Akhdar and Bani Jabir mountains that run parallel to the coast, the island of Masirah and the Southern coastal mountains of Dhofar. *Ex situ* gap analysis (Figure 6) identified four areas of high *ex situ* MP conservation priority, Musandam, the Northern range of Al Akhdar and Bani Jabir mountains that run parallel to the coast, the island of Masirah and the Southern coastal mountains of Dhofar.

Complementarity Analysis. The Complementarity analysis result predicted that 10 grid cells (putative genetic reserve locations) capture at least one population of 152 MP taxa (Figure 7), 85 grid cells were needed to capture all 316 MP taxa included in the analysis. It was interesting to note the polarity resulting from the complementarity analysis with eight of the top 10 sites were clustered relatively closely together in the Dhofar regions. The other two sites are in the Jabal Al Akhdar region and west of Muscat, clearly demonstrating the regional disequilibrium of plant diversity in Oman because of the pervasive dry conditions. Given the results of the previous analysis identified three areas of high MP presence it is slightly surprising that Musandam does not get a top 10-grid cell, but this may be explained by the MP taxa present being congruent with those found in the two Northern Oman sites selected.

Gap Analysis Result and Conservation Recommendations . The taxon richness (Figure 4), predictive distribution (Figure 5) and *ex situ* gap analysis (Figure 6) each indicated areas of high MP diversity in the Northern range of Al Akhdar and Bani Jabir mountains and the Southern coastal mountains of Dhofar. Although previous sampling has been focused in these areas further surveying and *ex situ* sampling is required throughout these areas, as possibly 135 other MP taxa may be present but have to be recorded. Within these areas, the Al Jabal Al Akhdar Scenic Reserve and the Jabal Samhan Nature Reserve particularly should be targeted for future surveying efforts, as there were a high number of taxa predicted to be found there. As well as the two hotspots in the Al Akhdar / Bani Jabir mountains and Dhofar, it is also recommended that *ex situ* collection is undertaken in Musandam, the island of Masirah and Janaba as these areas are remote from the two main hotspots and may contain complementary MP taxa.

Climate Change. To illustrate the procedure climate change modelling was initiated for *Medicago sativa* and *Aerva javanica*, both species were selected as they had a relatively large number of presence points available for the analysis (the higher the number of points the greater the accuracy of the prediction) and *M. sativa* because it is cultivated and *A. javanica* because it is wild. Current climatic data was obtained from WorldClim (*Hijmans et al*., 2005) and future climate projections were from CCAFS (www.ccafs-climate.org). The SRES-A2 emission scenario was selected as it is considered the most realistic and due to its use in similar studies (Ramirez-Villegas et al., 2014). The

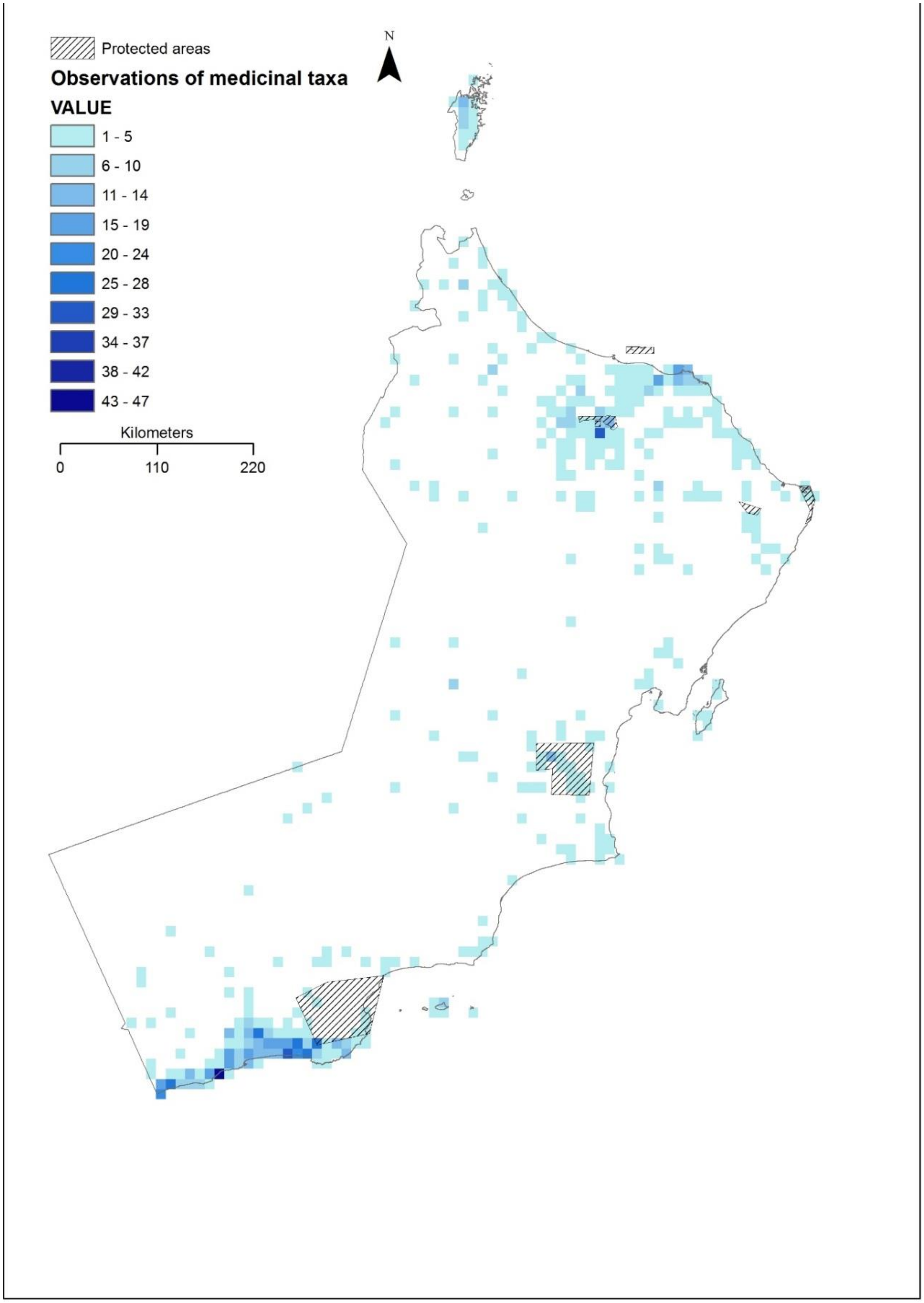


Figure 3. Map indicating numbers of medicinal plants observations in Oman.

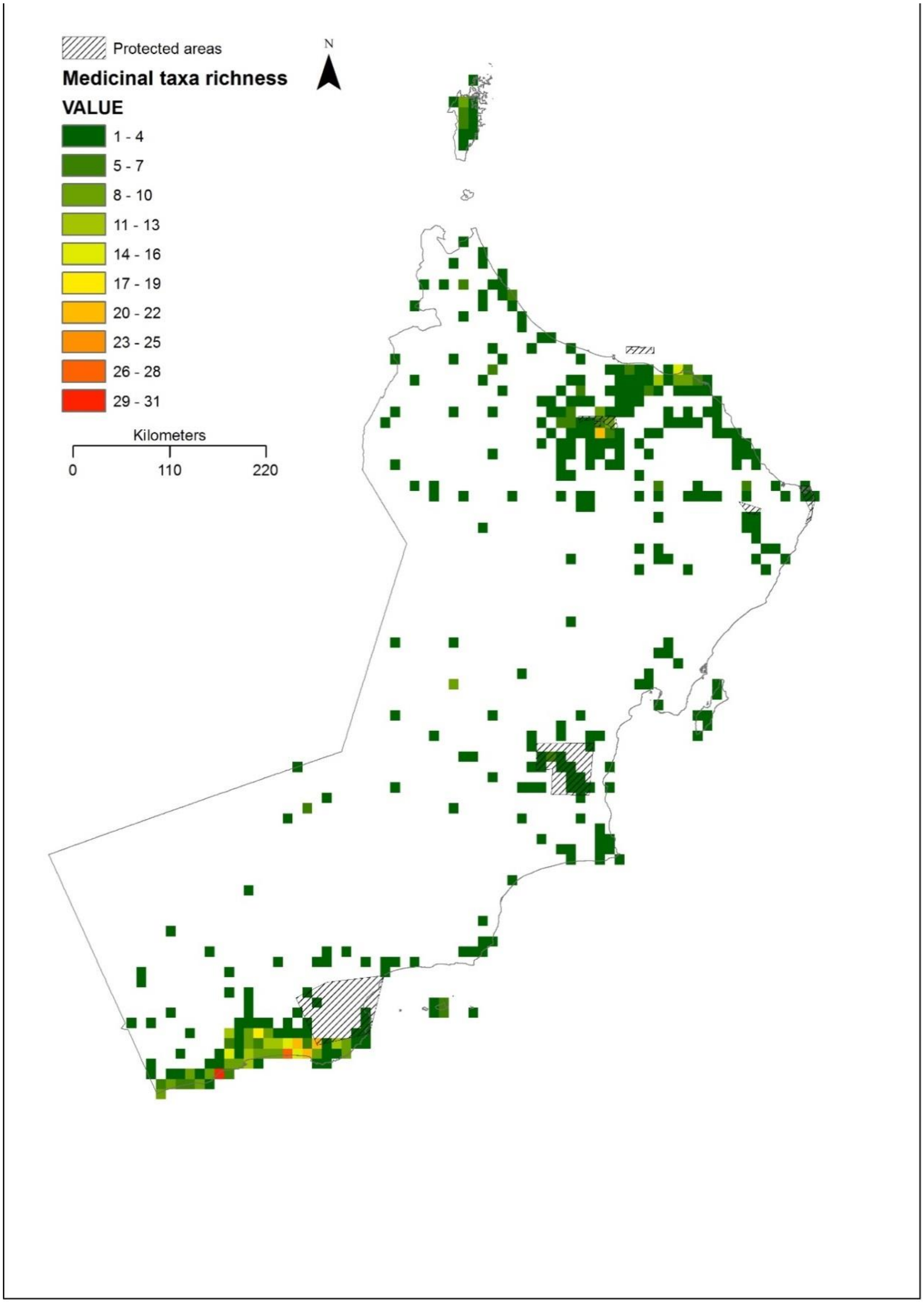


Figure 4. Map indicating taxon richness of medicinal plants in Oman.

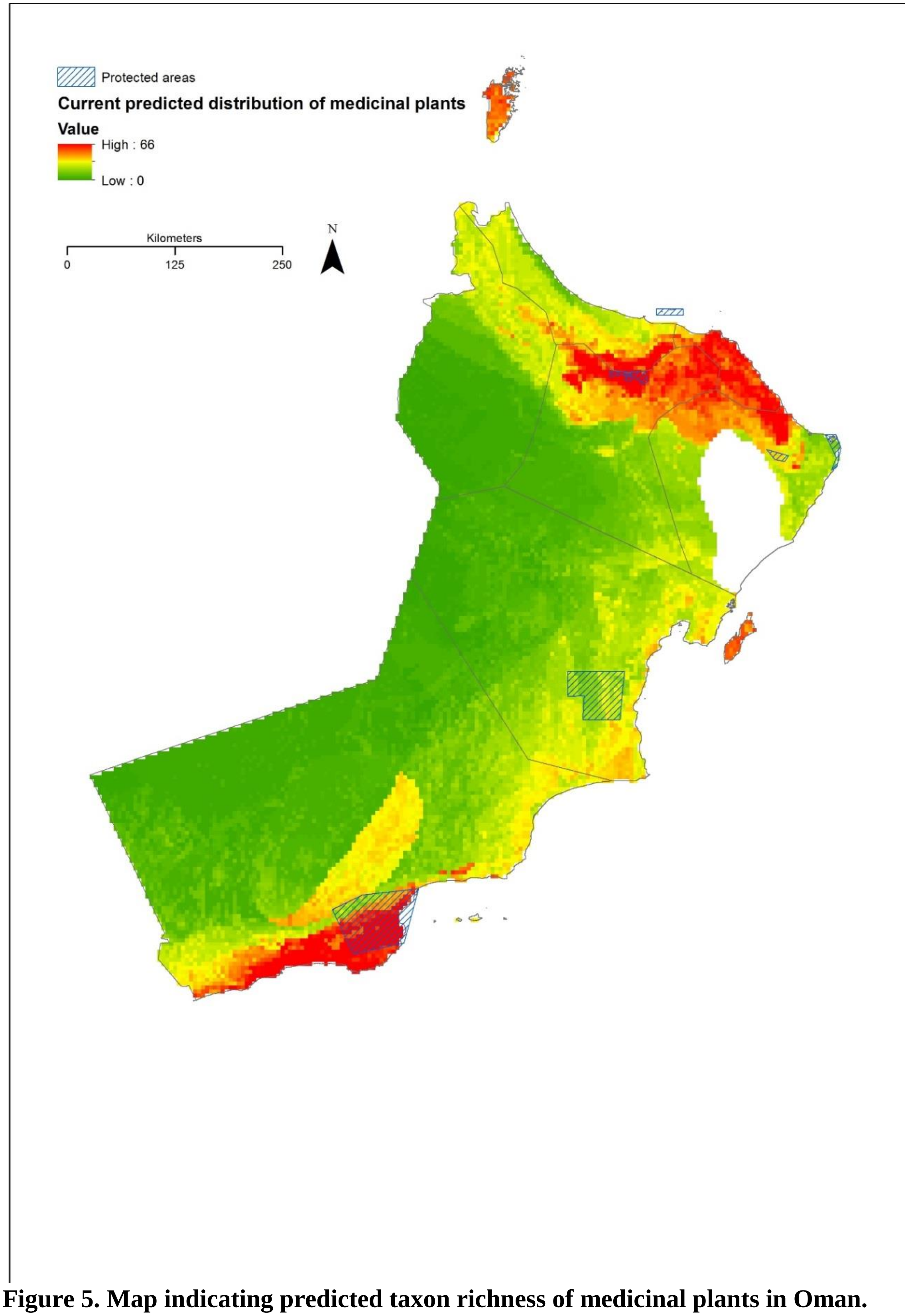


Figure 5. Map indicating predicted taxon richness of medicinal plants in Oman.

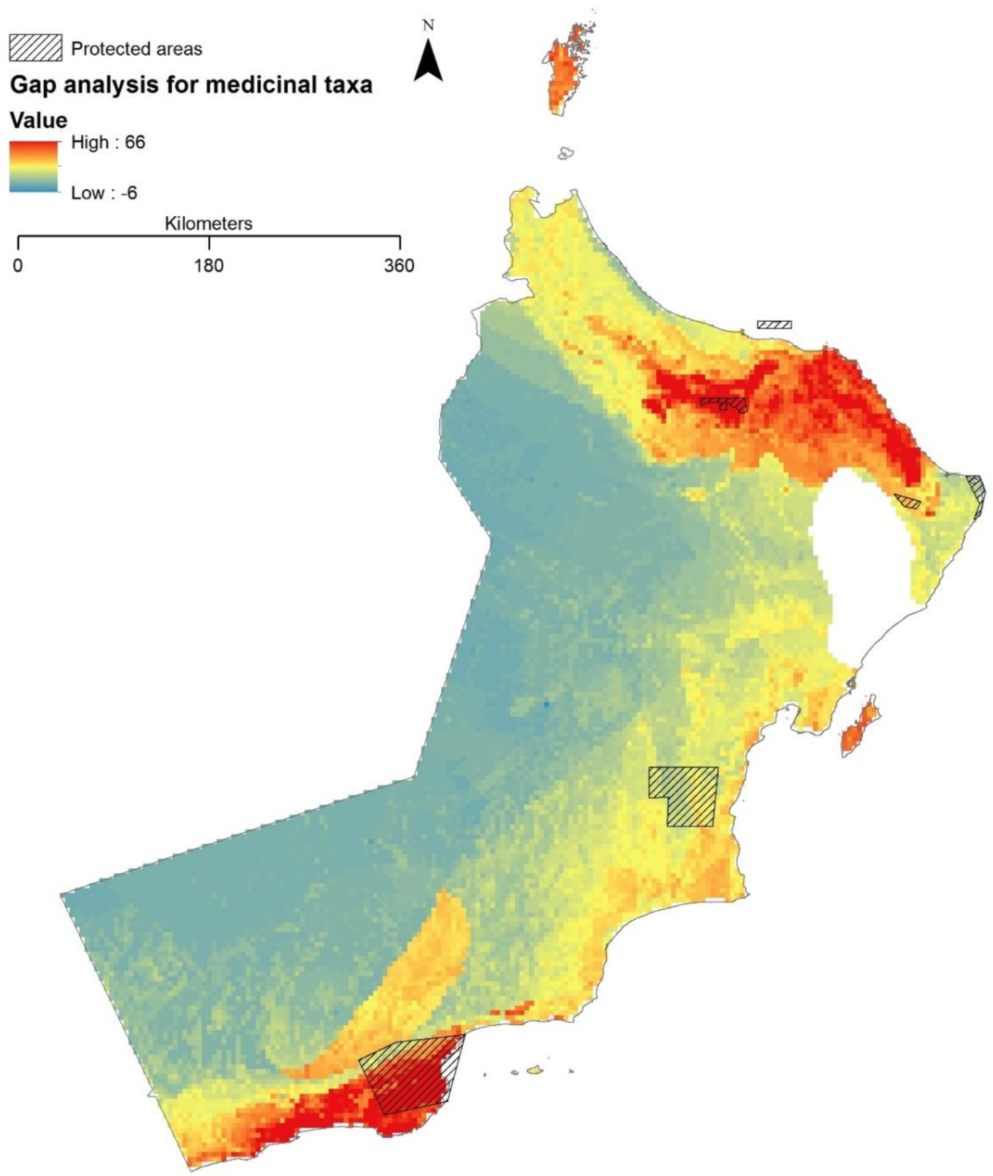


Figure 6. Map indicating overall results of ex situ gap analysis for medicinal plants in Oman.

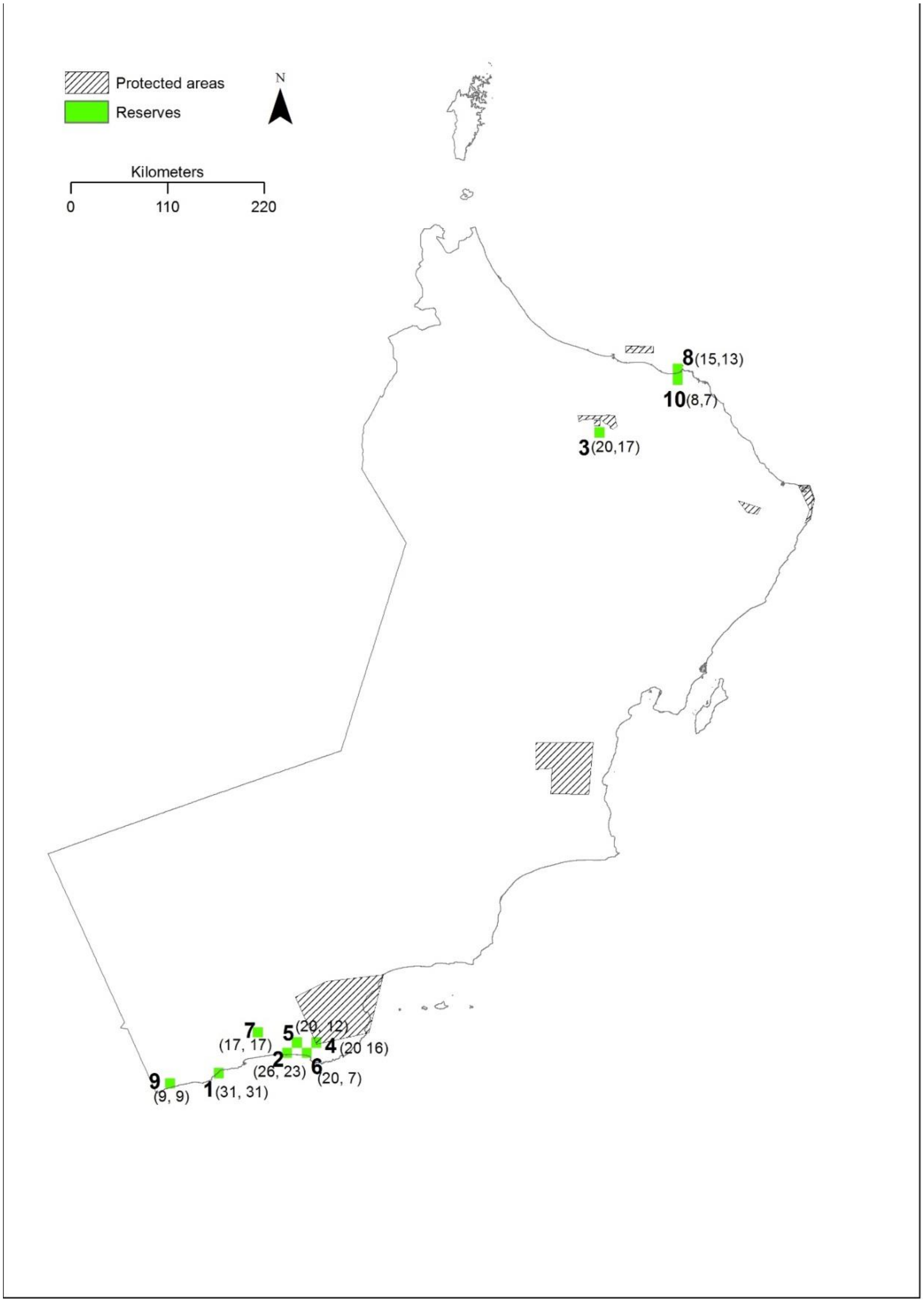


Fig. 7. Map indicating complementary grid cells for medicinal plants in Oman.

General Circulation Model (GCM) used was the UKMO-HadCM3 (http://www.metoffice.gov.uk/research/modelling-systems/unified-model/ climate-models/hadcm3).

The three time projections for *M. sativa* and *A. javanica* are shown in Figure 8a, b and c and Figure 9a, b and c respectively. Figure 8a shows the distribution of *M. sativa* under current climatic conditions with a distribution of 12% throughout the whole country area and predicted presence within three protected areas. Figure 8b shows the predicted distribution under climatic conditions in 2020 with 17.4% distribution area and presence inside one PA. Figure 8c shows the predicted distribution in 2050 with 2.3% distribution area and presence within one PA. To ensure *in situ* conservation of *M. sativa* it is recommended to set up active on-farm conservation management with local communities within the Ad Dakhliyah region. This area appears to be the 'core' of the *M. sativa* distribution as populations are predicted to be present here under all three time projections up to 2050. Figure 9a shows the distribution of *A. javanica* under current climatic conditions with a distribution of 10.7% throughout the whole country area and predicted presence within three protected areas. Figure 9b shows the predicted distribution under climatic conditions in 2020 with 35.2% distribution area and presence inside one PA. Figure 9c shows the predicted distribution in 2050 with 1.6% distribution area and presence within one PA. To ensure *in situ* conservation of *A. javanica* it is recommended to undertake active protected area management within the Al Jabal Al Akhdar Scenic Reserve in the Ad Dakhliyah region.

It would also be a priority to increase active management and protection of those populations of *M. sativa* found in the south near Salalah and *A. javanica* adjacent to the Yemen border because they both appear to persist over the prediction period and may contain important and different genetic diversity to the populations in the north. It will also be necessary to sample and store *ex situ* from the population that are projected to go extinct as a result of climate change.

Current Conservation Status. Oman currently has no *in situ* protected areas or genetic reserves that actively conserve MP diversity. Although MP taxa are undoubtedly conserved in the existing formally designated protected and non-protected areas in Oman, such as areas of roadsides, field margins, forest and national parks. However, in each existing protected area the existence of a particular MP taxon is likely to be coincidental because the site is managed for mega-fauna, habitat diversity or recreation, or is not managed in any active form. In terms of conservation, this is termed 'passive' conservation (Maxted *et al.*, 1997), 98 that in this context means 'healthy' MP populations may occur coincidentally within a location designated for conservation but where there is no active management of those populations by conservationists. To sustain MP populations long-term more 'active' management is required, which implies some form of dynamic intervention at the site, even if that intervention is simply limited to monitor the MP populations and adjusting the site management if target MP populations are declining.

In terms of *ex situ* conservation, it was estimated that Oman has 441 plant accessions held in *ex situ* collections and of these 292 were socio-economically important species and 136 (out of 448) MP taxa have *ex situ* samples. But it was unknown how many populations of each of these are conserved. The recommendation within a country for a non-endemic taxon is 10 populations per MP taxon (Maxted *et al.*, 2008b) as this was thought to provide an adequate sample of indigenous genetic diversity. As the precise number of *ex situ* collections per MP is unknown it is proposed that further sampling for *ex situ* conservation is required for all MP taxa in Oman.

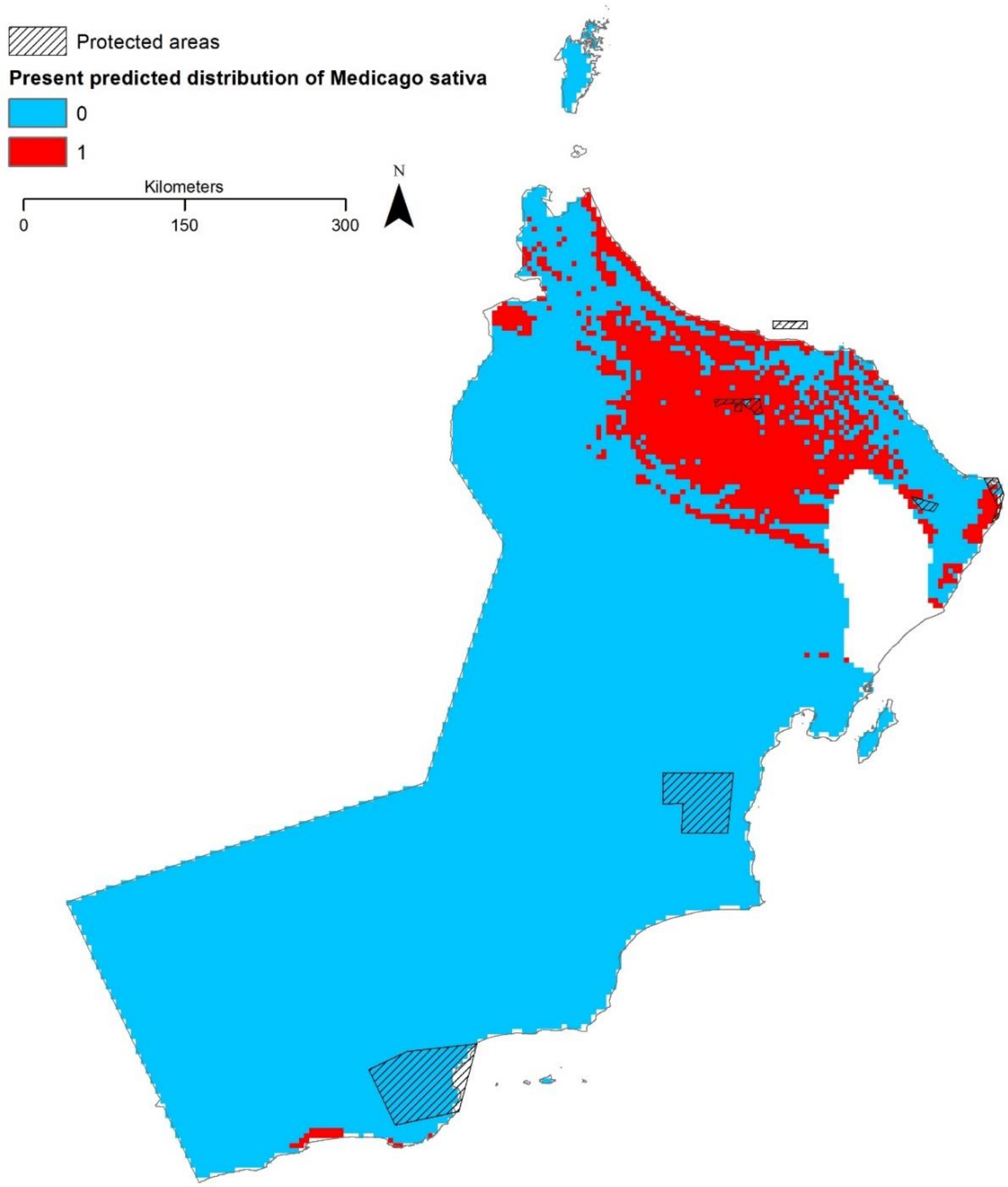


Figure 8a. Predicted distribution of *Medicago sativa* in Oman at present (2015).

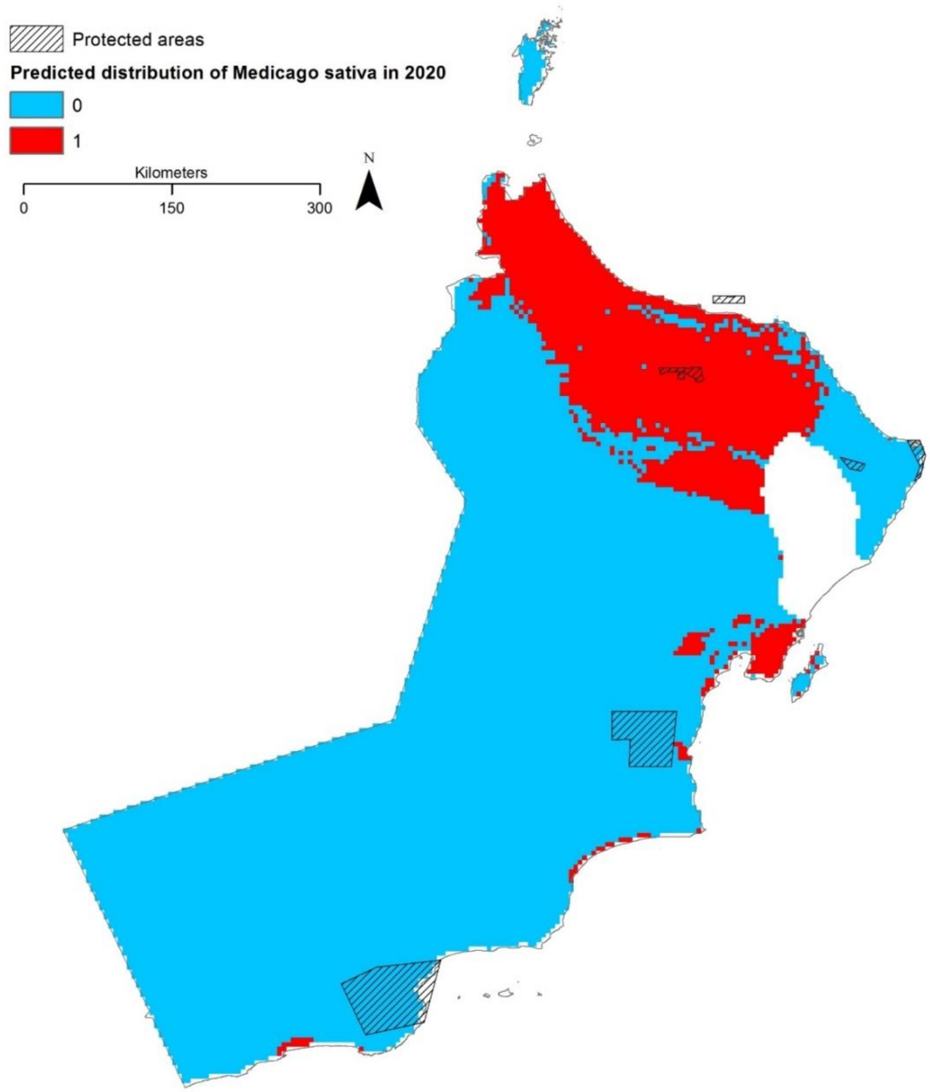


Figure 8b. Predicted distribution of *Medicago sativa* in Oman at 2020.

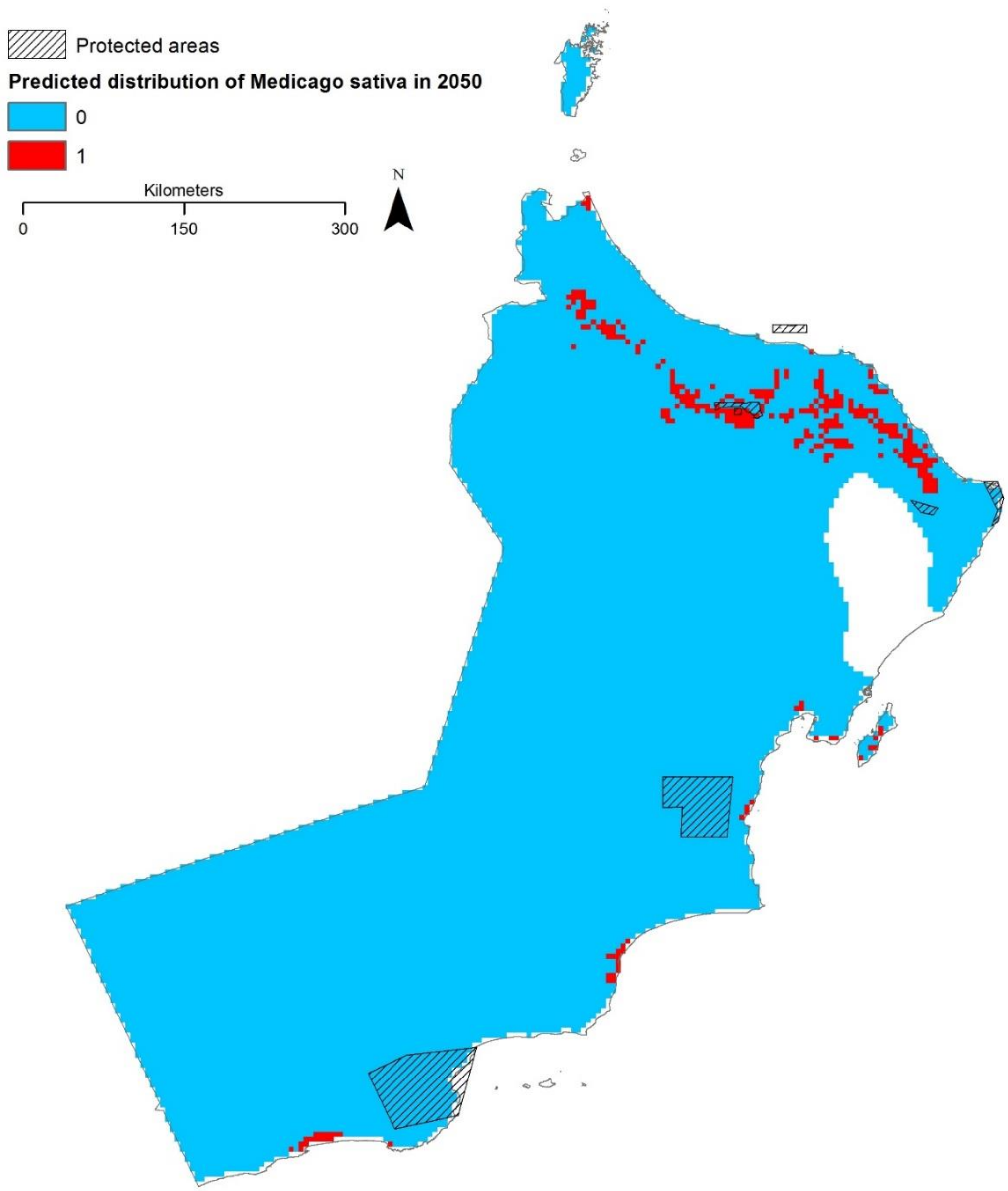


Figure 8c. Predicted distribution of *Medicago sativa* in Oman at 2050.

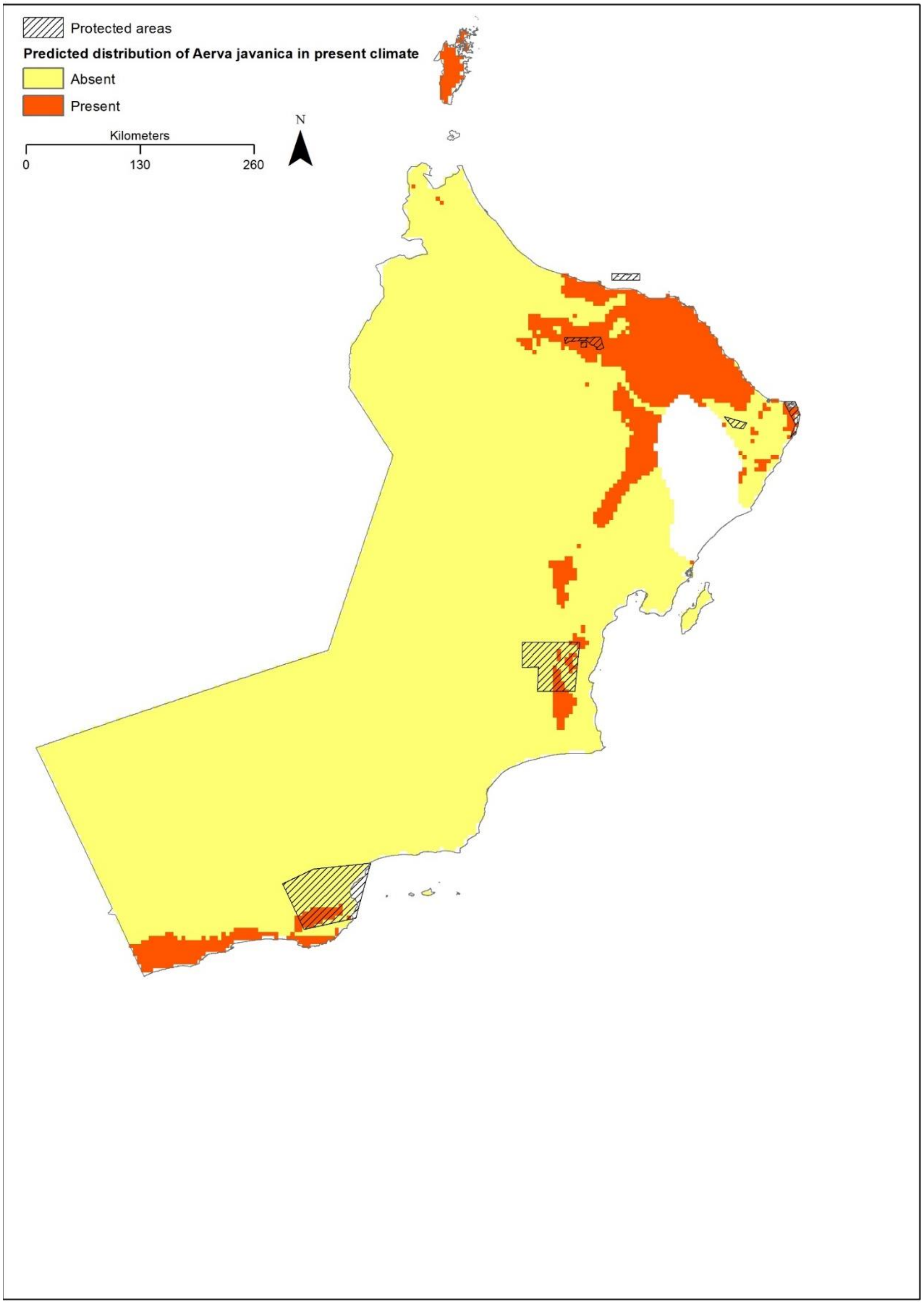


Figure 9a. Predicted distribution of *Aerva javanica* in Oman at present (2015).

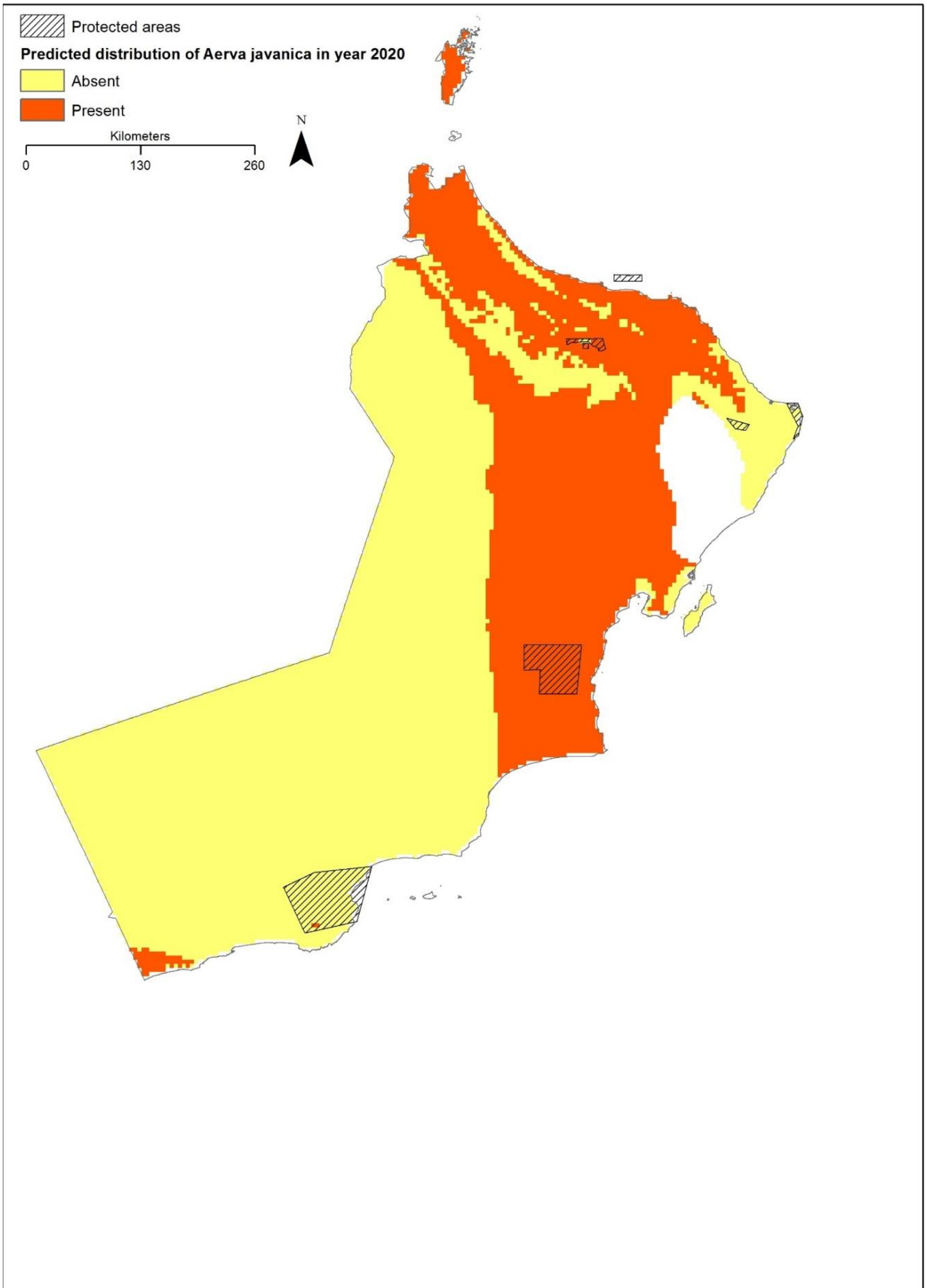


Figure 9b. Predicted distribution of *Aerva javanica* in Oman at 2020.

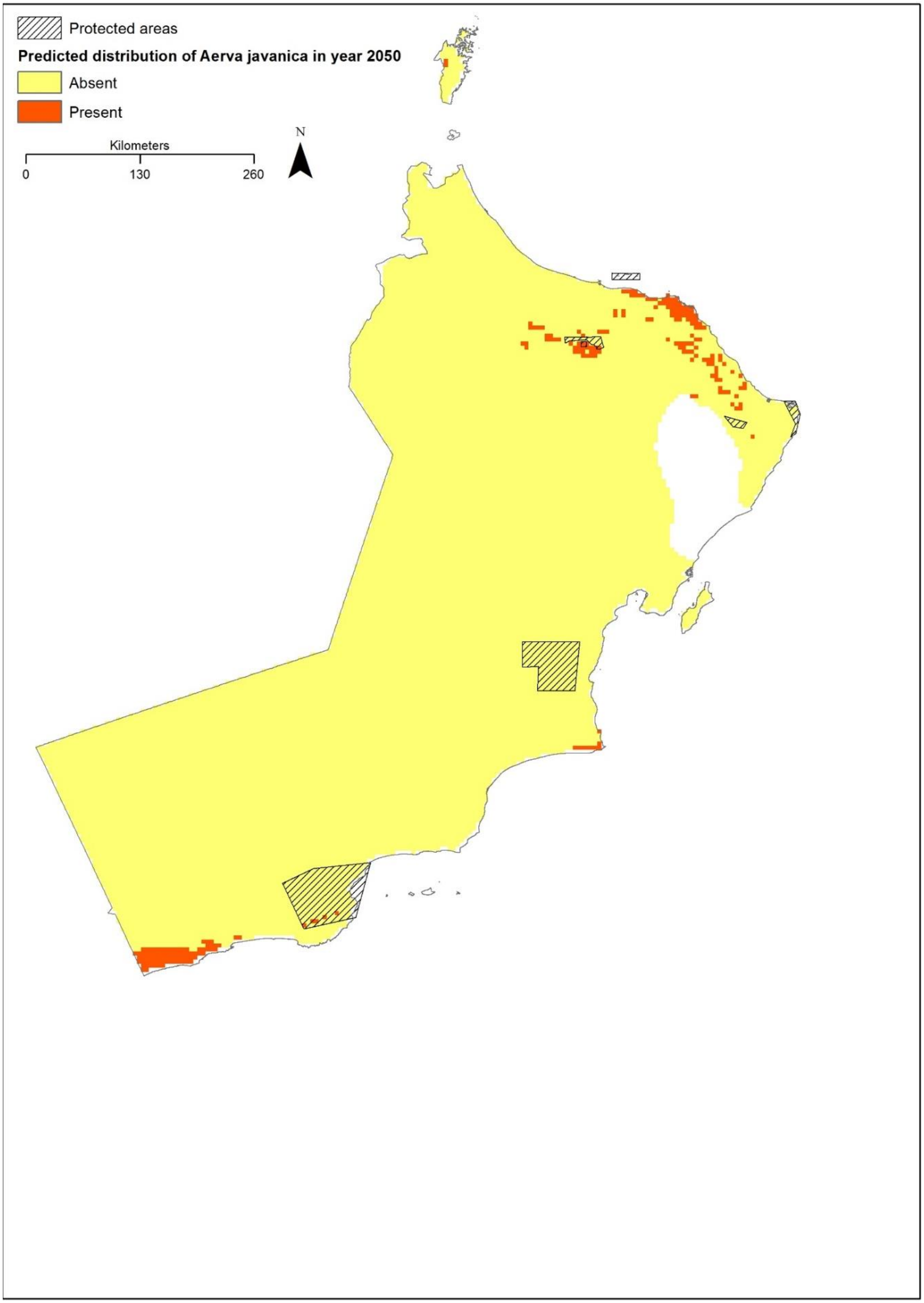


Figure 9c. Predicted distribution of *Aerva javanica* in Oman at 2050.

Conclusions

Oman is rich country for plant species that have socioeconomic importance such as medicinal plants. However, this study showed us that MP is not well conserved either in *ex situ* and *in situ*. In addition, diversity within species of MP collections is not effectively being genetically conserved using either *in situ* or *ex situ* conservation techniques. Impact of climate change model studied in this paper showed significant reduction in distribution of investigated species in coming 35 years.

References

Aburjai, T., Hudaib, M., Tayyem R, Yousef M, Qishawi M (2007) Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. *Journal of Ethnopharmacology*, 110: 294-304.Aburjai, 2010

Al-Assaf, A., Nawash, O. and Omari, M., (2014). Identifying forest ecosystem services through socio-ecological bundles: a case study from northern Jordan. *International Journal of Sustainable Development and World Ecology*, 21(4): 314-321.

Chapman, A.D., (2005). *Principles of Data Quality, version 1.0*. Report for the Global Biodiversity Information Facility, Copenhagen.

Daily, G. and Dasgupta, S., (2001). *Concept of ecosystem services*. Academic Press, San Diego, USA.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones P.G. and Jarvis, A., (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25: 1965-1978.

Hijmans, R.J., Guarino, G. and Macathur, P., (2012) *DIVA-GIS Version 7.5. Manual*. Rome, Italy, Bioversity International.

Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G., (1997). Complementary Conservation Strategies. In: *Plant genetic conservation: the* in situ *approach* (eds. Maxted, N., Ford-Lloyd, B.V. & Hawkes, J.G.), pp. 20-55. Chapman & Hall, London.

Maxted, N., Ford-Lloyd, B.V. and Kell, S.P., (2008a). Crop wild relatives: establishing the context. In: Maxted N, Ford-Lloyd BV, Kell SP, Iriondo J, Dulloo E and Turok J (Eds) *Crop wild relative conservation and use*. CABI Publishing, Wallingford. pp. 3–30.

Maxted, N., Iriondo, J.M., de Hond, L., Dulloo, M.E., Lefèvre, F., Asdal, A., Kell, S.P. and Guarino, L., (2008b). Genetic reserve management. In: Iriondo, J.M., Dulloo, M.E. and Maxted, N. (eds) *Conserving plant genetic diversity in protected areas: population management of crop wild relatives*. CAB International Publishing, Wallingford. pp. 65-87.

Maxted, N., Castañeda Álvarez, N.P., Vincent, H.A. and Magos Brehm, J., (2012b). Gap analysis: a tool for genetic conservation. In Guarino L, Ramanatha Rao V, Goldberg E (editors). *Collecting Plant Genetic Diversity: Technical Guidelines*. 2011 update. Bioversity International, Rome. Available online: http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=678

McNeely, J.A., Mittermeier, R.A., Brooks, T.M., Boltz, F. and Ash, N., (2009). *The wealth of nature: ecosystem services, biodiversity and human well-being*. Cemex, Arlington, WA, USA.

Millennium Ecosystem Assessment (MEA). (2005). *Ecosystems and Human Well-Being: Synthesis*. Island Press, Washington. 155pp.

Ministry of Agriculture and Fisheries (MAF), (2005). Establishment of field genebank of indigenous medicinal plant species of Oman. Annual Report of Agriculture Research-2003-2004 (Abstracts). *Directorate of Agriculture and Livestock Research. Ministry of Agriculture and Fisheries. Sultanate of Oman*. Pp 24-25 (Arabic).

Ministry of Agriculture and Fisheries (MAF), (2008). The state of Plant Genetic Resources for Food and Agriculture in Oman. *Directorate General of Agriculture & Livestock Research, MAF, Muscat, Sultanate of Oman*.

Parra-Quijano, M., Iriondo, J.M., Cruz, M. and Torres, E., (2012). Spatial and ecogeographical approaches for selecting genetic reserves in Europe. In: Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Frese, L., Iriondo, J.M. and Pinheiro de Carvalho, M.A.A. (eds.) *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces*. Pp. 20-28. CAB International, Wallingford.

Phillips, S.J., Anderson, R.P. and Schapire, R.E., (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190(3/4): 231-259.

Ramirez-Villegas, J., Cuesta, F., Devenish, C., Peralvo, M., Jarvis, A., Arnillas, C.A., (2014). Using species distribution models for designing conservation strategies of Tropical Andean biodiversity under climate change. *Journal for Nature Conservation*, 22: 391-404.

Rivera, D., Obn, C., Heinrich, M., Inocencio, C., Verde, A. and Fajardo, J., (2006). Gathered Mediterranean food plants ethnobotanical investigations and historical development. In: Heinrich M, Müller WE and Galli C (eds). *Mediterranean food plants and nutraceuticals.* Forum of Nutrition, Basel Karger. pp. 218-274.

Rebelo, A.G., (1994). "Iterative selection procedures: centres of endemism and optimal placement of reserves," In Huntley, B.J. (ed.) *Botanical Diversity in Southern Africa*. Pretoria: National Botanic Institute. pp. 231-257.

Scheldeman, X. and van Zonneveld, M., (2010). *Training manual on spatial analysis of plant diversity and distribution*. Bioversity International, Rome, Italy.

Takruri, H.R., Tukan, S.R. and Ahmad, M., (2008). Medicinal and edible wild plants used by the community of Northern Badia of Jordan. *Journal of Agricultural Research*, 4(4): 391-405.

Tukan, S.K., Takturi, H.R. and Al-Eisawi, D.M., (1998). The use of wild edible plants in the Jordanian diet. *International Journal of Food Sciences and Nutrition*, 49: 225-235.

Section 2

Collection, Processing and Storage of Seeds

Overcoming Seed Challenges to Address Landscape Restoration Needs in Lebanon and Jordan

Karma Bouazza, Mohammad Nsour, Khaled Sleem and Anthony S. Davis
Lebanon Reforestation Initiative (LRI), Hazmieh, Lebanon
* **e-mail**: kbouazza@lri-lb.org

Abstract

For the past few decades, Lebanon and Jordan have been facing an ongoing and rapid decrease in their natural spaces. Only 13% of forest cover is remaining in Lebanon and Jordan's Badia region (arid rangeland) is undergoing rapid degradation. Several factors including urbanization, grazing, climate change, land tenure, and unmanaged natural resources are contributing to this rapid loss. To mitigate the effects of this loss of ecosystem integrity, restoration projects are readily being launched in the region. One of the major challenges facing successful landscape restoration is access to, and the proper use of, native genetic resources. Two major projects, the Lebanon Reforestation Initiative (LRI) and Sustainable Environment and Economic Development – Jordan (SEED) are working at the national and regional levels to develop and expand sharing best practices to promote sustainable landscape restoration approaches. In Lebanon, efforts are focused on managing native genetic resources and improving propagation and field protocols to support forest restoration efforts; in Jordan those efforts are focused on Badia rangelands. With regional collaboration increasing, practices are being disseminated both within Lebanon and to Jordan. Thishas led to a prioritized use of native genetic material for native seedling propagation and landscape restoration. Both countries face challenges due to a lack of shared best practices, no national strategy for seed and genetic resource management, and the inability to share technologies and practices across relevant partners in a timely manner. Through research and collaborative partnerships on local and regional levels andbetween public and private sectors, a platform is being developed that integrates all related efforts with resources made public for all involved stakeholders. This paper outlines the approaches taken by LRI and SEED to address challenges related to seeds in landscape restoration, highlighting the major limitations faced and how collaborative partnerships on the local levels and between the two countries have helped in establishing a baseline forsustainable native genetic resource management.

Keywords: Land degradation, seedling production, Badia region, forest restoration, sustainable economic and environmental development project.

Introduction and Background

Landscape restoration is an important step toward increasing green cover, protecting watersheds, and alleviating existing pressures on degraded natural resources and ecosystems (Sabogal et al. 2015). A critical step towards success is building on regional experiences, whether successes or failures. However, in the Middle East, there is a clear lack of communication between countries on common issues they share. To challenge this shortcoming, the U.S. Forest Service International Programs (USFS IP) is adopting a regional approach to address landscape restoration practices. USFS IP aims to further expand sharing of best practices throughout the region to promote sustainable landscape restoration approaches. We discuss specifically in this paper the role of two USFS IP projects in Lebanon and Jordan that focus on the use of native genetic resources for ecosystem restoration.

For the past few decades, Lebanon has been facing an ongoing, and rapid, decrease in its natural spaces, highlighted by only 13% of forest cover remaining (FAO 2005). Several factors contribute to this rapid loss, including urbanization, grazing, climate change and unmanaged natural resources. One of the major challenges facing successful landscape restoration is the proper use of native genetic resources successfully and sustainably. The Lebanon Reforestation Initiative (LRI), a program funded by the United States Agency for International Development (USAID), and implemented by USFS IP, has been assisting these efforts since 2011. LRI promotes a participatory approach to reforestation, bringing together diverse committed municipalities, local stakeholders, Lebanese civil society organizations, and other actors, such as the private sector, to restore and replant degraded community lands (LRI 2014). In Lebanon, efforts are focusing on managing native genetic resources and improving propagation and field protocols to sustain restoration efforts. Through implemented research and collaborative partnerships on the national level, between public and private sectors, a data platform is being developed that integrates related efforts with resources made public for stakeholders. Lebanon faces some limitations, in large part due to a previously non-existing central/national management of native seed sources and seed collection and lack of developed practices. Thus, LRI is supporting disseminating practices within Lebanon, where there is a prioritized use of native genetic material for native seedling propagation and landscape restoration.

Jordan's Badia, the arid rangeland featuring low rainfall while supporting a wide range of biodiversity represents 80% of Jordan's landbase. The region is also characterized by a great diversity of unique habitats, harboring a diverse array of fauna and flora, including medicinal and rangeland native species. Intensive use of natural resources by communities, combined with poor land ownership policies and a lack of clear land tenure, and environmental factors, are resulting in rapid degradation of lands, leading to water scarcity in an area that is already water-poor and to loss of wildlife habitats. Landscape-level water conservation is an important aspect of water security in Jordan, as vegetation, soil and water are all interconnected (ICARDA 2012). The Sustainable Environment and Economic Development project (SEED) is funded by USAID and implemented by USFS IP. USFS IP cooperates with governmental and non-governmental organizations in Jordan to advance natural resource conservation, primarily by providing technical assistance and exchange opportunities for Jordanian professionals in the natural resource sector. The SEED project targets the enhancement of native plant nursery and outplanting practices to improve seedling quality and survival rates post-planting, in the framework of sustaining natural resources in the Badia.

LRI and SEED are supporting community nurseries in advanced seedling production practices, based on regional and international nursery trials and experience. This is done with an aim of refining practices that are easily adoptable and replicable in order to ensure sustainable production of high quality native plant seedlings. The Target Plant Concept, a theoretical framework that provides holistic consideration of all aspects of the plant establishment process, serves as the underlying basis for the regional approach being taken. This approach was introduced to regional partners and nursery growers to help build connectivity needed to to produce a fit and robust seedling that is adapted to local site conditions, thus resulting in higher survival rates post-planting. To achieve a target seedling, several factors are taken into consideration, including limiting factors related to the outplanting site, sources of planting material and timing of the outplanting window (Dumroese et al. 2016).

Currently, ecosystem rehabilitation and restoration projects throughout the world prioritize the use of native genetic resources, to conserve species richness and diversity. In this paper we review the current practices of ecosystem restoration, focusing on native seed management in Lebanon and

Jordan. The LRI and SEED projects also focus on developing technical capacities of local communities with minimal or no scientific backgrounds. Therefore, communities transfer the knowledge acquired through trainings and workshops to implement rehabilitation and restoration related activities, from seed management to site outplanting. The projects depend more on community based research with preliminary results that would lead to the development of more scientific research in the region, as it was primarly lacking, with no data to base new research on.

Lebanon and Jordan Approaches

In Lebanon, in order to address seed challenges as a component of its broader focus on landscape restoration, LRI has taken a science-based approach, based on collaborative partnerships and a global body of research, to help establish a baseline for a more sustainable native genetic resource for research and management. LRI, working with the Centre for Applied Research in Agroforestry Development (IDAF), sought to develop seed collection zones for reforestation purposes. Several principal native species used in reforestation in Lebanon were included in the study, emphasizing the importance of biodiversity in restoration projects. Taking into consideration the genetic make-up of the forest reproductive material will help develop guidelines for choosing the most suitable forest reproductive material (FRM) to grow good quality seedlings adapted to specific site conditions, thus increasing reforestation success rates. Developing a sustainable native seed network is achieved through the following objectives:

- Identification and description of the regions of provenance in Lebanon. For species or sub-species, the region of provenance is the area or group of areas subject to sufficiently uniform ecological conditions in which stands showing similar phenotypic or genetic characters are found.
- Identification and mapping of seed sources and stands for the main forest species in Lebanon.

LRI has also worked with several native nurseries and academic institutions to develop seed germination protocols based on international standards and local conditions. Given that one of the blockers to successful restoration is a lack of knowledge on germination protocols (CITE), this was viewed as a priority by LRI and partners. From an initial list of 41 protocols, Table 1 shows the native species that were germinated using different protocols which were subsequently shared across the Cooperative of Native Tree Producers of Lebanon.

In Jordan, the SEED project focused on developing germination protocols for native rangeland species for restoration of the Badia, while simultaneously developing contemporary nursery practives. In 2016, a pilot was developed to produce around 80,000 seedlings from six species native to the Badia region; *Atriplex halimus*, *Artemisia herba-alba*, *Salsola vermiculata*, *Retama raetam*, *Ziziphus jujuba* and *Thymus bovei*. Seed sources and dates of seed collection were unknown as seed was sourced from local partners with limited record keeping. Poor germination was common across all species and seeds were notably mixed with high loads of debris as well as insects, both of which were identified as contributing factors to the latter.

Building on that experience, the SEED project decided to partner with local experts to develop its own seed collection and treatment plans to improve the biological and administrative quality of seed processing (Table 2). This led to a 2017 growing plan of six species native to the Badia region; *Atriplex halimus*, *Artemisia herba-alba*, *Salsola vermiculata*, *Retama raetam*, *Achillea fragrantissim*a and *Thymus bovei*. These seedlings were produced in a community nursery, located in Sabha, Mafraq, Early results from this shift in practice indicate improved germination and seedlings are developing well (Table 3).

Table 1. List of Native Species used for Seed Germination Trials

Scientific Name	Common Name	Suitable Altitude
Abies cilicica	Cilician Fir	1200 – 2000
Acer syriacum	Syrian Maple	0 - 500
Acer tauricolum	Taurus Maple	1000 - 1900
Arbutus andrachne	Grecian Strawberry Tree	0 - 800
Cedrus libani	Cedar of Lebanon	1000 - 2500
Celtis australis	Mediterranean Nettle	500-2000
Ceratonia siliqua	Carob	0-500
Cercis siliquastrum	Judas Tree	0-800
Cupressus sempervirens	Mediterranean Cypress	500-700
Fraxinus syriacus	Syrian ash	300-800
Fraxinus ornus	Flowering ash	700-1200
Juniperus excelsa	Grecian Juniper	1000-2000
Laurus nobilis	Bay Laurel	0-600
Pinus brutia	Calabrian Pine	0-1800
Pinus halepensis	Aleppo Pine	500-1000
Pinus pinea	Stone Pine	0-1500
Pistacia palaestina	Palestinian Pistachio	500-1000
Prunus amygdalus	Almond	700-1700
Pyrus syriaca	Syrian Pear	1500-2000
Quercus brantii look	Brant's Oak	1000-1600
Quercus calliprinos	Kermes Oak	500-1000
Quercus cerris	Turkey Oak	1000-1600
Quercus infectoria	Aleppo Oak	500-1000
Rhus coriaria	Summac	0-1600
Sorbus flabilifolia	Fan-Leaved Service Tree	0-1600
Sorbus torminalis	Wild Service Tree	1400-1600
Styrax officinalis	Storax	500-1000

Table 2. Identified Seed Collection Zones and Time Per Native Rangeland Species

Species	Collection time	Seed zones
Atriplex halimus	August – October	North Badia: Rowayched, Sawgaa reserve South Badia: Bayer reserve
Artemisia herba alba	Mid-July to mid-September	South Badia: Shobak, Maan North Badia: Rowayched
Salsola vermiculata	August – October	North Badia: Rowayched
Thymus bovei	June	North Badia: Safawi, El Hashamiya reserve
Retama raetam	July – August	Wadi el Botom, Azraq
*Achillea fragrantissim*a	September	North Badia: Safawi

Table 3. Identified Seed Treatment and Sowing Time Per Native Rangeland Species

Species	Sowing time	Seed treatment
Atriplex halimus	March – April	4°C controlled environment until sowing
Artemisia herba alba	March – April	4°C controlled environment until sowing
Salsola vermiculata	March – April	4°C controlled environment until sowing
Thymus bovei	March – April	4°C controlled environment until sowing
Retama raetam	March – April	4°C controlled environment until sowing Before sowing, boiling for 2 minutes and soaking
*Achillea fragrantissim*a	March – April	4°C controlled environment until sowing

Outcomes and next steps

To enhance genetically-appropriate restoration practices in Lebanon, 48 seed collection zones across 13 regions of provenence are being used to capture altitude, topography, and climatic differences that result in highly diverse ecological conditions in a relatively small country (Figure 1) (IDAF 2013). The regions of provenance serve as communities of potential tree stocks with similar genetic compositions that are sufficiently large enough to provide sustainable seed quantities.

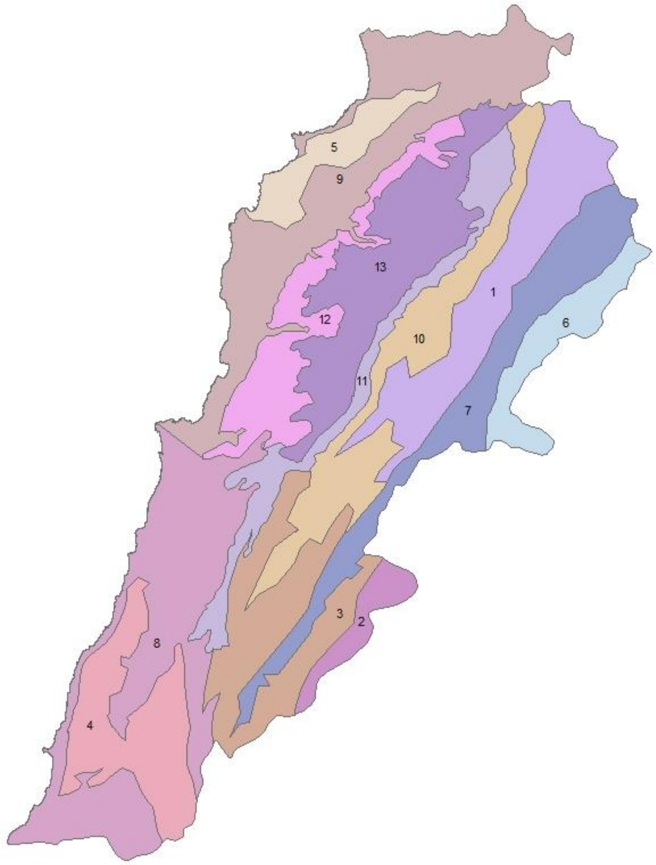


Figure 1. Regions of provenance in Lebanon (LRI-LB.org).

Trials acrsoss seedling nurseries have yielded general activities that lead to enhanced tree seed germination through investigations by LRI and partners. Table 4 indicates recommended sowing times per species and treatments required before sowing that were followed by LRI-supported native tree nurseries. Continuous testing on germination of native species should be implemented to improve database in Lebanon related to seed management.

In Jordan, during the 2016 growing season, there was a very low germination rate, and for some species, no germination at all. Table 5 shows approximiteley the germination for each native rangeland species under the conditions experienced. The trial was implemented on a small scale due to limited seed sources and poor quality of available seeds. Moreover, the unusually high temperatures observed in May 2016, 2-3 weeks after sowing (Figure 2) may have lead to further decreases in germination and seedling development as conditions became unfavourable for seeds to germinate and plants to develop. Temperatures fluctuated greatly throughout the growing season and could have affected germination rates and seedling growth.

Table 4. Recommended Sowing Time Per Species and Pre-sowing Treatments

Species	Sowing time	Treatment pre-sowing
Abies cilicica	February	2 months cold stratification
Sorbus flabellifolia	February-March	3 to 4 months cold stratification
Sorbus torminalis	February-March	3 to 4 months cold stratification
Styrax officinalis	February-March	Cold stratification > 3 months
Acer tauriculum	March	3 months cold stratification
Acer syriacum	March	3 months cold stratification
Arbutus andrachne	March	2 months cold stratification
Cedrus libani	March	1 month cold stratification
Celtis australis	March	3 months cold stratification
Juniperus excelsa	March	Citric acid scarification + 7 months cold and warm stratification
***Cercis siliquastrum*.**	March	3 months cold stratification
Laurus nobilis	March	1-2 months cold stratification
Ceratonia siliqua	March-April	2 weeks in water
Pistacia palaestina	March-April	Cold stratification 15 days
Alnus orientalis	April	No treatment needed
Cupressus sempervirens	April	1 month stratification
Pinus pinea	May – June	No treatment needed, pre-soaking for 24 hours
Pinus brutia	May	No treatment needed
Pinus halepensis	May	No treatment needed
Quercus brantii	December-January	No treatment needed
Quercus cerris	December-January	No treatment needed
Quercus calliprinos	December-January	No treatment needed
Quercus infectoria	December-January	No treatment needed

Table 5. Approximate Germination Rate Per Species, 2016

Species	Sowing time	Germination
Atriplex halimus	April 15 – 30	< 30%
Artemisia herba alba	April 15 – 30	0%
Salsola vermiculata	April 15 – 30	0%
Thymus bovei	April 15 – 30	< 50%
Retama raetam	April 15 – 30	< 30%
Ziziphus jujuba	April 15 – 30	< 30%

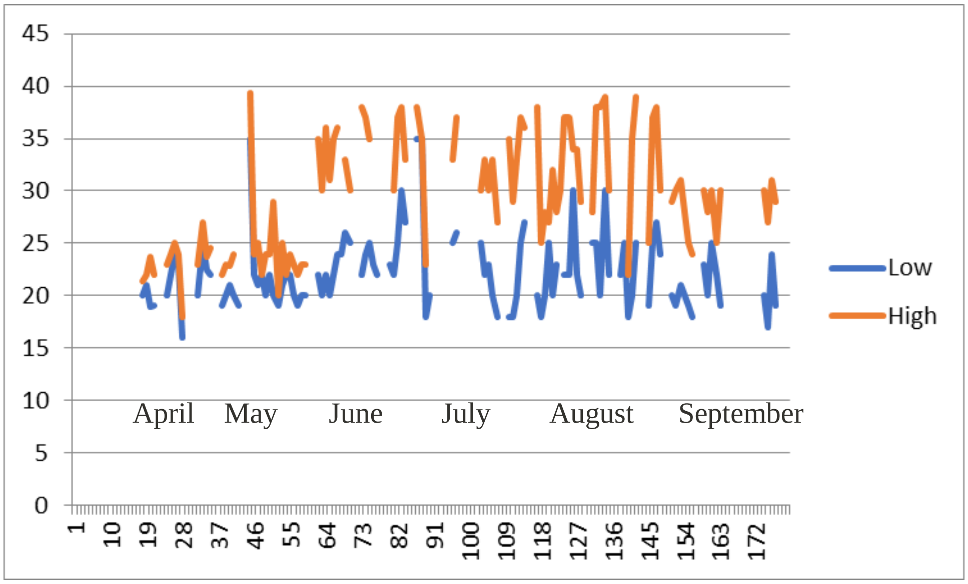


Figure 2. High (red) and low (blue) temperature in the greenhouse.

In Jordan, outplanting trials were implemented in December 2016 to assess the quality of seedlings in two sites, Majidiya and Sabha. Monitoring results that eneded in October 2017 showed that in Majidiya there was an 80% survival rate for *Atriplex halimus*, based on monitoring 380 seedlings, and 72% survival rate for *Retama raetam*, based on monitoring 92 seedlings. In Sabha, there was an 81% survival rate for *Atriplex halimus*, and 77% survival rate for *Retama raetam*. These compare favorably with the reported estimate of 25% first year survival for shrubs in restoration plantings.

During the 2017 growing season, sowing was done earlier in the season to avoid the high temperatures experienced in May 2016. As shown in table 2, seeds were collected from known zones and quality of seeds was assessed before. Priort to sowing, germination trials were done and showed high germination rates for most species. In 2016, *Artemesia herba alba* and *Salsola vermiculata* had 0% germination. In 2017, the germination rates were more than 80% and more than 60% respectively. This improvement is attributed to the improved collection and handling pratices that were developed and led to a total production of more than 80,000 seedlings in 2017 (100,000 containers were sown).

Table 6. Approximate Germination Rate Per Species, 2017

Species	Sowing time	Germination
Atriplex halimus	March 15 – 30	>60%
Artemisia herba alba	March 15 – 30	>80%
Salsola vermiculata	March 15 – 30	>60%
Thymus bovei	March 15 – 30	>30%
Retama raetam	March 15 – 30	NA
Achillea fragrantissima	March 15 – 30	>70%

The nursery staff, mainly composed of women from the neighbouring local communities (Figure 3), received technical trainings for seed collection and treatment, which led to a better seed management plan and seed quality for the propagation of native rangeland species from the Badia region. Such capacity building programs ensure the implementation of proper restoration techniques and improved results in the field. These nursery growers have developed a strong appreciation for the steps needed to grow high-quality seedlings and are recongnized within Jordan and across the region for their high-quality work in seed handling and seedling production.

Figure 3. Seed management Training for Community Nursery Staff

Conclusions

As Lebanon has been working to develop enhanced forest restoration practices, USAID has supported the development of these with the understanding that successful forest restoration is a key social and ecological driver. Quickly expanding succeful practices in Lebanon to regional partners such as Jordan provides the framework for increased communication towards success and decreased duplication of effort within the region. This will create stability in production and quality that will improve the likelihood for sustainability for ecosystem restoration projects in Lebanon and Jordan. Significant improvements in seed germination for selected native species was observed and coupled with advances in seedling production and outplanting approaches, the nursery developments will contribute to improved restoration efforts and successes in Lebanon and Jordan, and dessiminated throughout the region.

Acknowledgements

We would like to express our deepest appreciation to all those who provided us the possibility to complete this work and to continue developing and improving ecosystem restoration in the region. A special gratitude to the staff of LRI and SEED for playing a crucial role in the implementation process.

References

Food and Agricultural Organization of the United Nations (FAO). 2005. National Forest and Tree Assessment and Inventory TCP/LEB/2903, http://www.fao.org/forestry/15565-0f921641e230ef06f11d15b8856f2ff07.pdf

International Center for Agricultural Research in the Dry Areas (ICARDA). 2012. Land Degradation in Jordan – Review of Knowledge Resources, file:///C:/Users/Nursery/Downloads/OASIS_1_Jordan%20(1).pdf

Davis, A. S.; O.A. Kildisheva; M.M. Aghai. 2014. Lebanon Reforestation Initiative: a guide to container tree seedling production.

Dumroese, R. K.; T.D. Landis; J.R. Pinto; D.L. Haase; K.W. Wilkinson; A.S. Davis. 2016. Meeting forest restoration challenges: Using the Target Plant Concept. Reforesta. 1: 37-52.

Sabogal C, C. Besacier, D. McGuire. 2015. Forest and landscape restoration: concepts, approaches and challenges for implementation. Unasylva 245 66(3): 3-10.

Lebanon Reforestation Initiative (LRI). 2014. A Guide to Reforestation Best Practices, http://lri-lb.org/sites/default/files/AGuidetoReforestationBestPractices.pdf

Center for Applied Research in Forestry Development (IDAF). 2013. Establishment of a National Network of Forest Stands for Sustainable Native Seed Production in Lebanon.http://www.lri.lb.org/resources.php#resources

Section 3

Seed Germination and Seedling Production

The USA National Seed Strategy and Regional Native Plant Programs: Progress and Challenges

Nancy L. Shaw*[1] and Peggy Olwell[2]
[1] 4283 N. Marcliffe Ave., Boise, Idaho, USA 83702;
[2]USDI Bureau of Land Management, 20 M Street SE, Room 2134, Washington, DC, USA
***email**: nancyshaw@cableone.net

Abstract

Hurricanes and other extreme weather events in the eastern United States, serious drought conditions and altered fire regimes in the western states, and the fast spread of invasive species nationwide emphasize the need for restoration practitioners to respond quickly to landscape-scale ecological changes with appropriate seed resources. The National Seed Strategy provides a plan for stabilization, rehabilitation, and restoration of public and private lands. It guides coordination of efforts to achieve four major goals: (1) assess seed needs and ensure the reliable availability of genetically appropriate seed reserves, (2) identify and conduct research to aid users in seed sourcing for current and projected future conditions and to improve technology for seed production and ecological restoration, (3) develop science delivery tools and monitoring applications to inform decision making for ecological restoration, and (4) formulate plans for internal and external communication that engage all stakeholders. The Strategy benefits landscapes by supporting ecosystem services provided by healthy plant communities and supports additional benefits such as aesthetics, recreational opportunities, food, fiber and commercial products. The National Seed Strategy provides impetus, direction and support for new as well as established native seed programs across the country. Collaborative programs initiated by federal, state, local, city and Tribal governments, universities, and non-governmental organizations vary widely in scope, complexity and stage of development, but they share common goals of (1) developing and maintaining communication and collaboration among partners, (2) establishing priorities for development of native seed sources and supplies, (3) creating a seed supply chain from collection through seed increase and marketing, (4) identifying knowledge gaps and conducting research, and (5) increasing public awareness of the value of native plant communities, revegetation needs and the necessity for collaboration and funding to address these needs. Successful programs will aid in stabilizing native communities, conserving biodiversity, and meeting emergency needs.

Keywords: ecological restoration, native seed, plant conservation, revegetation, seed sourcing

The US National Seed Strategy: The right seed in the right place at the right time

The Native Plant Materials Development Program. Rehabilitation efforts following the extensive wildfires of 1999 and 2000 that burned more than 1 million ha in the western United States were limited by inadequate supplies of native plant materials (USDI & USDA 2002). To address this issue the Native Plant Materials Development Program was authorized by the U.S. Department of the Interior and Related Agencies Appropriations Act of FY2001. A Report to Congress (USDI & USDA 2002) prepared by the U.S. Department of the Interior (USDI) and Department of Agriculture (USDA) provided recommendations and strategies for implementation of this program to provide native seed required for restoration of degraded areas within the 182 million ha or about 30% of the nation's lands managed by agencies of these two departments. The stated mission of the national program is to “ensure a stable and economical supply of native plant materials for rehabilitation and restoration needs” and to “facilitate the development of a long-term program to supply and manage native plant materials for use on public lands” (USDI & USDA 2002). Key goals of the program are expanded support for federal, state and Tribal native plant materials research, production and use; seed storage facilities; public and private sector partnerships; and education and outreach programs.

Over the intervening 15 years, the Native Plant Materials Development Program, a variety of national initiatives and directives, and increasing public concern for the conservation of biodiversity have stimulated the initiation of new local to regional native plant programs and the growth of established ones. Programs are now scattered across the United States and differ widely with respect to developmental stage, scale and complexity. Program organization depends upon goals, local land ownership patterns and the availability and sustainability of funding. Native plant programs may be organized by independent entities or by partnerships of public agencies (federal, state or local), non-governmental organizations, universities, and private companies. Programs may simply involve volunteer groups that harvest and plant adapted native seed or seedlings in settings as disparate as empty city lots or degraded prairie falcon or golden eagle habitat. More complex regional programs may implement plans to provide seed for long-term requirements of multiple stakeholders, diverse disturbance types, and complex geographic areas.

The National Seed Strategy. Extreme weather events, continued invasion of exotic species, land overuse, energy development and the effects of increased human population, all contributing to and exacerbated by climate change, continue to impact the many and diverse ecosystems of the United States. Plant communities are being altered and even populations of species once considered common are being destabilized by ecosystem threats and habitat fragmentation. Native plant communities support more 16,000 native plant species, but nearly one-third are considered at risk. The native biodiversity of healthy ecosystems is critical to provide habitat for wildlife, including pollinators; contribute to stabilizing water supplies, soils, and nutrient cycles; impede the spread of exotic species; reduce the spread of wildfires and afford recreation and cultural experiences.

By 2014 expanding ecological threats highlighted the need to accelerate and better coordinate restoration efforts on public lands. Leaders of the 12 agencies represented on the Plant Conservation Alliance (PCA) Federal Committee met in Washington, DC to celebrate the 20th anniversary of the committee’s formation and to discuss the need for an over-arching, multi-agency national seed strategy to address these challenges. A recognized need was to increase collaboration within and among agencies and with external partners to more effectively and economically achieve the long-term goals of the Native Plant Materials Development Program. Representatives of these agencies served as a steering committee for development of the Strategy, which was released in 2015 (PCA 2015).

The Strategy encourages, but does not mandate actions, and it does not set policy. It does outline goals and objectives for increasing native seed supplies and strengthening all aspects of seed-based ecological restoration. It provides a framework for promoting the growth of networks among seed collectors, seed producers, nurserymen, researchers, managers and restoration ecologists. Locally adapted seed sources are critical for restoration success and longevity. Resources are in danger and funds poorly expended when non-local seed is used. Success of the Strategy requires a very major commitment to collaboration within and among the 12 federal agencies, the more than 400 partners of the non-federal PCA Committee (NGOs, universities, non-federal government agencies, and the private seed and restoration industry), and with other cooperators to develop priorities, share knowledge and resources and restore the health of damaged ecosystems.

The mission of the National Seed Strategy is "To ensure the availability of genetically appropriate seed to restore viable and productive plant communities and sustainable ecosystems." Goals (PCA 2015) are to:

1. Assess seed needs and ensure the reliable availability of genetically appropriate seed (Seed supply).
   - Increase infrastructure investments.
   - Escalate wildland seed collection and production.
   - Expand cooperation and partnerships, particularly among seed collectors/growers and restoration practitioners.
   - Create a network of native seed reserves and storage facilities.

2. Identify research needs and conduct research to provide genetically appropriate seed and to improve technology for native seed production and ecosystem restoration (Research).
   - Develop and test seed zones and seed transfer guidelines to plan for seed sourcing for current and future climatic conditions.
   - Formulate reliable protocols for seed testing, storage and production.
   - Identify effective restoration and monitoring strategies.

3. Develop tools that enable managers to make timely and informed decisions for ecological restoration (Decision tools).
   - Offer training programs on the selection of genetically appropriate seed for restoration.
   - Develop web-based seed sourcing tools that facilitate inclusion of climate change considerations into restoration planning and provide training on their use.
   - Emphasize professional restoration ecologist certification and continuing education.
   - Develop web-based tools and databases to aid planning and implementation of restoration projects.
   - Identify, modify, or develop monitoring methodology for restoration projects.

4. Formulate strategies for internal and external communication (Communication).
   - Develop and implement communication plans and strategies to foster Strategy implementation and progress as a collaborative, multiagency effort.
   - Incorporate the Strategy's goals and key messages into ecological restoration initiatives.
   - Involve the Plant Conservation Alliance in communicating the importance of healthy native communities and native plant restoration to increase participation in the Strategy and inform the general public.
   - Report on progress and recognize achievements through awards and press releases.
   - Provide for feedback, reviews and revisions to provide a dynamic National Seed Strategy.

The National Seed Strategy recognizes the progress and accomplishments of federal agencies and urges collaboration to maximize the impact of their collective expertise, facilities and functions. Examples include the Agricultural Research Service's National Plant Germplasm System, the Natural Resource Conservation Service's Plant Materials Program with its 27 Plant Materials Centers distributed across the country, the Forest Service's Native Plant Restoration Program and the Bureau of Land Management's Interagency Native Plant Materials Development Program with its Seeds of Success Program. Interns in this program for natural resource graduates has made more than 16,500 native seed collections of more than 5000 taxa for ex situ conservation and for use in restoration (Haidet and Olwell 2015). Native plant programs to meet agency needs are also conducted by The National Park Service, Fish and Wildlife Service, Federal Highway Administration and other land management agencies.

The aim of the Strategy is not to create duplication, but to encourage coordination among federal agencies to better manage seed supplies. Public agencies and private land owners with similar plant communities and disturbance types on contiguous lands require similar seed supplies, equipment and facilities as well as knowledge and expertise to address restoration goals. Increased user coordination on local and regional seed needs and more effective communication with the native seed industry will better inform seed collectors and growers of types, origin and quantities of plant materials required. Improving these communication lines will enable more rapid and economical responses to existing or planned restoration projects as well as to unplanned landscape-scale disturbances such as wildfire across all land ownerships. Smaller scale restoration projects will also benefit from public investment in native seed production and research and expansion of the native seed industry.

The incorporation of National Seed Strategy goals and objectives into recent major national initiatives serves both to reinforce the Strategy and strengthen the role of native plant material development and use in implementation of these initiatives. Among these are the 2015 National Strategy to Promote the Health of Honey Bees and Other Pollinators and the 2015 Department of Interior Secretarial Order 3336 on Rangeland Fire and Invasives, which calls for restoration of sagebrush steppe impacted by wildfires in the western United States. Development of sustainable seed supplies as outlined in the National Seed Strategy was cited as a component of restoration plans requiring plant materials by the International Standards for the Practice of Ecological Restoration – Including Principles and Key Concepts (McDonald et al. 2016).

Implementation of the National Seed Strategy is ongoing. At present, native plant programs are funded by individual agency budgets; funding to meet Strategy goals and objectives is dependent upon future Congressional budget appropriations. A 5-year business plan prepared by the PCA federal committee provides cost estimates for accomplishing Strategy goals and facilitates development of an interagency budget initiative. A communication plan has been completed and contributed to development of reports, webinars, presentations and special sessions at key venues and workshops to update stakeholders on Strategy progress. An implementation team was chartered in 2016. The National Native Seed Conference held in Washington, DC in 2017 provided a venue for further developing the implementation plan and for sharing recent research, progress and challenges. Plans are underway to organize a national assessment of seed needs, capacity and infrastructure.

Regional Native Plant Programs

Native plant programs that address repair of degraded lands at regional scales have been organized at a number of locations in the United States. Program organization, vegetation types, and the nature of disturbances addressed vary widely. Shared goals are to increase native seed supplies and improve their management, expand infrastructure, encourage expansion of native seed collection and production, provide the knowledge required to increase restoration success and improve communication and cooperation. Examples of these include New York City’s Greenbelt Native Plant Center and Mid-Atlantic Regional Seed Bank, the Texas Native Seeds Program, the Tallgrass Prairie Center’s Natural Selections Project, the USFS Pacific Northwest Native Plant Program, and many others.

The Great Basin Native Plant Project. Approximately 70% of the land in the 11 western states is owned by the federal government, with the USDA Forest Service and USDI Bureau of Land Management having major responsibility for managing these lands. Bureau of Land Management lands are primarily arid and semi-arid cold shrublands and woodlands. Their native plant programs have been organized along ecoregional lines. Three major programs are the Great Basin, the Mojave Desert and the Colorado Plateau Native Plant Projects.

The first established BLM ecoregional program, the Great Basin Native Plant Project, was organized in collaboration with the US Forest Service in 2001 and is used as an example here. The Great Basin, a vast region of more than 54 million ha, about the size of France, is a cold desert dominated by salt desert shrublands and sagebrush steppe, characterized by woody *Artemisia* L. species. The sagebrush ecosystem, one of the largest ecosystems in the United States, is also ranked as one of the most imperiled. Excessive livestock grazing, beginning with European settlement, opened the area to exotic invasive plants, principally *Bromus tectorum* L. Continued spread of this highly flammable and competitive annual grass across millions of hectares has reduced fire return intervals and depleted or replaced native communities and seedbanks. Protecting existing sagebrush communities and developing native plant materials and effective techniques for restoring diverse, resilient communities adapted to current and projected future climatic conditions poses major challenges.

Objectives of this program are to 1) select native species appropriate for restoring successional processes that will contribute to the recovery of degraded ecosystems; 2) develop cultural practices for producing seed and seedlings; 3) work with seed growers to increase the commercial availability of genetically diverse, regionally adapted native plant materials, particularly native forbs; 4) provide decision tools for selection of plant materials and restoration strategies and equipment required to re-establish diverse native communities; and 5) conduct educational programs for all stakeholders on the value of healthy native plant communities. Science delivery is an essential component of each objective. Over time the project has grown to include practitioners, researchers from many disciplines, native seed collectors and growers and personnel at seed regulatory agencies.

The initial step was selection of priority species for restoration by a multi-disciplinary group of field personnel from across the Great Basin (Shaw et al. 2012, Shaw and Jensen 2014). Native forbs were emphasized due to their limited availability and their importance in providing community diversity and food for chicks of sage-grouse, a species of concern. The importance of forbs has only increased over time in response pollinator conservation efforts. Screening prospective revegetation species by collecting seed and growing it under agricultural conditions permits identification of potential seed technology, production and use bottlenecks relative to species utility in restoration. Such bottlenecks may become the focus of research studies, or in extreme cases may preclude use of the species.

Post-fire rehabilitation efforts in the Great Basin have focused on the use of widely seeded exotic grass cultivars developed to provide soil stabilization and forage for livestock. Transition to greater use of native materials requires recognition and conservation of genetic diversity to provide material adapted to restoration site conditions. Seed sourcing was addressed through development of species-specific seed zones based genetic data for most major widespread bunchgrasses (WWETAC 2017). Provisional seed zones based on climatic variables considered important to plant adaptation were provided for use in sourcing seed of the many species lacking genetic data (WWETAC 2017). The online Seedlot Selection Tool allows practitioners to incorporation climate change considerations in seed sourcing (Howe et al. 2017). Seed technology, seed production and seeding and planting strategies have been addressed by researchers from multiple disciplines (Shaw et al. 2012, Shaw and

Jensen 2014, see publications listed on GBNPP 2017). In addition, seed regulatory agencies involved in seed testing and certification have contributed by developing testing procedures for additional native species and addressing questions regarding certification of wildland collections.

Importantly, research has included 225 plant taxa and added more than 30 species to commercial production. Outputs from the Great Basin Native Plant program, in addition plant materials and tools for sourcing seeds, include more than 200 publications (GBNPP 2017). Importantly, in addition to refereed journal articles, these include manuals, plant guides, protocols and online databases for native seed cleaning and testing, seed production, and nursery seedling propagation. New equipment and equipment modifications developed by project cooperators enable cleaning, harvesting and planting diverse seed types. The project has hosted numerous webinars, workshops, symposia, and conferences as well as field days for practitioners, seed industry personnel, the general public and international visitors.

Work in all project areas continues and challenges are numerous. Research is required to aid in selecting adapted species and genetically diverse materials. Genetic diversity of these materials must be maintained to provide for future adaptation in response to climate change and other disturbances and contribute to community resilience (Wood et al. 2015). Planning restoration projects in consideration of projected changes in species distributions as well as community composition and fragmentation pose additional challenges to practitioners.

Establishment of seedings on arid and semi-arid lands is risky and dependent upon timely precipitation and water harvesting techniques when planting. In the Great Basin an added complication is the pervasiveness and competitiveness of *B. tectorum* and other exotic annuals that frequently contribute to seeding failures (Shaw and Jensen 2015). Reduction of *B. tectorum* seed banks following wildfire alone or combined with mechanical, chemical and biocontrol techniques can reduce seed density and permit seeding establishment. However, results are not consistent and additional research and field trials are required to improve methods for controlling the species.

Cultivars of exotic grasses, particularly wheatgrasses (e.g. *Agropyron cristatum* [L.] Gaertn.) have long been widely planted to provide soil stabilization, forage for livestock, and competition with *B. tectorum* with the result that hundreds of thousands of hectares are now occupied by exotic forage grass monocultures to the exclusion of most native species. Although research results vary, there is a continuing perception that native species cannot perform these functions (Wood 2915). Exotic and native cultivars selected for agronomic traits including high seed and forage production may be incompatible with native species and may also exhibit loss of ecologically adaptive traits (see Wood 2015 and references therein). Recent federal directives including the Pollinator Strategy, Secretarial Order 3336 and earlier policies (see Johnson et al. 2010) call for restoration using native materials to provide diverse and resilient communities, rather than prioritizing economic goals. Increased use of native materials requires a shift in traditional approaches and science-based revegetation decisions to address land management priorities.

Widely planted cultivars can be grown at relatively large scales, cultural practices are well known and yields and markets somewhat predictable. Increasing use of native plant materials developed through an ecological approach considering adaptation and genetic diversity rather than an agricultural approach of selection for seed and forage production poses a number of new issues for seed collectors and growers (Leger and Baughman 2015). Growing a wider variety of species, particularly forbs, for specific seed zones requires acquisition of additional equipment, and increased time, labor and knowledge of cultural practices. Seed fields are smaller, planning to provide required isolation essential, and yields sometimes difficult to predict. A key concern is market demand, which fluctuates widely. Growers are reluctant to take on new species without assurance that their crop will be purchased. Thus there is a need for users to conduct forward planning, combine requests and contract or otherwise assure payment is essential, at least initially, when adding new materials to seed mix prescriptions. For native shrubs, there is increasing interest in conserving key native stands for seed production or planting shrub seed production areas on wildland or agricultural sites to insure production of key populations.

Future Direction

Recognition of the environmental, economic and cultural importance of healthy native plant communities and calls for restoration using native plant materials are increasing across the country. The National Seed Strategy along with other federal directives provides impetus and direction for scaling up native plant restoration. Emerging and established native plant programs are striving to provide the science and germplasm required to meet this objective. Numerous challenges remain. For federal projects there is a need for clear policy regarding native seed use, sustained funding and increased coordination among agencies and non-federal entities. Public and private programs require personnel trained in ecology, seed technology and ecological restoration and research and thorough understanding of buyer needs in order to develop long-term seed collection and production plans. Continued efforts to coordinate and expand all aspects of ecological restoration will be essential to meet future challenges.

References

Great Basin Native Plant Project [GBNPP]. 2017. The Great Basin Native Plant Project. http://www.greatbasinnpp.org/, (accessed on October 10, 2017).

Haidet, M. and P. Olwell. 2015. Seeds of Success: A national seed banking program working to achieve long-term conservation goals. *Natural Areas Journal* **35:** 165-173.

Howe, G.; B. St. Clair; and D. Bachelet. 2017. Seedlot selection tool. https://seedlotselectiontool.org/sst/, (accessed on October 6, 2017).

Johnson, R.; L. Strich; P. Olwell; S. Lambert; M. E. Horning; and R. Cronn. 2010. What are the best seed sources for ecosystem restoration on BLM and USFS lands? *Native Plants Journal* 11: 117-131.

Leger, E. A. and O. W. Baughman. 2015. What seeds to plant in the Great Basin? Comparing traits prioritized in native plant cultivars and releases with those that promote survival in the field. *Natural Areas Journal* **35:** 54-68.

Plant Conservation Alliance [PCA]. 2015. National seed strategy for rehabilitation and restoration 2015-2020. BLM/WO/GI-15/012+7400. USDI Bureau of Land Management, Washington, DC. www.blm.gov/seedstrategy, (accessed on October 6, 2017).

Shaw, N.; S. Jensen. 2014. The challenge of using native plant materials for sagebrush steppe restoration in the Great Basin, USA. Chapter 4.2. In K. Kiehl; A. Kirmer; N. Shaw; and S. Tischew (Eds.). Guidelines for native seed production and grassland restoration. Newcastle upon Tyne, UK: Cambridge Scholars, p. 141-159.

Shaw, N.; M. Pellant; M. Fisk; E. Denney. 2012. A collaborative program to provide native plant materials for the Great Basin. *Rangelands* **34:** 11-16.

McDonald, T.; G. D. Gann; J. Jonson; and K. W. Dixon. 2016. International standards for the practice of ecological restoration – including principles and key concepts. Society for Ecological Restoration, Washington, DC. 47 p. http://www.ser.org/?page=SERStandards, (accessed on October 11, 2017).

U.S. Department of the Interior & U.S. Department of Agriculture [USDI & USDA]. 2002. Report to Congress: Interagency program to supply and manage native plant materials for restoration and rehabilitation on federal lands. 17 p. https://www.nps.gov/plants/npmd/Native%20Plant%20Materials%202002%20Report%20To%20Congress.pdf, (accessed on October 6, 2017).

Western Wildland Environmental Threat Center (WWETAC). 2017. TRM Seed zone applications. https://www.fs.fed.us/wwetac/threat-map/TRMSeedZoneMapper.php, (accessed on October 8, 2017).

Wood, T. E.; K. Doherty; W. Padgett. 2015. Development of native plant materials for restoration and rehabilitation of Colorado Plateau ecosystems. *Natural Areas Journal* **35:** 134-150.

Seed Quality Testing of Native Species

Rini R. Thomas, Sheena Jacob, Majda K. Suleiman and Narayana R. Bhat
Kuwait Institute for Scientific Research, Kuwait
***e-mail:** rthomas@kisr.edu.kw

Abstract

A healthy seed plays a vital role in native plant establishment. In any restoration program, assessment of seed quality is an essential measure for the success of the program. One of the main attempts towards the recovery of degraded areas in Kuwait is re-vegetation using native plant species. Hence, viable seeds ensure efficient accomplishment of the purpose. There are several methods for assessing the viability of seeds, which vary amongst various species. It is important that the procedure used for assessing the viability should give reliable and reproducible results. Previous work on the establishment of a protocol for the assessment of region and species-specific seed viability procedure is scarce; hence, it must be extensively studied for the restoration activities taking place in Kuwait and the region. This paper discusses suitable procedure for assessing the quality of native seeds and estimation of seeding rate based on seed quality. This is done to establish an efficient protocol on the quality testing of native plant species to ensure effective mass propagation.

Keywords: Re-vegetation, conservation, endangered species, seed viability, seed germination.

Background and Findings

Seed is the biological component of a plant and is responsible to sustain the genera of the species. The structure of a seed varies from species to species but the main function of it is to sustain the regeneration of the species. The health of a seed plays a vital role in this process as only a healthy seed can grow into a healthy plant, which is true to its variety and producing healthy seeds for future uses. Seed testing technologies were developed to minimize the risk of loss and to ensure steady supply of high-quality seeds in any regeneration program.

Seed quality is characterized by trueness to variety, germination percentage, purity, vigor and appearance (Ferguson et. al., 2017). Achieving high seed quality is the base for an ensured high yield. Various technologies for testing the seed quality has been developed based on the applicability and reproducibility of the procedure.

The structure of the seed, its maturity during the harvest and its physiological condition is a major aspect for the quality testing method. The knowledge of seed structure and its response to various procedures is important to ascertain a standardized quality testing procedure.

A seed comprises of three basic structures namely: the seed coat or testa; embryo or the baby plant and; cotyledons or supporting tissues. Based on the number of cotyledons, seeds are classified into monocots (monocotyledons) and dicots (dicotyledons) (Ferguson et. al., 2017). The seed coat is a protective covering that protects the embryo from injuries, deterioration and pathogen attack. The location of seed structure also have a major role in determining seed health and its resistance to adverse conditions. Any damage to the embryo will result in abnormal seedlings or even the death of the seed. Therefore, a complete knowledge about the morphology and physiology of the test seed should be known before subjecting it to any pre-treatments. Seed internal morphology and size also contributed towards the basis of classification by Martin (1946) and Baskin and Baskin (2007).

A seed is capable of germinating into a healthy plant only if it is viable. Not all the seeds produced by a plant be viable. A seed can be considered viable if it is alive when subjected to any viability test and capable of reproducing themselves in an appropriate growing condition (Gosling, 2001). Viability of a seed is affected by various factors such as:

- Seed coat: the protective covering of the seed known as the seed coat varies in its structure and thickness. The seed coat is also responsible for the permeability of the seed and hence affects the viability of the seed. The seed requires an optimum level of moisture to keep itself alive and the seed coat plays a vital role in this.
- Moisture content: as mentioned above the level of moisture inside the seed determines the viability and longevity of a seed. Moisture content of a seed can be altered not only by the permeability of the seed coat but also by various factors such as exposure to direct sunlight, elevated storage temperature and even excess moisture deteriorate the seed.
- Harvesting conditions: External environmental conditions and internal seed conditions before and after harvest contributes towards the viability of a seed. Fluctuating environmental conditions and extreme storage conditions are also responsible for decrease of seed viability.
- Oxygen pressure: an optimum level of oxygen pressure is also important for a seed to keep itself alive. Any decrease or increase in this level is a hindrance for the seeds to remain metabolically active and alive.

There are many other factors which serve against the viability of a seed such as attack of organisms such as fungi, bacteria and viruses, apart from attacks from rodents, insects and mites. So in short, we can conclude that the life of a seed can be well safeguarded only if these factors are carefully controlled.

A healthy plant is grown from a fully developed viable seed, which contains living, healthy embryo with enough stored food to support the plant until they are capable to prepare their own food. This capability can be termed as Germination. Apart from the internal favorable conditions, a seed also seeks the presence of optimum environmental conditions that can favor its growth in a healthy manner. As mentioned previously optimum levels of temperature, moisture, oxygen and light is the basic requirement of a seed to germinate. Any condition above or below the optimum range will affect or delay the growth of the plant. Absence of optimum germination conditions, directs a seed to a resting condition termed as dormancy. Certain seeds tend to enter dormancy even at the presence of favorable conditions to keep seedling safe from possible bursts of bad weather or herbivores that feed on them(Penn State Extension, 2016). To retrieve a dormant seed, the factor leading the seed to dormancy (physical or chemicals) has to be identified and precise pre-treatments specific for that dormancy factor has to be administered. A dormant seed may be viable but certain factors prevent it from germination. However, to confirm if the seed is viable or not and is capable of germinating at the return of optimal condition, the seeds needs to undergo viability testing.

The success of any restoration and re-vegetation program is determined by the quality of the seed lot used to carry out the program. Utilization of unhealthy, non-viable seed will only lead to an uncertain and unsuccessful outcomes accompanied with monetary loss, work force and time wastage. Quality seeds can ensure the maximum production of genetically pure, true to type, resistant, healthy, well-adapted crop that will be capable to grow rapidly, vigorously, uniformly and with minimal maintenance.

There are many procedures tested and standardized for checking the viability of seeds. Each procedure has its own methodology and requirements. A thorough knowledge of the seed and its morphology has to be known to select the most appropriate technique suitable for a species. Among various viability testing techniques a few which are commonly used are: cut test, excised embryo test, germination test, electrical conductivity measurement, vital stain, Triphenyl Tetrazolium Chloride (TTC) testing and X-radiography (Gosling, 2001; Hampton, 1995; ISTA, 2007). As the names suggest, the first two tests requires the seed to be cut open skillfully, without damaging the embryo and examining the filling of the seed and the development of the embryo. These techniques require patience, skill and good identification expertise to come up with a conclusion if the test seed is viable or not (Gosling, 2001; Hampton, 1995). Germination test is used for non-dormant seeds, which easily grow when soaked in water or under favorable conditions. This method is a non-destructive technique, which is easy and less time consuming. The test seeds are soaked in water and then place on filter paper. Viable seed germinate and non-viable fail to do so and the viability percentage is calculated based on the number of germinated seeds against non-germinated ones. On the contrary the electrical conductivity (EC) test is based on the principle that the tissues of a dead or dying seed is not very intact and functional and allows easy exchange/release of electrolyte when soaked in water. Hence, the EC of the non-viable seed is intended to be higher than that of viable seed (Steere et al., 1981). The staining methods such as the vital stain method and Triphenyl Tetrazolium Chloride testing are formulated on the fact that the live and dead tissues of a seed react to the subjected stains in different manner. Therefore, the change in color can be used as an indicator for assessing the health of the seed (Gosling, 2001). The X-ray image method is also a non-destructive method of testing the health of the seed. The internal X-ray images of the seeds are examined for any infestation, deformity etc. However, the images can provide with the information on the filling of the seed but cannot be relied for assessing the viability of the seed.

Among the various techniques detailed the germination test and the TTC test is the most common techniques used for most of the non-dormant and dormant species respectively. In the TTC test the colorless chemical reacts with the respiratory enzymes (dehydrogenase) released by the live tissues of the seed staining them red. Non-viable seeds do not respire so no reaction happens hence, no staining occurs. In this method, the embryo needs to be exposed and then treated with the TTC solution. The number of stained seeds against the non-stained ones determines the viability percentage (Ramos et al., 2012). This is a quick method as the results are obtained within 24 hours.

Utilization of native plant species in a restoration and re-vegetation program is the most advantageous choice as the species are well adapted to the native climatic conditions and it promotes the conservation of the heritage of the region. Based on these ideas various research projects in this area have been implemented in Kuwait and Kuwait Institute for Scientific Research (KISR) has also contributed in this cause. In the past few years, the focus on the native plant species has increased as many of the species are at the verge of extinction and hence it has aroused alarm in the minds of most of the researchers and research institutions. The main hindrance faced by most of the researchers in the usage of Kuwait native plants in these projects is the lack of enough literatures on the details of the seed morphology, physiology and the handling measures of these species. This information is important for the effective implementation of research activities.

The objective of this paper is to detail such information on few of the selected native plant species that our research team has utilized in the past few years namely *Haloxylon salicornicum*, *Halothamnus iraqensis*, *Salsola imbricata*, *Farsetia aegyptia*, *Nitraria retusa*, *Lycium shawii*, *Ochradenus baccatus*, *Horwoodia dicksoniae*, *Rhanterium epapposum*, *Acacia pachyceras*, *Convolvulus oxyphyllus* and *Peganum harmala*. The focus will be mainly on the viability testing using germination and TTC test. The methodology detailed by ISTA (2007) was followed for carrying out these techniques of viability test. It is to be noted that the findings were derived by continuous hand-on trials and hence, were valuable for the success of the projects. Such findings on few of the test species is detailed in this paper.

As detailed before, germination test is the easiest technique for testing the viability of a seed lot. This method can be used for seeds such as *Haloxylon salicornicum*, *Halothamnus iraqensis*, and *Salsola imbricata*, that do not have any type of dormancy. The prominent fact that was revealed to us during the test trials was that these three species had very short shelf life and performed well when used immediately after the harvest. Additionally, as part of the TTC, these seeds germinate after the one day soaking period indicating viability and hence TTC test can be avoided.

- *Haloxylon salicornicum* - is a native shrub of Kuwait found towards the northern region of the country (Omar et al., 2007). It produces winged seeds (Figure 1a) and these wings facilitate easy seed dispersal. The seeds are produced during the month of November – December. The seeds have a very short shelf life hence it needs to be planted immediately after harvest or else the viability of the seed is lost. This clearly indicates that the seed does not have any dormancy and the viability of the seed lot can be measured by germination test.
- *Halothamnus iraqensis* – it is a perennial shrub found in deserts and semi-deserts areas, 0 to 2800 m above sea level (Omar et al., 2007). Similar to *Haloxylon* this species also produces winged seeds (Figure 1 1b) and germinate easily on soaking, when used immediately after harvest.
- *Salsola imbricata* – It is a perennial low shrub of desert areas adapted to dry soil conditions (Omar et al., 2007). The winged seeds are presented in Figure 1c. Similar to above two species the viability of this species is also assessed by germination test as it does not address any dormancy.

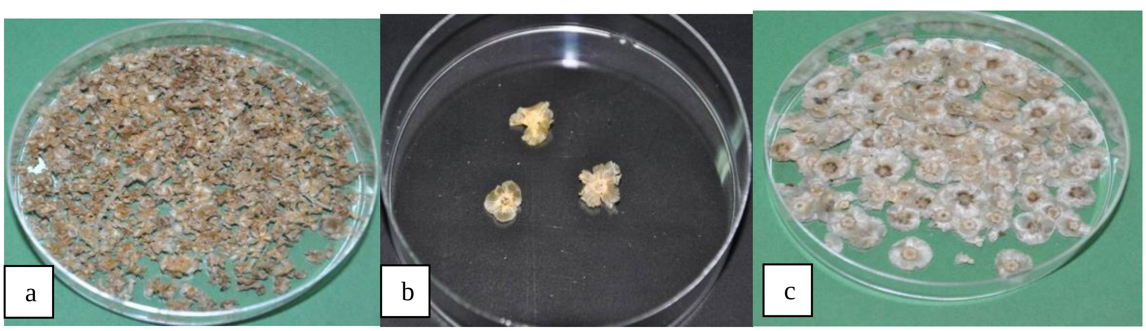


Figure 1. Dried flowers of (a) *Haloxylon salicornium;* (b) *Halothamnus iraqensis;* (c) *Salsola imbricate*.

Viability of these three species can be estimated by germination test i.e. soaking in water for a day and placing the seeds in moistened filter paper to observe germination. If TTC has to be conducted on these seeds, a transverse cut on the upper portion of the ovary is made to expose the embryo. It is very difficult to handle the seeds of this species. Seed filling is also a major problem in these species and it is very difficult to predict.

- *Farsetia aegyptia*: It is one of the native gray-green woody perennial about 30 cm in height that belongs to the Cruciferae family (Figure 2). It has slender, smooth and multibranched stems. The flowers are creamy brown with four petals. Two rows of seeds are formed in an oval-shaped seed pod. It flowers in April. (Omar, et al., 2007) and the propagation is through seeds (Figure 3). The winged seeds of farsetia are light orange in colour. Pre moistening of the seeds makes them slimy and handling them without damage becomes difficult. Hence, germination tests are convenient to test the viability of this species.

Figure 2. Seeds of *Farsetia aegyptia*.

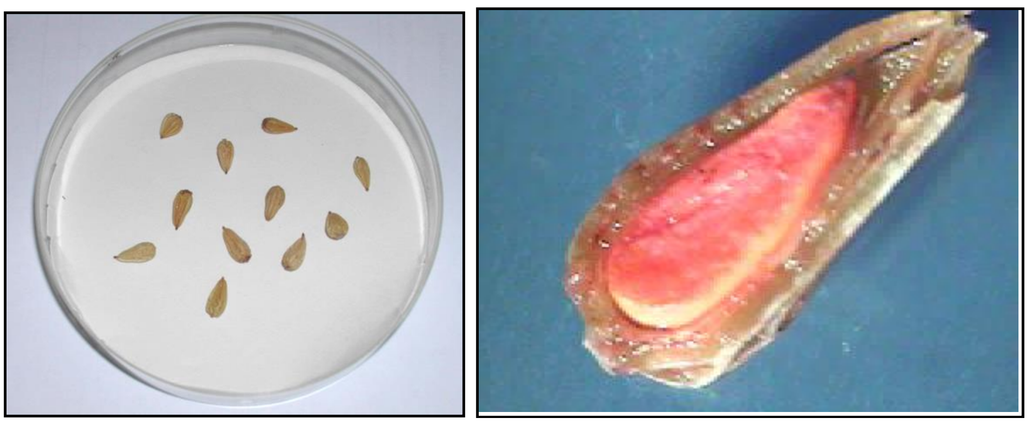


Figure 3. Seeds of *Nitraria retusa*.

The tetrazolium test is a biochemical test which is mainly used when a rapid assessment of viability of the seeds have to be done. This is a reliable test method for seeds that are hard or slow to germinate (ISTA, 2007) especially for seeds, which are known to have certain type of dormancy. For native species like *Nitraria retusa*, *Lycium shawii*, *Ochradenus baccatus*, and *Rhanterium epapposum* germination might extend upto 3-4 weeks. Hence, it is suggested to give preference to TTC test rather than germination test to save time. This test is performed by using 2,3,5 triphenyle tetrazolium chloride at a concentration varying from 0.1 % to 1.0%. The test seeds (100 Nos.) are soaked in distilled water for 24 hours for softening the tissues to facilitate ease in cutting the seeds. Once soft, seeds are cut open to expose the embryo and then treated with the TTC solution by placing the exposed seed in a petri dish containing the solution. The petri dish is then closed and wrapped with aluminum foil, as the solution is light sensitive. Staining of the seeds is observed after 24 hrs.

- *Nitraria retusa* – This is a salt-tolerant bush found in the coastal areas of Kuwait and on Mutla ridge. It is a large shrub about 150 cm high. The branches are woody and thorny, and grazed upon heavily by animals. It has bluish-green, leathery leaves, and greenish yellow flowers in the spring followed by red berries (Omar et al., 2007). The main propagation is through seeds but they are slow grower. The viability test best suited for this species is TTC. The seeds were pre-moistened in water for 24 hours and then the imbibed seeds were cut longitudinally through cotyledons exposing the embryo. These were soaked in 0.1% TTC for 24 hrs. Pattern of staining was evaluated.

- *Lycium shawii:* This is a very spiny shrub that can reach up to 150 cm in height. It has purple, sometimes white, trumpet-like flowers and sharp thorns that are used by the shrike to impale its prey. The leaves are elliptical and congested in close clusters. Seeds (Plate 4) are produced in the summer (Omar, et al., 2007). The flowers are produced during March to April in its natural environment and throughout the year in irrigated soil. The fruits are globular, many seeded, red to orange berries which are edible and somewhat sweet. The TTC procedure is same as that of *Nitraria retusa.*

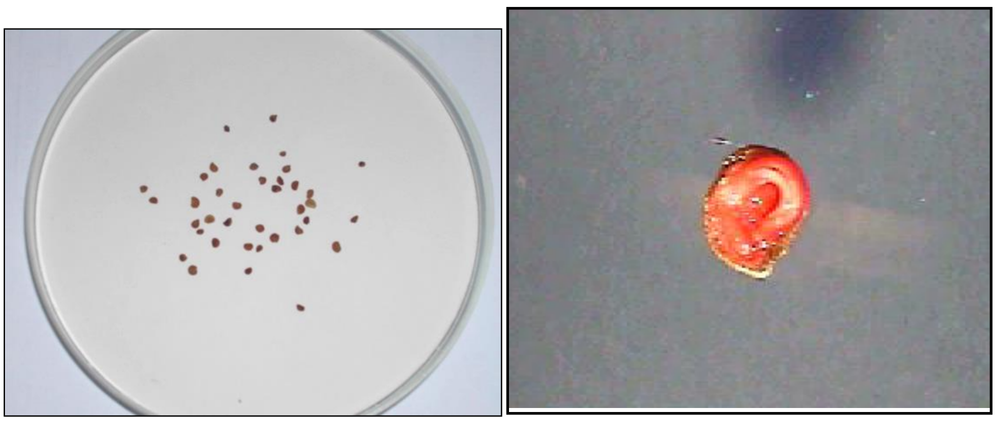


Figure 4. Seeds of *Lycium shawii*.

- *Ochradenus baccatus* This is a large dense shrub found in sandy, stony areas, approximately two meters tall, with grey-green linear leaves (Figur 5). It blooms in yellow flowers, appearing in spring, followed by whitish berries containing black seeds (Omar et al., 2007). The TTC procedure is same as that of *Nitraria retusa.*

Figure 5. Seeds of *Ochradenus baccatus*.

- *Horwoodia dicksoniae*: This is a sweet-scented annual herb; named after Dame Violet Dickson who is locally known as Umm Saud. It is a leafy plant with stems that are ascending to prostrate and slightly hairy (Figures 6). It has deep lilac flowers and large, round and winged fruit (Plate 13). It is abundant in sandy soil in Sulaibiya area (Omar, et al., 2007). The hard pericarp of *Horwoodia dicksoniae* fruit acts as a physical barrier for germination. As this species has exhibited physiological dormancy TTC tests plays an important role in assessing the viability. The hard pericarp was removed prior to the TTC test. The seeds were cut longitudinally and the same procedure as mentioned above was followed.

Figure 6. Seeds of *Horwoodia dicksoniae*.

- *Rhanterium epapposum:* This is the national plant of Kuwait. It is a very bushy shrub approximately 80 cm high with many stems branching out from the base. The leaves are small and narrow, and in late spring, it is covered with straw-yellow flowers about 1.5 cm wide (Omar et al., 2007). It is a C_3 desert shrub that can form monotonous stands covering vast areas of north-eastern Arabia (Brown, 2001). It flowers from April to May and produces numerous fruits which form in late spring and fall off the branches after maturity. It accumulates under the shrub and remains dormant until favorable conditions for germination to prevail. Each fruit (capitulum) contains about 6-8 seeds that are transported by wind or water. The capitulum is the unit of dispersal for *Rhanterium epapposum.* The seeds were extracted from the capitullum prior to TTC and the above-mentioned procedure was used to test viability (Figure 7).

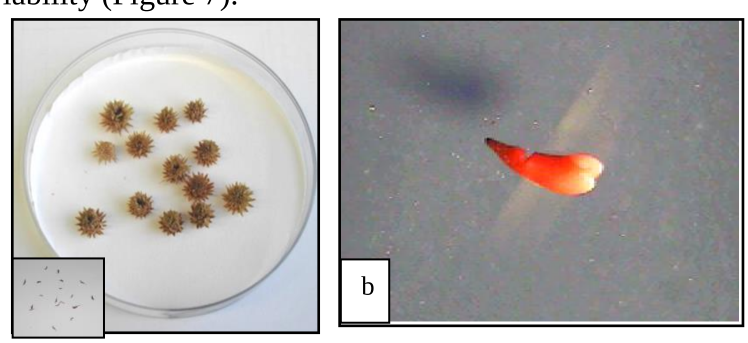


Figure 7. *Rhanterium epapposum* (a) capitulum and seed (b) stained embryo.

Some seeds have hard seed coat, which prevents imbibition of water and thus hindering germination (physical dormancy). For those seeds with physical dormancy scarification (mechanical/acid) of seed coat should be performed prior to TTC test. *Acacia pachyceras* and *Convolvulus oxyphyllus* are known to have physical dormancy.

- *Acacia pachyceras: Acacia pachyceras* O. Schwartz. (Synonyms- *Acacia gerrardii* Benth, *Acacia iraquensis* Reu.), only native tree species of Kuwait, is an important component of the desert wadis and hilly areas. It is a perennial tree which grows upto 10 m height, charecterised by pale yellow to white flowers, single or clustered globular heads, a pubesent pods and paired hornlike spikelets (Omar et al., 2007). For *Acacia pachyceras*, 100 seeds were mechanically scarified using sand paper and soaked in water for 24 hours to overcome the physical dormancy and the viability was evaluated through TTC test (Figure 8).

Figure 8. Seeds of *Acacia pachyceras*.

- *Convolvulus oxyphyllus* - It is a perennial shrub which can grow up to 60- to 70-cm height. The main branches arise from the base, bearing numerous straight lateral branchlets becoming spiny at apices. The leaves are mostly elliptical to linear in shape and flowers mostly forming on the lateral branchlets (Omar et al., 2007). Seeds of *Convolvulus oxyphyllus* are black (Figure 9) and do not germinate easily, and mechanical or acid scarification is helpful to increase germination.

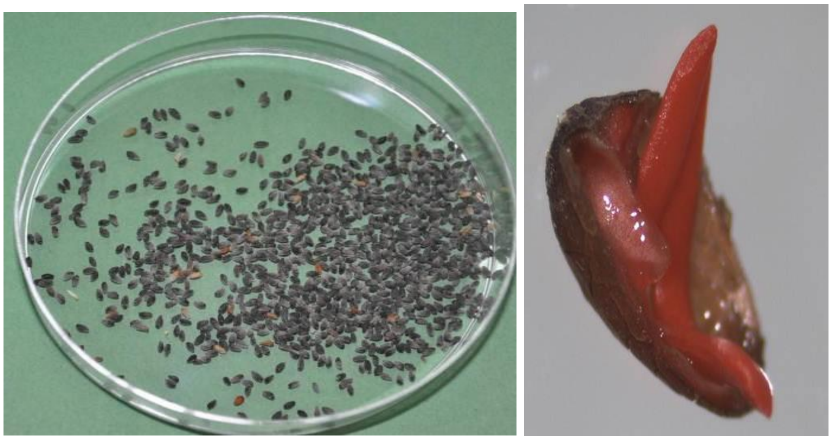


Figure 9. Seeds of *Convolvulus oxyphyllus*.

- *Peganum harmala*: It is a leafy perennial shrub about 40 cm high with yellowish-white flowers about two cm across. Its stems are woody at the base and multi-branched. The leaves are dark-green and linear (Omar et al., 2007). The seeds (Figure 10) are formed in small capsules. The seeds are extracted from the capsule, and the pre-moistened seeds are subjected to TTC tests as mentioned above. The results can also be confirmed by germination tests.

Figure 10. Seeds of *Peganum harmala*.

Conclusions

To conclude, these are the preliminary findings of viability tests on these rare native seeds on which adequate data is not available on the se areas of practical knowledge. However, these procedures have to be standardized to determine the pre-moistening time, TTC concentration and duration of soaking for TTC. However, this information will be highly useful for researchers involved in the quality testing of native plants of Kuwait.

Acknowledgements

We would like to thank the Kuwait Foundation for the Advancement of Sciences for their continuous financial support for our projects and the management of the Kuwait Institute for Scientific Research for their support, interest and encouragement. I would also like to extend my deepest gratitude towards all my team members especially my supervisor Dr. Majda Khalil and Ms. Sheena Jacob for their efforts, support and encouragement in all the works, compilation and preparation of the paper.

References

Brown, G. 2001. Vegetation Ecology and Biodiversity Of Degraded Desert Areas in North-Eastern Arabia.-Habilitation Thesis. Rostock University. P.9-92

Ferguson, J.M; R. D. Keys; F. W. McLaughlin and; J. M. Warren. 2017. Seed and Seed Quality – AG 448. NC State Extension Publication. North Carolina State University.

Gosling, P.G. 2001. Viability Testing. Workshop on Seed conservation: Turning science into practice, Royal Botanic Gardens, West Sussex, UK.

Hampton, J.G. 1995. Methods of viability and vigor testing: A critical appraisal, Seed quality: Basic mechanisms and agricultural implications, (Ed.) A. S. Basra, Food Products Press, Binghamton, New York.

ISTA. 2007. International rules for seed testing, (The International Seed Testing Association: Bassersdorf).

Omar, S.A.S.; Y. Al-Mutawa; and S. Zaman. 2007. *Vegetation of Kuwait*, Kuwait: Kuwait Institute for Scientific Research. P. 47-89.

Penn State Extension. 2016. Seed and Seedling Biology. http://extension.psu.edu/business/start-farming/vegetables/factsheets/seed-and-seedling-biology, (accessed on October 29, 2017).

Ramos, K. M. O.; J. M.M. Matos; R. C. C. Matins; I. S. Martins. 2012. Electrical conductivity testing as applied to the assessment of freshly collected Kielmeyera coriacea Mart. Seeds, International Scholarly research network. *Agronomy*, **12**: 1-5.

Steere W.C., W.C. Levengood, J.M. Bondie (1981). An electronic analyst for evaluating seed germination and vigor. *Seed Science and Technology* **9:** 567-576.

Ecophysiological requirement for the germination of *Stipagrostis ciliata* Desf, candidate species for ecological restoration in arid environment

L. Mnif Fakhfakh and M. Chaieb
Faculty of Sciences, University of Sfax, (Tunisia)
U. R. of Biology and Ecophysiology of Arid Ecosystems, Tunisia
Mchaieb133@gmail.com

Abstract

The study evaluated the effects of water and temperature limitation on the germination of perennial grass *Stipagrostis ciliata* seeds collected from the park of Central South Tunisia. The hydrothermal time model was used to describe *S. ciliata* seed germination under different water potential and temperature incubation periods. The germination response of seeds in darkness was evaluated over a range of temperatures (15, 20, 25, 30 and 35°C) and polyethylene glycol (PEG 6000) solution-based different osmotic potentials (0, -0.2, -0.6, -1.2, -1.6 and -2.0 MPa) at each temperature level. Among the temperatures tested, 25°C was found optimal to the germination of *S. ciliata* seeds. The highest germination (75%) was obtained with distilled water. However, the progressive increase of the solution-osmolality, inhibited seed germination. Less than 10% of seeds germinated at -1.2 MPa. PEG treatments significantly decreased the mean temperature for germination ($P<0.001; F=268.07$). The linear regression analysis was used to determine the relationships between final germination percentage and different osmotic potential levels. Hydrothermal time models that fitted data for *S. ciliata* seeds, showing high R^2 values, indicated a high degree of congruency between predicted and observed germination time course curves. θ_{HT} values were different between tested temperatures. $\Psi b_{(50)}$ values for seeds were highly negative, particularly at the lowest (15°C) incubation temperature. In conclusion, *S. ciliata* exhibited a significant adaptation capacity for water limitation and high temperature.

Keywords: Germination; temperature; water potential; *Stipagrostis ciliata*

Introduction

The degradation of arid ecosystems is typically linked to two factors namely climatic change and human activities (Le Houérou, 2001). It is likely that these factors have caused major changes in the vegetation cover; and therefore, of living natural resources. This degradation was mainly attributed to stressful environmental conditions, land clearing and overgrazing. It has also caused a rapid regression of grazing lands and erosion of biodiversity. In addition to declining of grasses (mainly the perennial) many other grass species of the arid zones have been submitted by other chamaephytes having a very low pasture value (Maestre et al., 2015). The studies mainly on the behavior of native species, in particular their responses to drought and aridity have been considered important for restoring vegetation in these degraded zones. Seed germination behavior in relation to thermal and water stress is very important to determine the colonization capacity of the species. Earlier, extreme temperature and moisture conditions were reported to significantly affect the seed germination in arid and semi-arid regions (Mseddi et al., 2002). Nevertheless, gathering information about temperature-effects on the germination was considered useful for evaluating the germination characteristics or the establishment potential among different species *Stipagrostsis ciliata*, a perennial and C_4 plant of the *Poaceae* family has a wide spread distribution in the hotter and drier parts of the tropical world (Kellogg 2001). The geographical distribution of this plant covers the deserted regions of South and

North Africa (Hosney et al., 2009). It shows strong ecological abilities to tolerate arid bioclimate, gypsy soil and wind erosion; hence, is useful grass for fixing sand in shifting and semi-fixed sandy areas (Daur 2012).

Considering the multiple roles of *S. ciliata* in arid regions, it would be interesting to study its ecophysiological characteristic and it field requirements. Given the above, the present study hypothesizes that *S. ciliata* seed germination may increase with increasing temperature and decreasing water potential. It was aimed to examine the effect of constant temperature (between 15°C and 35°C) on the germination; to analyze the response of *S. ciliata* seeds to a wide range of osmotic potential levels obtained with polyethylene glycol (PEG_{6000}) at each temperature level; and to describe the patterns of seed germination response to incubating temperature regimes in terms of the hydrothermal time parameter.

Material and Methods

The effect of temperature on germination was examined by incubating seeds in a 90 mm glass Petri dish with 10 ml of distilled water at constant temperatures 10, 15, 20, 25, 30 and 35°C. The incubation was made for 20 days in a dark incubator (LMS Cooled Incubator). Eight replicates of 50 seeds were used for each treatment. The germinated seeds were counted and removed every 2 days during 20 days of exposure periods. A seed is proven to germinate at the emergence of the radical. Different concentrations of Glycol Polyethylene (PEG_{6000}) to determine the effect of water potential (ψ) on the seed germination. PEG is none penetrating, inert osmoticum and forms a colloidal solution, which exerts effects similar to the matric properties of soil particles. This method gives a good estimate of germination behavior in relation to soil moisture under field conditions. As detailed below, a number of relevant germination tests were conducted in dark conditions at constant temperatures of 15, 20, 25, 30 and 35°C. Final germination percentage (FG) was calculated as the cumulative number of germinated seeds with normal radicles. Number of days to first germination (delay of germination) was determined as mean time to germination (MTG) following the formula $MTG = \sum (ni \times di) / N$. n is the number of seeds germinated at day i, d the incubation period in days and N the total number of seeds germinated. After the germination tests of *Stipagrostis ciliata*, the germination times and temperature-after relation parameters were analyzed by regressions based on the thermal time, hydro time and hydrothermal time to estimate the temperature and water relations parameters. Thermal time combines the initial germination time-courses in water at five temperatures. Germination data were analyzed based on the thermal time concept (Dahal et al., 1990) according to formula $\Theta_T (g) = (T - T_b) t_g$. Θ_T is the thermal time (degree-days) to radicle emergence of percentage g, T is the actual temperature at which the germination test conducted, $T_{\mathbf{b}}$ is the base temperature for germination and t_g is the actual time to germination of percentage g. Hydrotime model was followed to analyze the germination time-courses in water and osmotic solutions at 15, 20, 25, 30 and 35°C. This model is similar to the thermal time model, except that the base water potential (Ψb) values $\Theta_H = (\Psi - \Psi_{b(g)}) t_g$. Θ_H the hydrotime constant (MPa h), Ψ is the water potential of the imbibition medium, $\Psi_{\mathbf{b(g)}}$ is the base Ψ just preventing ridicule emergence of percentage g and t_g is the actual time to germination of percentage g. Finally, equations 1 and 2 were combined and the hydrothermal time model was adopted to analyze the seed germination rates across T and Ψ conditions were conducting according to the hydrothermal time model (Bradford 1995). Hydrothermal time was expressed as $\Theta_{HT} = (\Psi - \Psi_{b(g)}) t_g (T - T_b) t_g$. $\Theta_{\mathbf{HT}}$ is the hydrothermal time constant. The results of the germination experiments were analyzed using SPSS for Windows, version 13. Tukey test (Honestly significant differences, HSD) was used to estimate the least significant range between means.

Results and Discussion

Temperature Stress Effects on Germination. Temperature regimes significantly affected the germinative percentage of *Stipagrostis ciliata* (P<0.001). The germination curves were sigmoid at 25 and 30°C. However, the germination rates were higher than 70% at 25°C. *S. ciliata* seeds were able to germinate at temperature between 25 and 30°C and the optimal temperature was 25°C. Germination was significantly inhibited at 15, 20 and 35°C with final germination respectively 10, 39 and 34% (Figure 1). To better understand the effect temperature on germination of *S. ciliata* seeds, the relationship between MTG and the final germination percentage was established. The MTG also varied significantly with temperatures. The highest MTG was in correspondence with the low germination percentage. The temperature 25°C was characterized by a low MTG (2.47±1.5), indicating rapid germination and high final germination percentage.

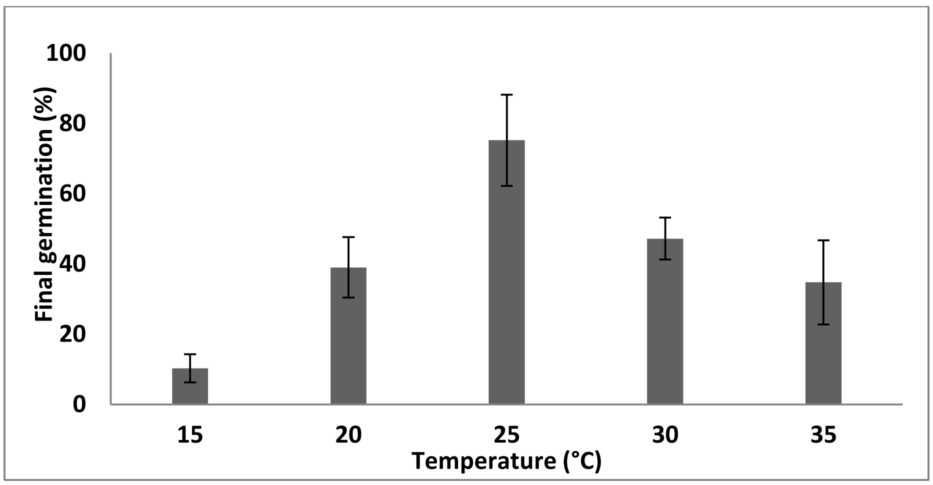


Figure 1. Final Mean germination percentages (%) of *Stipagrostis ciliata* seeds at different temperatures (15, 20, 25, 30 and 35°C).

Hydrothermal Time Analyses. *S. ciliata* seed germination responses to temperature were well described by the thermal time, hydrotime and hydrothermal time at both sub-optimal and supra-optimal temperatures. Three cardinal temperatures generally characterized germination responses to the temperature: the minimum, optimum and maximum. The minimum (Tb) and maximum Tc is temperatures below or above which germination will not occur. The optimum temperature T_0 is the temperature at which germination is most rapid. The variation of Median germination rates against germination temperature showed the trend of change in germination rates with temperature (Table 1). These rates exhibited a continuous increase at sub-optimal temperatures (from 15°C to 20°C). However, the curve rates decreased at supra-optimal temperatures (from 30°C to 40°C). Germination rates of *S. ciliata* seeds were correlated strongly at sub-optimal and supra-optimal temperature ranges with R^2> 0.70. However, the ceiling temperature Tc value was 43°C at supra-temperatures.

Table 1: Germination parameters of *Stipagrostis ciliata* seeds at constant temperatures based on a thermal time model analysis

Temperature range	**Model parameters**	***Stipagrostis ciliate***
Sub-optimal temperature	Tb (°C)	12
	$\theta T_{(50)}$ (°C days)	72
	σ Tb	0.54
	R^2	0.84
Supra-optimal temperature	Tc (°C)	43
	$\theta T_{(50)}$ (°C days)	44.46
	σ Tc	0.06
	R^2	0.72

Water Stress Effects on Germination. The kinetics of germination under osmotic stress conditions at different temperatures, optimum temperature (25°C), high temperature (30 and 35°C) and bass temperature (15°C and 20°C) reflects the sensitivity of a plant species to water stress. Germination of *S. ciliata* seeds was significantly (P<0.001) affected by water stress, where the highest final germination (FG=75.2 %) percentage was found in distilled water at 25°C. The increase in PEG_{6000} solutions of different osmotic potentials resulted in a gradual decrease in both the rate and percentage of germination with respectively 0.25 % and 2% at -2 MPa at optimal temperature 25°C (Fig 2). With a reduction of osmotic potential, the number of days to first germination was delayed. The MTG was significantly (P<0.001; F=268.067) decreased by PEG_{6000} treatments based on the results of one-way ANOVA. The depressive effect of water stress on germination occurred during one or all of these three phases, depending on the degree of the lowering of water potential linear regression analysis was used to determine the relationships between final germination percentage and different osmotic potential levels. Hydrothermal time models fit to data for *S. ciliata* seeds had high R^2 values, indicating a high degree of congruency between predicted and observed germination time course curves. θ_{HT} values were different between tested temperatures. $\Psi b_{(50)}$ values for seeds were highly negative, particularly at the lower (15°C) incubation temperature. $\Psi b_{(50)}$ increased linearly with incubation temperature. The final germination was highest at 25-30°C, intermediate at 35°C and lowest at 15 and 20°C.

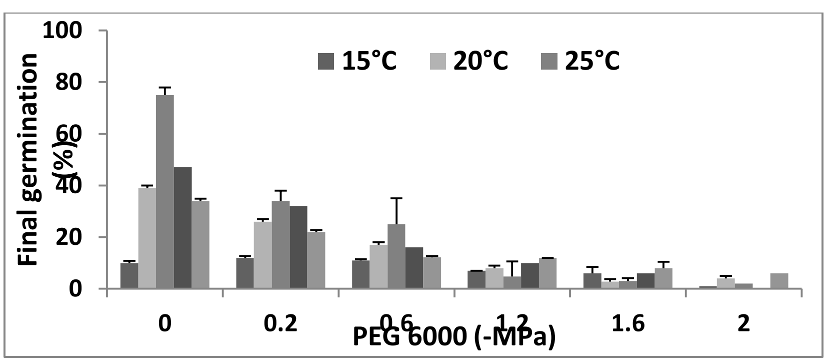


Figure 2. Final Mean germination percentages (%) of *Stipagrostis ciliata* seeds at different PEG_{6000} solutions of different osmotic potentials (0 to -2 MPa), at different temperatures (15, 20, 25, 30 and 35°C).

Discussion

The improvement of the methods based on seed dispersal and seed sowing would enable to know more about seed response to main environmental factors especially drought stress in arid land. Under these conditions, seed germination monitoring in relation to water is very important to determine the colonization capacities of species. Results obtained herein suggested that *S. ciliata* seed germination occurred over a range of temperature between 25 and 30°C in darkness. The optimum temperature of germination of this species was 25°C with germination percentage 75%. This behavior may constitute a mechanism of adaptation to the ecological conditions of the arid environment characterized by relatively high temperatures. It was also suggested that the success of germination recorded at elevated temperatures allows seeds to escape the risks of rapid desiccation of the upper soil horizons during the period of germination and that higher temperatures 25°C are considered to be very favorable to the species of the photosynthetic type $C_{4.}$ This species germinates in dry climate, where it tolerates high temperatures and water stress. Our data strongly supported the assumption that germination of *S. ciliata* is mainly controlled by ambient climatic factors. The germination of *S. ciliata* was the best at temperature from 20 to 25°C.

This study has also demonstrated that mean time of germination can be affected by the changes in the temperature regimes. The lowest MTG was recorded at 25°C. At this temperature, the seeds took a little time to germinate. This is probably due to the decrease of water absorption associated to a low diffusion of respiratory gas. Temperature and water potential are considered as the most important environmental signals regulating species germination (Chauhan and Johnson 2008). In the arid environment, the water necessary for germination is available only for short periods (winter) and therefore successful establishment of species depends not only on rapid and uniform germination, but also the ability of the seed to germinate under low water availability. Our results suggested that germination of *S. ciliata* continued despite the increase of osmotic potential. Up to -1.6 MPa germination rate was completely inhibited. This germination capacity may be related to its better adaptation to water deficits. This behavior is a typical strategy of saharo-mediterranean plants with optimal temperatures ranging between 15 and 30°C. The highest germination percentage of studied specie was obtained for non-stressed seeds. The variation of PEG_{6000} concentration has significantly affected the mean time of germination and also germination rate. Gutterman (2002) showed that in the areas with dry and hot summers, seeds germination should be mainly triggered by interactions in temperature and precipitation to prevent germination occurring shortly after seed maturation following late rainfall in the rainy season before the summer. Hassan et al., (2009) assumed that water availability, rather than temperature, is a more important factor driving germination in Jordanian *Stipa* species in the field condition**.**

Seed germination is strongly influenced by temperature (T) and water potential (Y). Accumulation of progress toward radicle emergence under various combinations of T and Y can be described using hydrothermal time. The thermal time and hydrotime models provide insight into how physiological and environmental factors interact to regulate the germination behavior of seed populations. It has been clearly demonstrated that seed dormancy is a reflection of high (more positive) values of the Ψb (g) threshold, and that conditions that break dormancy (after-ripening, hormones, etc.) shift the Ψb (g) distribution to lower values (Meyer et al., 2000). Subsequently, *S. ciliata* had a low Ψb that may be a condition that break dormancy and had a high Tc value. Ψb(50) increased linearly with incubation temperature. Further, Bradford (1994) reported that the low Tc values are often associated with seed dormancy, as in relative dormancy or thermo-inhibition. It is clear that the species may be in dormancy at high temperatures.

Conclusions

This study concludes that *S. ciliata* seeds can germinate under temperature between 25 and 30°C, with an optimum temperature at 25°C; whereas, 15°C temperature can limit the germination. *S. ciliata* can be considered tolerant to water stress, and can also present an adaptive capacity to aridity. Overall, it can be concluded that this perennial grass is an excellent and promising pastoral species for the ecological restoration, and enhancement of ecosystems biological productivity in the areas with arid bioclimate.

References

Bradford, K.J. 1995. Water relations in seed germination. In: Kigel J, Galili G, editors. Seed development and germination. Marcel Dekker: New York. P. 351–395.

Chauhan, B.S. and D.E. Johnson. 2008. Germination ecology of Southern crabgrass (*Digitaria ciliaris*) and India crabgrass (*Digitaria longiflora*): two important weeds of rice in tropics. *Weed Science* **56**: 722-728.

Dahal, P. and K.J. Bradford. 1990. Effects of priming and endosperm integrity on germination rates of tomato genotypes. II. Germination at reduced water potential. *Journal of Experimental Botany* **41**:1441–1453.

Daur, I. 2012. Plant flora in the rangeland of western Saudi Arabia. *Pakistan Journal of Botany* **44**: 23-269.

Gutterman, Y. 2002. Survival Strategies of annuals desert plants: Adaptation of desert organisms, Springer, New York, NY, USA.

Hassan, R. and H. Hamasha Isabell. 2009. Seed germination of four Jordanian *Stipa* spp: differences in temperature regimes and seed provenances. *Plant Species Biology* **(24)**: 127-152.

Hosny, A.; A. Mosallam Amal; A. Morsy; A. Youssef; and A. Ahmed. 2009. Structure of the common plant population along Alamain-WadiEl- Natrun Desert Roat. Australian *Journal of Basic and Applied Sciences* **3 (1)**: 177-193.

Kellogg, E.A. 2001. Evolutionary history of the grasses. *Plant Physiology* **125**: 1198-1205.

Le Houérou, H.N. 2001. Biogeography of the arid steppe land north of the Sahara. *Journal of Arid environment* **48**: 103-128.

Maestre, F.T.; M. Delgado-Baquerizo; T.C. Jeffries; V. Ochoa; B. Gozalo; et al. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *PNAS* **112**:15684–89.

Mseddi, K.; M. Visser; M. Neffati; D. Rehaul; and M. Chaieb. 2002. Seed and spike traits from remnant populations of *Cenchrus ciliaris* L., in South Tunisia: high distinctiveness, no ecotypes. *Journal of Arid Environment* **50**: 309–324.

Meyer, S.E.; S.B. Debaene-Gill; and P.S. Allen. 2000. Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in *Elymus elymoides*. *Seed Science Research* **4**:1-10.

Rhanterium Seed Biology and Germination Study for Large-scale Plant Production

L. Al-Sabah, C. Sudhersan, S. Jibi and S. Al-Melhem
Kuwait Institute for Scientific Research, Kuwait
e-mail: lsabah@kisr.edu.kw

Abstract

Rhanterium eppaposum locally known as Arfaj is a native perennial shrub growing in Kuwait and neighboring Saudi Arabia. The flower of Rhanterium is considered as the national flower of Kuwait. The Rhanterium population in Kuwait is decreasing due to various anthropological factors and natural calamities. This plant species produces thousands of flowers and seeds annually. However, natural regeneration is very poor due to various factors that affect the seed germination and plant establishment in the open field. It is one of the major plant species that has been included in the Kuwait's environmental rehabilitation program (KERP) and a large number of seedlings of this species is required for the rehabilitation purpose in Kuwait. Large-scale plant production process is very slow due to the lack of knowledge about the Rhanterium seed biology and germination. Therefore, a study on seed biology and germination is necessary for the mass plant production. Recently, an experimental study was undertaken in our plant tissue culture laboratory to find out the factors that are controlling the seed germination, plant growth and development in this species. KISR station for research and innovation (KSRI) in Kabd was used for the field study and seed collection. The main objective of the study was to maximize the Rhanterium seedling production for the rehabilitation program. The results of the study indicated that each well-established Rhanterium shrub can produce 4,000-6,000 flowers and an average of 50,000 seeds annually. The poor percentage of seed germination is due to several factors including seed viability, type of seed germination media, planting depth, planting position etc. Through the present study, a plant production protocol has been developed and standardized to enhance the percentage of Rhanterium seed germination towards large-scale plant production for the rehabilitation program in Kuwait.

Keywords: Arfaj, seed germination, environmental re-habilitation, large-scale plant production.

Introduction

Desert ecosystem of Kuwait has damaged due to the natural calamities such as drought, low and erratic rainfall, frequently occurring sand storms and anthropological activities. Natural rehabilitation has failed due to the harsh climatic conditions. Desert rehabilitation in Kuwait require large number of native shrubs and human intervention on seedling production, planting and care. *Rhanterium epapposum*, belongs to the botanical family Asteraceae is a native perennial shrub which is included in the list of native species for rehabilitation program in Kuwait.

Rhanterium epapposum is the national plant of Kuwait. It is a bushy perennial shrub having fibrous taproot system. It grows 80 to 90 cm high with multiple irregular and twisted branches from the basal woody stem giving a good appearance. The leaves of this plant are small, narrow with serrated margin. Each Rhanterium bush produces thousands of yellow colored inflorescence called capitulum during April to May. It is a forage plant for camels and sheep.

Understanding the seed biology of native plant species used in revegetation program is vital for ensuring establishment of seedlings on large-scale. Seed biology of many native plants are not available. In the case of Rhanterium, studies have undertaken already (Sulaiman et al., 2009; Zaman et al., 2010). However, information on Rhanterium seed production and germination is too scanty.

During the summer months and winter months, native seeds stay dormant in the ground. Seed germination occurs during the season of highest water availability and optimal germination temperature. Native seeds of Kuwait germinate usually after the summer rain and winter rain. The optimum range of germination temperatures is 20–30°C and majority of the native seeds germinate under favorable condition. Previous studies on native seed germination proved that some species require external stimulant for seed germination.

Research on seed biology is limited for many native plants of Kuwait and there is no information available on seed germination for many native plant species of Kuwait. .*Rhanterium* produces a large number of seeds annually while the natural germination and field establishment is very poor. Thus, the aim of the present study was to investigate the seed biology and germination in *Rhanterium*. In the present study we determined: flowering and seed production characteristics, seed germination and factors that control the seed germination in *Rhanterium.*

Materials and Methods

Natural populations of *Rhanterium epapposum* bushes growing in the KISR station for research and innovation (KSRI) were used for the study. Flowers collected from the bushes were dissected out under a stereoscopic binocular microscope in the plant tissue culture laboratory. Average number of ray and disc florets were counted. Flower morphology was studied for the ray and disc florets and the characteristic features were recorded.

Seeds for germination tests were collected from plants of *Rhanterium epapposum* growing in the KSRI at Kabd during July-August 2017 in the form of capitulam. The capitula were collected, spread on plastic trays, maintained at 25°C until dried and stored at the same temperature for the experimental study. Capitula collected directly from the bush and fallen ones from the soil were maintained separately.

Seeds (achene) were manually removed from the capitulum, homogenized in a tray and visually selected in order to eliminate the wilted or sterile seeds, and undamaged and healthy ones were manually selected for germination test. Both the isolated seeds and the entire capitulum were used for the germination experiments.

Experiments were conducted to test: 1. Different soil types (sand, clay and potting mix), 2. Types of capitulum (directly from the bush and fallen ones from the soil), 3. Planting depth (deep, shallow and surface) and 4. Planning position (horizontal, vertical and irregular) using the capitulum at constant temperature (25°C). Isolated seeds (achene) were also used for the germination study. All the experiments were carried out at the KISR plant tissue culture laboratory. Each experiment had 25 replicates and the experiment was repeated twice. Mean values with SD were calculated and tabulated.

Results and Discussion

Floral Morphology. The inflorescence (capitulum) of *Rhanterium epapposum* was surrounded at the base by a group of scale leaves called involucres. Each capitulum had a flat receptacle on which the flowers (florets) were arranged. The flowers were heterogamous having two types: disc and ray florets (Figures 1a -d). The radiate head had disk flowers in the centre surrounded by one marginal row of ray flowers, which had an irregular corolla. The corolla was tubular at the base but prolonged on the outer side into a generally flat projection, the ray, or ligule in the case of ray florets. The ray flowers in radiate heads were pistillate (female). The disk flowers in a radiate head had both sexes, but they were functionally staminate, with anthers having fertile pollens and without a functional ovary.The disc florets were tubular,6-10 mm long with bract and located at the central part of the receptacle. The tubular disc florets are bisexual and actinomorphic. Each capitulum (head) had up to 80 disc florets and 15 ray florets. Flowers were sessile and epigynous. Calyx was poorly developed and represented in the form of scales. Corolla was tubular with 5 lobes in the case of disc florets and ligulate with 3-4 teeth in the case of ray florets. There were five stamens, epipetalous, syngenesious (filaments free and anthers united) forming a tube around the style. Carpel was bicarpellary and syncarpous. The stigma was bifid and ovary inferior.

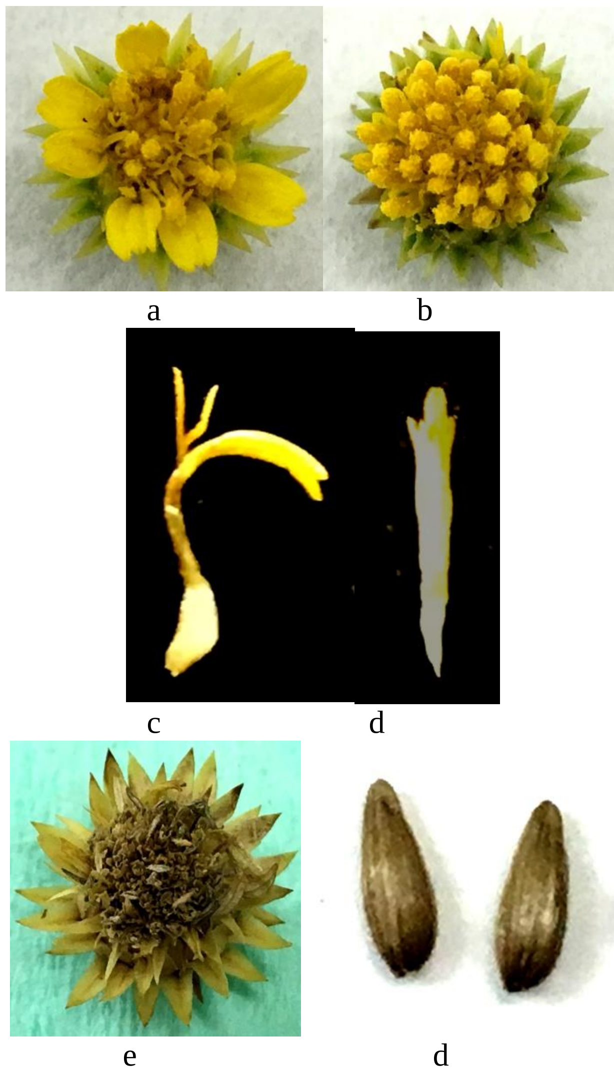


Figure 1. *Rhanterium epapposum* Oliv. Flower. a. Capitulum with ray and disc florets; b. Capitulum with disc florets; c. Ray floret; d. Disc floret; e. Mature capitulum; f. Achene.

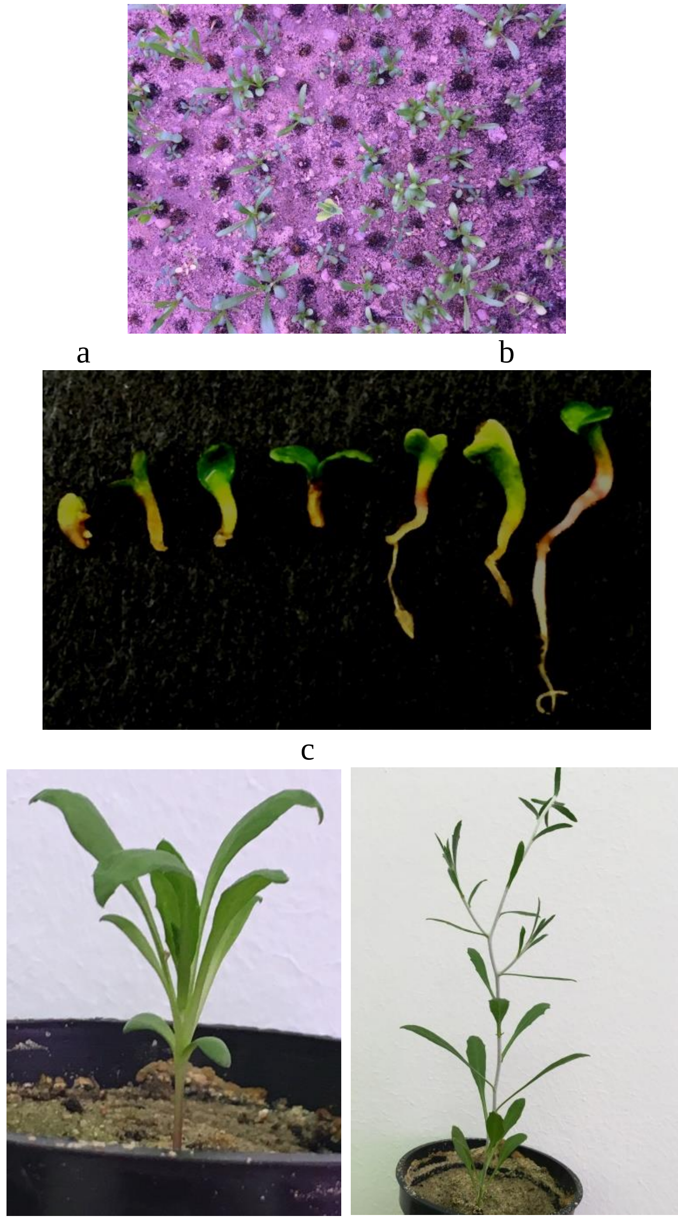


Figure 2, *Rhanterium* seed germination. a. Isolated seed germination; b. seed germination from capitulum; c. Stages of germination; d. 20 d 0ld seedling; e. 45 d old seedling.

Seed Biology. Fully mature *Rhanterium* bush produced an average of 5,000 flowers (capitula) and about 50,000 seeds. However, natural seed germination and seedling establishment in the open desert was found to be less than 3%. Fruits of *Rhanterium* were called cypsela (Marzinek et al., 2008) or achene in which the fruit and seed are joint together. Each mature capitulum had an average of 10 achenes and all the achenes were attached with the receptacle after maturity (Figures 1e, f). The fruit wall became the seed hull and gave false appearance as seed coat. The seeds were without endosperm and the reserve food was stored in the cotyledons of the embryo. The embryo was linear type, radicle facing down and cotyledons facing upward position surrounded by a thin seed coat. Unlike the other members of the *Asteraceae*, the seeds (achene) of *Rhanterium* were attached within the capitulum during the seed dispersal and germination (Figure 2b). Isolation and collection of individual seeds from the capitulum was found to be time consuming, difficult and expensive procedure.

Seed Germination. Germination is the process by which a dry, seed imbibe and increases in its metabolic activity, initiates the conversion of embryo into a seedling and culminates in the protrusion of the radicle (Aliotta and Cafiero, 2001) and dormancy is the biological phenomenon that blocks the viable seed from germination (Finch-Savage and Leubner-Metzger, 2006). In the *Rhanterium epapposum* seed germination study, the fresh seeds collected from the capitulum germinated within 10 d duration without showing any dormancy (Figure 2a). However, several factors affected the seed germination. The achene of *Rhanterium* had an outer dark hull and inner lite colored kernel the true seed. In the true seed, the embryo was covered by a thin membranous seed coat. The embryo was linear type and the narrow end of the embryo (radicle) was positioned towards the receptacle of the capitulum and the blunt end (cotyledons) facing up. The embryo was about 2 mm in length. During germination first the radicle part swelled and protruded out of the seed coat followed by the cracking of the outer hull. Latter on the fleshy cotyledons turned greenish in color and spread apart bilaterally after the compete germination. The *Rhanterium* seed did not produce any hypocotyl hook during germination like the other dicot seed germination (Figure 2c). The protrusion of the radicle into the soil was affected by the hard receptacle and the involucre around the capitulum. Due to this reason seed germination and seedling establishment was noticed very low when compared to the other members of *Asteraceae*. The factors that affected the seed germination were involucre around the capitulum, temperature, soil type, planting position and depth of planting. The highest germination was observed when the capitulula were planted vertically in sandy soil exposing 50% of the capitula above the soil media. Fresh capitula harvested from the shrub, planted vertically facing the receptacle end down and 50% exposed condition under the controlled temperature germinated 98% (Table 1). The clay soil did not supported the germination. Potting mix (sand, peat moss and compost at 1:1:1 ratio) supported germination but, did not support the well establishment of the seedling. Deeply planted seeds and planted upside down (inverted) did not germinated (Table 1). Irregularly placed capitula showed poor germination. The capitula collected from the ground also showed low percentage of germination. This may be due the embryo damaged by the insects or embryos damaged due to heat from the hot sand. The selected viable seeds separated from the capitulum when placed on the moist sandy soil also germinated 100% at controlled temperature. The seed germination took 10-20 days and the seedling establishment took 45 d (Figure 2a-e). From out study, it is concluded that the *Rhanterium* seed has no dormancy and fresh capitula harvested directly from the shrubs up on proper drying and storage germinate when planted in the sandy soil vertically exposing 50% outside of the soil media.

Table 1. *Rhanterium epapposum* Seed Germination*

Soil type	Planting position**	Germination %
Clay	Vertical	1 ± 0
	Horizontal	0
	Inverted	0
Sand	Vertical	98 ± 0.7
	Horizontal	4 ± 0.1
	Inverted	0
Potting mix	Vertical	96 ± 0.14
	Horizontal	6 ± 0.07
	Inverted	0

± Standard Deviation; *Capitulum used as seed material.

Acknowledgement

The financial support (PR17-12SL-04) from KFAS and full support and encouragement from the KISR's management are hereby acknowledged.

References

Aliotta, G. and G. Cafiero. 2001. “Seed bioassay and microscopy in the study of allelopathy: radish and purslane responses.” In Handbook of plant ecophysiology techniques. M. J. Reigosa, ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Finch-Savage, W. E. and G. Leubner-Metzger. 2006. Seed dormancy and the control of germination. Newphytologist, 171: 501-523.

Marzinek, J., De-paula, O.C., Oliveira, D.M.T. 2008. Cypsela or achene? Refining terminology by considering anatomical and historical factors. Revista Brasileira de Botânica, 31: 549-553.

Suliman, M. K., Bhat, N. R., Abdal, M. S., Zaman, S., Jacob, S. and R. R. Thomas. 2009. Germination Studies in *Rhanterium epapposum* Oliv. World Applied Sciences Journal, 7: 468-471.

Zaman, Z., Padmesh, S. and H. Tawfiq. 2010. Germination Ecology of *Rhanterium epapposum* Oliv. American Journal of applied Sciences, 7: 1321-1326.

Effects of Planting Techniques on Seedling Establishment in Native Species for Sustainable Revegetation

Sheena Jacob, Majda K. Suleiman, Rini R. Thomas, Narayana R. Bhat and Mini Sivadasan
Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research, Kuwait
e-mail: sjacob@kisr.edu.kw

Abstract

Rapid natural regeneration of native plants in the degraded land of Kuwait is implausible, considering the typical harsh weather conditions of arid region characterized by minimal erratic rainfall. Although not impossible, it requires a longer period, probably decades to witness a reasonable vegetation cover. Hence, the focus is on implementing revegetation programs in an efficient manner. Success of any revegetation program depends on the planting technique used, which varies with species and with significant effects on plant survival and growth. From least expensive seeding method to labor-intensive transplanting method constitute various techniques currently used for revegetation. However, additional information on the effective techniques for the species, site, and climate- specific activities are much in demand. In this study, establishment of plants from primed and non-primed seeds and transplanted seedlings of *Rhanterium epapposum* and *Haloxylon salicornicum* were evaluated. The preliminary results indicated higher establishment of *Rhanterium epapposum* plants from primed seeds (91%) when compared to non-primed seeds (71%) and transplanted seedlings (80%). Transplanted *Haloxylon salicornicum* seedlings had higher survival chances (160% and 355%) than plants from primed and non-primed seeds, respectively during the first year after planting. However, continuous monitoring of plant survival and performance is essential for outlining effective planting techniques and the timeline of cultural activities suitable for native plants.

Keywords: Native plants, seed priming, direct sowing, transplantation of seedlings, *Rhanterium epapposum*, *Haloxylon salicornicum*

Introduction

Arid, semiarid and dry-sub humid areas dominate the Arab region and it covers approximately 9% of the world's land area (Asma et al., 2002). Climate of Kuwait can be categorized as hyper arid to arid (Middleton and Thomas, 1997), and it is characterized by extremely hot dry summers with long, intense sunshine hours and brief winters with moderately cool climate with occasional rain. The average daily maximum temperatures varied from 18.9 °C during January to 46.8°C in July (Annual Statistical Report, 2006). The rainy seasons extends from October to May, and mean annual rainfall is 113 mm (Omar et al., 2007). The rate of evaporation exceeds precipitation and is approximately 16.6 mm per day. The average annual desertified land in Kuwait is estimated to be 285 km^2 (Al-Awadhi et al., 2003).

Kuwait's land resources are threatened by nature (long hot dry period) and human activities (Shahid et al., 2003). Land degradation is accelerated by many factors especially soil erosion by wind, loss of vegetation cover by severe grazing, soil compaction, crusting and sealing, and salinization (Al-Awadhi et al., 2005). The destruction of natural grass and woody vegetation cover in dry areas affects the topsoil temperature and air humidity, and consequently influences atmospheric temperature and rainfall (Abahussain et al., 2002). Natural vegetation regeneration is likely to proceed extremely slowly under climatically severe conditions that prevail in the hyperarid regions (Brown, 2007). Hence, continuous and systematic research is essential in the area of soil stabilization, biodiversity conservation, and desert rehabilitation activities. In the recent years, many research activities were initiated in Kuwait to formulate efficient revegetation methods, standardization of propagation methods, and other unexploited region-specific study areas. Evaluation and standardization of planting techniques is essential for the effective implementation of any revegetation program. Evaluation of direct seeding of primed and non-primed seeds and transplanting of *Rhanterium epapposum* and *Haloxylon salicornicum* were evaluated in the current study.

In the arid regions, mortality during initial stages after germination is high. However, the results may vary with species and planting technique. Direct seeding is a cost-effective way of reestablishing native vegetation by sowing seeds directly into the prepared soil with or without pretreatment. The main objective of this study was to evaluate and compare the survival rate and performance of direct-seeded and transplanted *Rhanterium epapposum* and *Haloxylon salicornicum* plants grown under the same field conditions.

Materials and Methods

An experimental area of 3000 m^2 at Agricultural Research Station, Sulaibiya, was used for this study. In each species there were three treatments (primed, non-primed seeds, and transplanted seedlings), which were replicated thrice. The plants were drip irrigated. In the direct seeded trials, the primed or non-primed seeds of selected plants were sown manually in the prepared site at a depth of 5-10 cm, at pre-located seed spots. *Rhanterium epapposum* seeds were soaked in water (hydro-primed) for 12 hours followed by surface drying. *Haloxylon salicornicum* seeds were hydro-primed for 6 hours followed by surface drying. Control seeds were directly sown in the field as mentioned earlier without priming.

Accordingly, 0.5 g of *Rhanterium epapposum* (approximately 10 seeds) and 1.5 g of *Haloxylon salicornicum* (approximately 105 seeds) were sown in each planting hole in the first week of March 2016. The plant spacing was 2 m x 2 m and interspace between plots was 2 m. In each replication, individual treatments were conducted in 10 x 10 m plot.

Plant germination and survival percentage were recorded monthly, and plant growth parameters (plant height, number of branches, and root collar diameter) were recorded periodically. Growth in plant height, number of branches, and root collar diameter were calculated using equation 2:

Relative Growth (%) = ([(Final value - Initial value) / Initial value] X 100)…………….(2)

The collected data on survival percentage and growth in plant parameters were analyzed using Analysis of Variance Procedure (ANOVA) of IBM® SPSS® software, version 22, and Duncan's Multiple Range test was used to ascertain the significant differences among treatments (Little and Hills, 1978). The non-transformed data were presented in the graphs. The survival percentage data was arcsine transformed and the growth data was log transformed prior to analysis (McDonald, 2009).

Results and Discussion

The survival rate was recorded every month and is illustrated in Figures 1 and 3. Initial germination and the survival of the plants grown through various planting techniques during different seasons varied with species. Assessment on the growth performance of the plants was done based on plant parameters such as plant height, number of branches, and root collar diameter for each species (Figures 2 and 4).

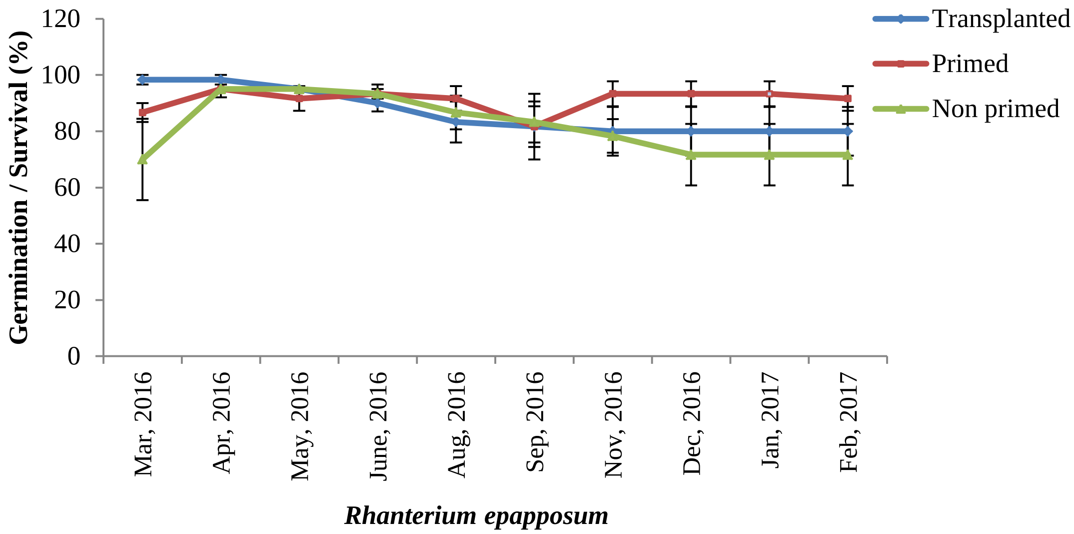


Figure 1. Germination or survival data of *Rhanterium epapposum*.

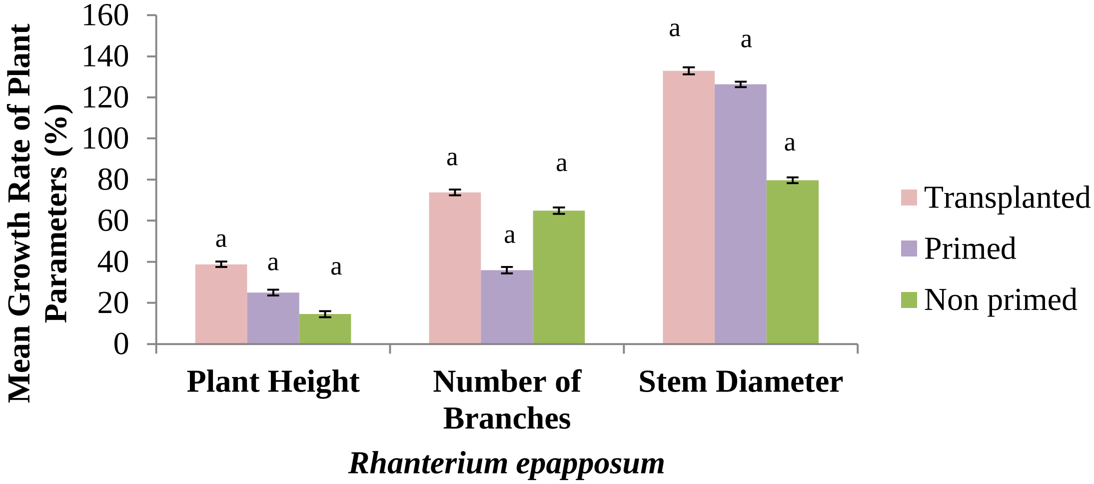


Figure 2. Mean growth rate of plant parameters in *Rhanterium epapposum*.

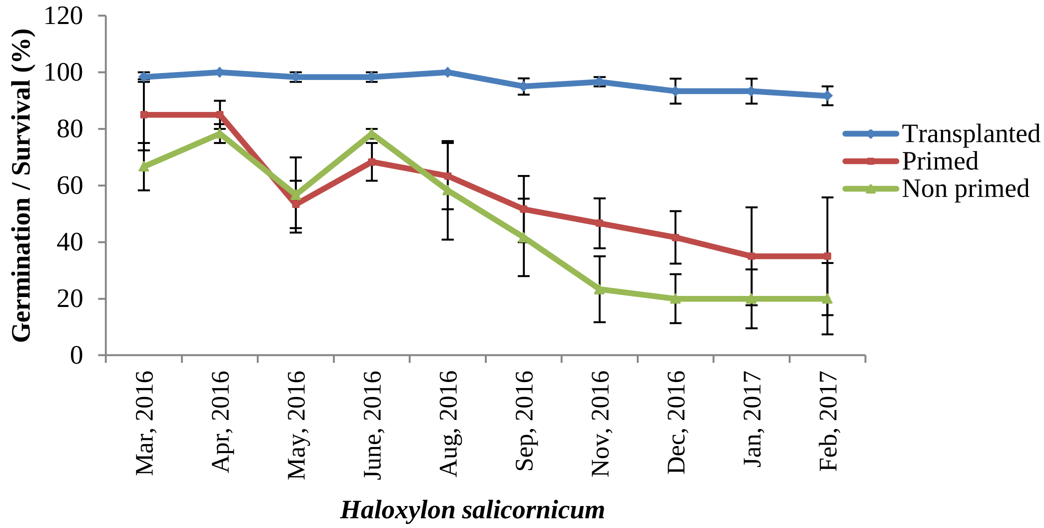


Figure 3. Germination or survival data of *Haloxylon salicornicum*.

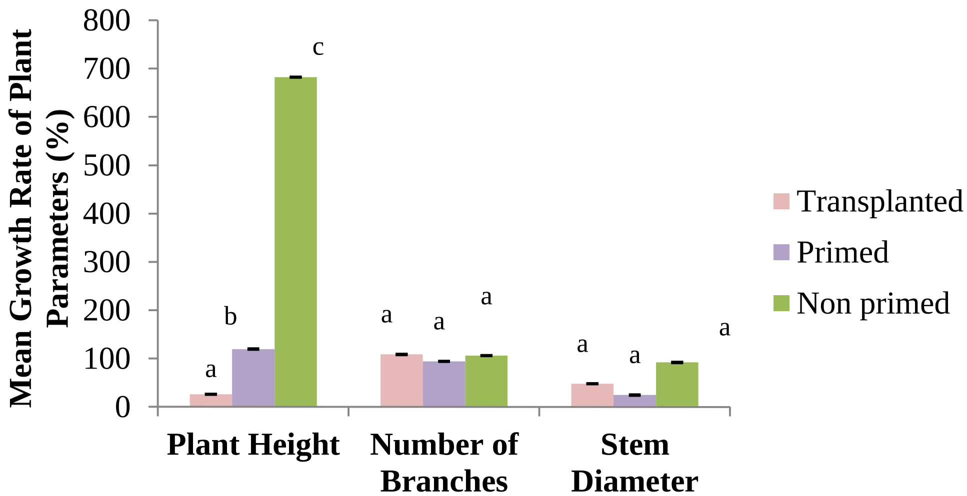


Figure 4. Mean growth rate of plant parameters in *Haloxylon salicornicum*.

In *Rhanterium epapposum*, there was no significant difference (p≤0.05) in the survival rate of seedlings from various planting techniques during one year duration after planting. Seedlings from primed seeds exhibited a superior performance (92%) when compared to non-primed and transplanted seedlings, though the differences were statistically insignificant. The team observed 80% survival of transplanted *Rhanterium epapposum* seedlings, while Islam et al. (2015) reported ~20% survival of the same species in a similar arid environment. Growth in the plant height, number of branches, and root collar diameter of transplanted seedlings was higher than those in the other two treatments though not significantly. Flowering was observed in all the treatments from March 2017.

In *Haloxylon salicornicum*, there was significant difference (p≤0.05) in the survival percentage of seedlings from various planting techniques during the study period. Survival in the seedlings from primed and non-primed seeds consistently declined after initial germination, and the reduction in survival was significantly lower than the transplanted seedlings. This tendency indicates that transplanting is the preferred planting technique for *Haloxylon salicornicum* in harsh climatic conditions. In contrast, the growth rate in plant height and number of branches of seedlings from primed and non-primed seeds were significantly higher than those of transplanted seedlings. The few plants that managed to survive from primed and non-primed seeds exhibited vigorous growth. Flowering (November 2016) and seed set (January 2017) were also observed in 35% of transplanted plants. No flowering was observed in seedlings from primed and non-primed seeds.

Conclusions

Survival response of each species was varying. While priming of *Rhanterium epapposum* seeds resulted in better survival at the end of one year, transplanting technique was found to be superior for *Haloxylon salicornicum*. However, observation on survival and growth behavior of these species for a longer period is required to reach a broader conclusion.

Acknowledgement

The authors would like to thank the Kuwait Foundation for the Advancement of Sciences (KFAS) and Kuwait Institute for Scientific Research for providing funds and encouragement during the investigation.

References

Abahussain, A. A., A.S. Abdu, W.K.Al-Zubari, N. A. El Deen and M. A. Raheem. 2002. Desertification in the Arab region: analysis of current status and trends, *Journal of Arid Environments* **51**: 521-545.

Al-Awadhi, J. M., S.A. Omar, and R.F. Misak. 2005. Land degradation indicators in Kuwait. *Land Degradation and Development* 16:163-176.

Annual Statistical Report. 2006. Ministry of Planning. The State of Kuwait.

Asma A.A., Anwar Sh.A, Waleed K. A, Nabil A. E and Mahmmod A. R. 2002. Desertification in the Arab Region: Analysis of Current Status and Trends, Journal of Arid Environments 51:521-545.

Brown, G., 2007. Desertification in the Arabian Peninsula and Perspectives for Ecosystem Restoration, In: Desertification in Arid Lands: Causes, Consequences and Mitigation, Proceedings of the International Conference on Desertification Control in the Arid Region, May 12-15, 2007, Kuwait.

Islam, M.A., Bhat, N.R., Suleiman, M.K. Jacob, S., Al-Mulla, L and R.R. Thomas. 2015. Standardization, Mass propagation of native and naturalized exotic plants and establishment of field plantations. Phase II – mass propagation and establishment of field plantations. Kuwait Institute for Scientific Research. Final Report. KISR 13097, Kuwait.

Little T.M.,.Hills F.J., 1978, *Agricultural Experimentation Design and Analysis*. U.S.A: John Wiley & Sons Inc, pp 63-65.

Macdonald, B. 1990, *Practical Woody Plant Propagation for Nursery Growers*, Oregon: Imber Press.

Middleton, N. and D.Thomas. 1997. World Atlas of Desertification. United Nations Environment Program/ Amold pp, 182.

Omar, S.A.S., Y. Al-Mutawa, and S. Zaman. 2007. *Vegetation of Kuwait*, Kuwait: Kuwait Institute for Scientific Research.

Shahid, S. A., S. A. S. Omar, R. Misak and H. A. Rizq, 2003. Desertification in the Third Millennium. Edited by A.S. Alshaahan, W.W. Wood, A. S. Goudie, A. Fowler and E. M. Abdellatiff, Swets &Zeilinger Publishers, Lisse, The Netherlands, P. 351-360.

Effect of Seeding Methods on Seedling Establishment of Mangroves in Kuwait

Bison M. Thomas, Narayana R. Bhat, Majda K. Suleiman and Laila Almulla
Kuwait Institute for Scientific Research, Kuwait
* **e-mail**:bmthomas@kisr.edu.kw

Abstract

Mangroves are halophytic trees inhabiting the intertidal zones and enriching the coastal biodiversity and marine ecosystems in the tropics and subtropics. Recognizing the potential of mangroves to improve the delicate coastal ecosystem of Kuwait, the Kuwait Institute for Scientific Research (KISR) introduced mangroves in the 1990s and standardized the growing techniques for grey mangrove (*Avicennia marina*). Despite concerted efforts made over the years, the seedling mortality of mangroves in Kuwait has remained to be unacceptably very high (over 50%) against the acceptable rate of less than 20%. Considering these facts, a study was conducted in September 2016, to evaluate six seeding methods in order to reduce seedling mortality and improve the seedling establishment success in the early nursery stage. The seeding treatments comprised of direct seeding in marine mudflats with regulated inundation, direct seeding in marine mudflats with unregulated inundation, seeding in nursery trays with seawater irrigation, seeding in nursery trays with freshwater irrigation, seeding in nursery pots with seawater irrigation, and seeding in nursery pots with freshwater irrigation. The results of the study indicated that seeding in nursery trays and nursery pots under freshwater irrigation could provide acceptable seedling establishment (above 80%) in mangroves at 30 d after sowing (DAS) under Kuwait's agro-climatic conditions. However, other treatments exhibited high rate of seedling mortality starting 20 DAS, and proved unfeasible for raising mangrove seedlings in Kuwait.

Keywords: Arid region, coastal rehabilitation, propagules, seed germination, salinity, mass propagation, ecological restoration.

Introduction

Mangroves are halophytic trees growing in the intertidal zone between the mean sea level (MSL) and the highest spring tide in the tropical and subtropical regions of the world. They are distributed between approximately 32^{o}N and 38^{o}S latitude (Alongi, 2009a; Lewis and Flynn, 2014). Their global distribution is believed to be limited by ocean currents, the 20^{o}C isotherm of seawater in winter, salinity of seawater, substrate charecteristics, and hydrology of intertidal mudflats (Alongi, 2009a). Establishing mangrove plantations in suitable intertidal areas is considered a sound approach to increase marine productivity and biodiversity in the Arabian Gulf. Planting projects to restore mangrove ecosystems have been conducted in most of the Arabian Gulf countries. Attempts to introduce mangroves to Kuwait were first made in the 1960's through cooperation with Food and Agriculture Organization, and again between 1978 and 1980, but were not successfull established (Firmin, 1968; Kogo and Tsuruda, 1996). Concerted efforts were made to introduce Avicennia marina in Kuwait (1992–1993, 1999–2000), Saudi Arabia (1980–1991), Oman (1983–88), United Arab Emirates (1983–88), and Qatar (1987–88) with some fully successful, while others partially or completely failed owing to several reasons (Kogo 1988; AbdelRazik, 1990; Abou El-Nil 1994: Saenger et al., 2002; Bhat et al., 2002b).

Geographically, Kuwait lies between 28º 30′ and 30º 05′ North and 48º 33′ and 48º 35′ East with a nearly 290 km long coastline, comprising an elleptical engulfment protruding westward from the Gulf waters, which facilitates highly sedimentary environment/mudflats congenial as mangrove habitat. Afforestation with mangroves in the intertidal flats would increase green cover, enrich marine fauna, and enhance the aesthetic and recreational value of the coastal areas. Mangrove habitats of the Arabian Gulf support a variety of important species of fish, shrimps, turtles, and birds, and significantly contribute to the coastal productivity (Al-Maslamani et al., 2013). Mangrove forests are among of the most productive and biologically vital marine ecosystems as they provide important goods and services to society and coastal and other marine ecosystems (Giri et al., 2011; Hutchison et al., 2014; Lee et al., 2002, 2014). From the environmental point of view, mangrove community and intertidal sediments, sequester approximately 22.8 million metric tons of carbon each year (Dittmar et al., 2006).

Success of mangrove rehabilitation in arid areas is limited by the low rate of growth, the limited number of suitable mangrove species, and the lack of sufficient numbers of ideal sites for mangrove rehabilitation (Bhat and Suleiman, 2014). *Avicennia marina* is the hardiest of all mangrove species and KISR's previous studies have clearly shown that *A. Marina* is the only species that can be grown successfully under the harsh arid environmental settings of Kuwait. Mangroves are propagated using difeerent techniques like natural recruits, direct sowing of fruits and transplantation of the mangrovelings (Rasool and Saifullah, 2002). Rearing of *A. Marina* seedlings in the nursery for some time before planting improved the survival chances than sowing the propagules directly in the field (Ravishankar and Ramasubramanian, 2004). The nursery-raised seedlings normally have a well-established root system before being transplanted in the field. Therefore, nurseries are considered as an essential component of large-scale mangrove introduction or restoration program as they prevent the risk of displacement or washing-off of propagules by receding tidal water, non-germination of propagules in the field or predation by crabs or other predators (Untawale, 1993; Bovel, 2011). In places like Kuwait, where the maturation and natural availability of seeds and propagules (August – September) in the region do not coincide with the regular planting season (October), there is no choice but to use seedlings raised in the nursery.

Establishment of mangrove nurseries is also necessary in places like Kuwait where no large-scale natural mangrove plantations that can supply sufficient numbers of propagules exist. There are still major challenges that need to be addressed to improve the success of rehabilitation efforts for realizing full potential of mangrove ecosystems. The primary problem that remains to be solved include the unacceptably high seedling mortality in the seedling establishment stage. Overcoming this shortcoming requires further refinement in seeding methods to ensure desirable seedling establishment. Mangrove seedling establishment being a function of germination, growth and development of propagules, and the hardening process employed; this study was conducted to evaluate different seeding methods to assess the germination and seedling establishment of mangroves under Kuwait's agro-climatic conditions.

Materials and Methods

The study comprised six methods of sowing viz. direct seeding in marine mudflats with regulated inundation (T_1), direct seeding in marine mudflats with non-regulated inundation (T2), seeding in nursery-trays with seawater irrigation (T_3), seeding in nursery-trays with freshwater irrigation (T_4), seeding in nursery-pots with seawater irrigation (T_5), and seeding in nursery-pots with freshwater irrigation (T_6). Direct seeding with regulated inundation was carried out in the marine mudflat, protected with an earthen embankment to prevent strong water currents during high tides (Figure 1a). Undisturbed and relatively flat mudflats in Kuwaisat area was selected for direct seeding

a. Site with regulated inundation **b. Site with unregulated inundation**

Figure 1. Direct seeding of mangrove propagules for seedling establishment.

Figure 2. Mangrove seeding in nursery trays with seawater and freshwater irrigation.

a. Seeding with seawater irrigation **b. Seeding with fresh water irrigation**

Figure 3. Seeding of mangrove propagules in nursery pots.

with non-regulated inundation (Figure 1b). Seeding in nursery-trays with seawater irrigation (45 to 50 $dS.m^{-1}$) and freshwater irrigation (0. 5 to 0.9 $dS.m^{-1}$) was carried out in GI trays [2.25 m (L) x 1.0 m (B) x 0.10 m (D)], filled with native soil collected from the beach. The experimental site was adjacent to KISR's mangrove nursery (Figure 2). Polyethylene nursery-pots of 6 cm diameter, filled with the native beach soil, arranged in polyethylene ponds established in the beach and in GI trays in KISR premises were irrigated with seawater and fresh water respectively (Figure 3a and 3b).

Mature and healthy mangrove seeds (*Avicennia marina*, UAE ecotype) obtained from the Ministry of Environment and Climate Change, United Arab Emirates (UAE), were used in the study. Mangrove seeding procedures standardized by KISR (Bhat et al., 2007) were adopted to establish the treatment units. Each treatment unit comprised 100 seeds and each treatment was replicated five times. The propagules were irrigated on alternate days in order to maintain desirable water level in the rhizosphere. Germination and establishment of the propagules under different seeding methods were studied for 30 days from seeding, at 10 days interval to identify the best method for mangrove seedling establishment. Observations were recorded on seedling establishment and displacement and drying of propagules.

Results and Discussion

Among the six seeding methods evaluated, propagules seeded in nursery-trays under fresh water irrigation (T_4) and in nursery-pots under fresh water irrigation (T_6) exhibited significantly higher seedling establishment of mangroves than other methods under Kuwait's climatic conditions. All other seeding methods recorded around 75% mortality at 20 days after seeding (DAS) and 100% mortality at 30 DAS (Table 1).

Table 1. Average Number of Mangrove Seedlings Established under Different Seeding Methods

Treatments	Direct Seeding		Seeding in Trays		Seeding in Pots	
	Regulated Inundation T1	Non-regulated Inundation T2	Seawater Irrigation T3	Fresh Water Irrigation T4	Seawater Irrigation T5	Fresh Water Irrigation T6
10 DAS[x]	40.2d	51.2c	60.2b	84.4a	62.4b	89.8a
20 DAS	17.4c	18.0c	25.8b	83.6a	24.0b	85.6a
30 DAS	0.0	0.0	0.0	80.8a	0.0	82.4a
Significance	**	**	**	**	**	**

Identical letters within a row indicate differences that are not significant.
[x] Days After Seeding, NS: Non-significant;**: Significant at P≤0.01; *: Significant at P≤0.05

It is evident from the study that decline of the propagules were more pronounced (37% to 60%) under seawater irrigation, irrespective of the seeding location/medium, at 10 DAS; against a low 15% under fresh water irrigation. This may mostly be attributed to the displacement of propagules during strong water currents, and drying of the propagules due to high rate of evapotranspiration and subsequent buildup of hyper salinization under seawater irrigation (Figure 4 and 5).

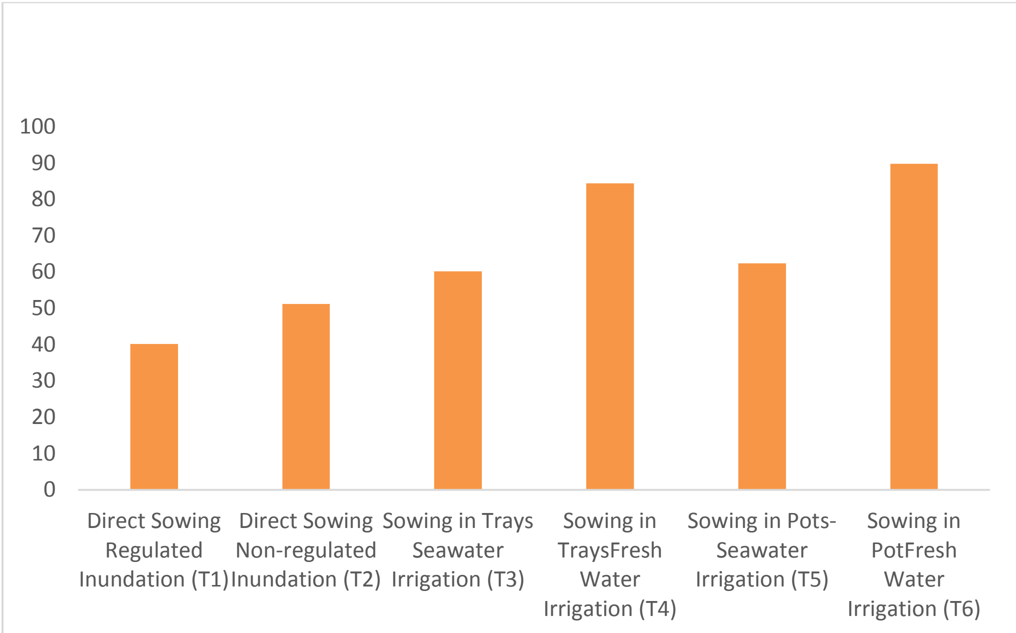


Figure 4. Mangrove seedlings established under different seeding methods at 10 days after seeding.

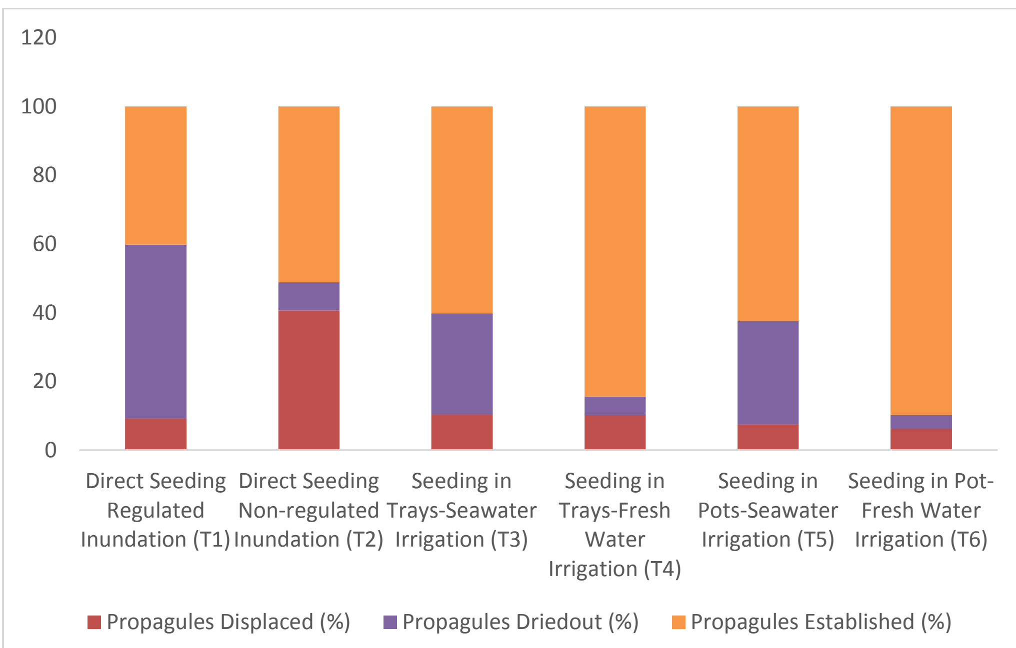


Figure 5. Proportion of Mangrove seedlings established, displaced, and dried-out under different seeding methods at 10 days after seeding.

Among the direct seeding methods evaluated, seeding under non-regulated inundation exhibited better establishment than seeding under regulated inundation at 10 DAS. However, seedlings under both the treatments declined completely at 30DAS. Strong water currents during the high tides dislocating the germinated propagules and prohibiting their anchoring (Figure 4a), and resultant deterioration and decay (Figure 4b) could be the main reasons for the decline of near established seedlings in the direct sown site with non-regulated inundation.

a.

Displaced propagule **b. Declining propagule**

Figure 4. Displacement of germinated propagule due to strong water currents and subsequent deterioration in non-regulated inundation seeding method.

Less frequent inundation coupled with high solar irradiation and rapid evaporation/evapotranspiration during the summer months in the regulated inundation treatment resulting in hyper-salinization (Figure 5a) and salt encrustation (Figure 5b) may be other important

a. Hyper-salinized site **b. Salt encrusted propagule**

Figure 5. Hyper-salinization and salt encrustation in directly seeded regulated inundation seeding method.

factors, which attributed to the decline of directly seeded propagules in the regulated inundation treatment.

The propagules seeded in the GI nursery trays and nursery pots under seawater irrigation (T3 and T5 respectively), despite the germination and initial establishment, recorded 100% decline towards the end of the study at 30DAS (Figure 6). This total decline may be attributed to the salinity build-up in the standing water in the holding trays (55 to 75 $dS.m^{-1}$), the then prevalent high ambient temperature and evapotranspiration.

Figure 6. Total decline of mangrove seedlings in nursery pots under seawater irrigation.

However, mangrove propagules seeded in nursery-trays and nursery-pots under fresh water irrigation (T_4 and T_6, respectively) exhibited above 80% survival at 30 DAS (Figure 7).

Conclusions

The overall goal of this study was to develop seeding methods to reduce seedling mortality in mangroves to an acceptable level (around 20%) during the nursery establishment stage. Seeding the propagules in trays or nursery pots with fresh water irrigation proved to be the best seeding method to obtain an acceptable stand (around 80%) of *Avecennia marina* UAE ecotype in the nursery under Kuwait conditions. However, the seedlings established under fresh water irrigation need to be sufficiently acclimatized with seawater irrigation, before planting in marine mudflats. High rate of evapotranspiration and subsequent buildup of hyper-salinization in the nursery trays/ponds being the possible reason for the sharp decline of mangrove seedlings raised under seawater irrigation; flow through nursery system coupled with adequate shading to manage the salinity build-up and reduce heat load needs to be evaluated to achieve better seedling establishment of mangroves in Kuwait.

Figure 7. Established Mangrove seedling in nursery pots under seawater irrigation.

Acknowledgements

The authors would like to thank the leadership of KISR and the Kuwait Foundation for the Advancement of Science (KFAS) for the financial support and encouragement, and the Ministry of Environment and Climate Change, United Arab Emirates (UAE), for supplying propagules for the execution of the project.

References

AbdelRazik, M. 1990. Towards a prospective transplantation of the local mangrove, Avicennia marina (Frossk.) Vierh. Growing in coast of Qatar. Qatar University Bul. 10: 199-211.

Abou El-Nil, M. 1994. Mangrove introduction along Kuwait's seashore. Proceedings of the Workshop on the Use of Seawater in Irrigation. Kuwait Institute for Scientific Research, Kuwait, October 1994, pp. 68-101.

Al-Maslamani, I.; M. Walton; H. Kennedy; M. Al-Mohannadi; and L. Le Vay. 2013. Are mangroves in arid environments isolated systems? Life-history evidence of dietary contribution from inwelling in a mangrove-resident shrimp species. *Estuarine, Coastal and Shelf Science* **124**: 56-63.

Alongi D. M. 2009a. The Energetics of Mangrove Forests. Dordrecht, The Netherlands: Springer. pp 213.

Bhat, N. R., and M. K. Suleiman. 2014. Coastal rehabilitation through sustainable management of mangrove community in Kuwait. Paper presented at Conference on Ecological and Ecosystem Restoration, July 27 – August 1, 2014, New Orleans, USA.

Bhat, N. R., H. Al-Menaie and S. A. Shahid. 2002b, Establishment of mangrove plantations for protection and enrichment of Kuwait coastlines. Proceedings of the International Symposium on Mangroves and Salt Marsh Ecosystems. Environmental Research and Wildlife Development Agency, Abu Dhabi, UAE.

Bhat, N. R.; A. Al-Nasser; M. K. Suleiman; and L. Al-Mulla, 2007. Growing Mangroves for Enrichment of Kuwait's Coastline (Guidelines and Recommendations), 2nd ed. Kuwait: Kuwait Institute for Scientific Research.

Bovel, O. 2011. Guyana Nursery Manual. Guyana Mangrove Restoration Project.

Dittmar, T; N. Hertkorn; G. Kattner; and R. J. Lara. 2006. Mangroves, a major source of dissolved organic carbon to the oceans. Global Biogeochemical Cycles 20: 1012-1026.

Firmin, R.1968. Forestry trials with highly saline or seawater in Kuwait. In Saline Irrigation for Agriculture and Forestry. Editted by H. Boyko and Dr. W. Junk, Hague, The Netherlands.

Giri, E.; Ochieng; L. L. Tieszen; , Z. Zhu; A. Singh; T. Loveland; J. Masek; and N. Duke. 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecology and Biogeography. 20: 154-159.

Hutchison, J., M. Spalding and P. zu Ermgassen. 2014. The role of mangrove in fisheries enhancement. The Nature Conservancy and Wetlands International, 54 pages.

Kogo, M. 1988. Studies on mangrove plantation in Arabia and geobotanical research of mangroves in the Middle East (1977-88). In Research Related to the UNESCO's Man and Biosphere Program in Japan, 1988-1989.

Kogo, M. and K. Tsuruda. 1996. Species selection for mangrove planting in Restoration of Mangrove Ecosystems. Field. (ed.). The International tropical Timber Organization Yokohama, Japan, and The International Society for Mangrove Ecosystems, Okinawa, Japan pp.197-208.

Lee, S.Y., and P. W. Kwok. 2002. The importance of mangrove species association to the population biology of the sesarmine crabs Parasesarma affinis and Perisesarma bidens. Wetlands Ecology and Management, 10: 215-226.

Lee, S.Y.; J. H. Primavera; F. Dahdouh-Guebas; K. McKee, K.; J. O. Bosire; S. Cannicci; K. Diele; F. Fromard; N. Koedam; C. Marchand; I. Mendelssohn; N. Mukherjee; and S. Record. 2014. Ecological role and services of tropical mangrove ecosystems: A reassessment. Global Ecology and Biogeography. 23: 726 -743.

Lewis R. R. III and L. L. Flynn. 2014a. Mangrove Zone Ecology. Oxford Biographies. The Oxford University Press, London.

Rasool, F. and S. M. Saifullah. 2002. Establishment of the field nursery for cultivation of mangroves at Miani Hor, Pakistan. In Prospects for Saline Agriculture. Ahmed, R. and K. A Malik (Eds.). Netherlands: Kluwer Academic Publishers, pp. 439-446.

Ravishankar, T., and R. Ramasubramanian. 2004. Manual on mangrove nursery techniques. M.S. Swaminathan Research Foundation; Chennai, India, pp 48.

Saenger, P.; F. Blasco; A. M. M. Yousseff; R. A. Loughland; and S. Wrydani. 2002. 'The mangrove vegetation of the United Arab Emirates, with particular emphasis on those of the Abu-Dhabi Emirate'. In: Proceedings of the 2nd International Symposium and Workshop on Arid Zone Environments: research and management options for mangrove and saltmarsh ecosystems. Javed, S. and A. G. de Souza (Eds.). ERWDA, Abu Dhabi, pp. 36-49.

Untawale, A.G. 1993. Development of an intertidal mangrove nursery and afforestation techniques along the Indian Coast. In Towards the Rational Use of High Salinity Plants. Lieth, H. and A. Al Masoom (Eds.)., London: Kluwer Academic Publications: 371-378.

High Value By-products Obtained During Native Seed Collection and Processing

S. Al-Melhem, C. Sudhersan, S. Jibi and L. Al- Sabah
Kuwait Institute for Scientific Research, Kuwait.
E-mail: smilhm@kisr.edu.kw; schellan@kisr.edu.kw

Abstract

Native plant seed collection is getting popularized for the environmental rehabilitation and desert greenerydevelopment program worldwide. While extracting the seeds form fruits of certain native perennial shrubs, we have obtained fruit juice and fibers as by products. These by products could be used as raw materials for high value industrial products and their recovery may be economically attractive. In this study, we have collected fruit juice from *Lycium shawii*, *Nitraria retusa* and fiber from *Calotropis procera* while extracting the seeds from the fruits as by products. These native plant fruit juice are the sources of sugars, minerals, vitamins, flavonoids and phenolic that have a wide range of applications as antioxidants, antitumoral, antiviral, antifungal, and antibacterial and cardio protective activities. The fibers collected from the *Calotopis procera* pods could be used as stuffing material, in textiles and in thermal insulations. Further research on converting these native plant by products obtained during the fruit processing for the seed extraction in to value added products could be beneficial and bring more economic returns to the native seed producers. Conversion of these native seed by products in to high value industrial products will attract more people in the field of native seed collection which will support and speed up the desert rehabilitation activities.

Keywords: By-products, native seed, phytochemicals, fibers, value added products.

Introduction

Kuwait is a small arid country located at the Northern corner of Arabian Gulf having 18,000 km^2. The flora of Kuwait comprises a total of 256 native plant species. Native vegetation of Kuwait is damaged due to several factors such as over grassing by sheep and camels, failure on natural plant regeneration through seeds due to harsh climatic conditions and many other anthropological activities. Desert re-vegetation is supported and encouraged by the Government of Kuwait. Human intervention on vegetation development in the desert is unavoidable nowadays. Desert re-vegetation activity in Kuwait requires large quantities of native seeds. There are two options to obtain the seeds: either buy it from the local market or collect the seeds from the wild populations available in the desert. Native seeds or native plant seedlings are not available in large quantities for the re-vegetation purpose in the local market and seed collection from the desert is very difficult and expensive. Many government and private organizations are involved in native seed production, collection and storage for desert re-vegetation and conservation purpose. Recently, in the KISR plant tissue culture laboratory several perennial native shrubs were propagated (Sudhersan et al., 2003) and grown under protected environment for seed production.

Native perennial shrubs such as *Lyciumshawii*, *Nitrariaretusa*, *Ocradenusbaccatus* and *Calotorpisprocera* were mainly concentrated on seed production in our laboratory. All these plants were initially multiplied by tissue culture method and maintained in the field for seed production. While extracting the seeds form fruits of these native perennial shrubs, we have obtained fruit juice and fibers as by products. These by products could be used as raw materials for high value pharmaceutical and industrial products, and their recovery may be economically attractive. The native plant fruit juice are the sources of sugars, minerals, vitamins, flavonoids and phenolic that have a wide range of applications as antioxidants, antitumoral, antiviral, antifungal, and antibacterial and cardio protective activities. The fibers collected from the *Calotopisprocera* pods could be used as stuffing material, in textiles and in thermal insulations. The details of the study is presented in this paper.

Materials and Methods

Calotropis procera, Licium shawii, and Nitraria retusa growing inside the KISR campus Shuwaikh and KISR station for research and innovation (KSRI) at Kabd were used as plant material.

Lycium shawii. *Lycium shawii* Roem. is an erect, spreading, intricately branched, very spiny perennial shrub belongs to the botanical family Solanaceae. It is called in Arabic as Awsaj. It grows up to 3 m height, exceptionally becoming a small tree up to 4 m. It is a desert plant grown as a hedge for protection to other plants. It provide food and shelter to many birds and animals. Leaves are thin, elliptical to ovoid with smooth edge, tapering at base with a short stalk. During summer months they shed the leaves completely and sprout immediately after the summer. The flowers are solitary, small, tubular or trumpet shaped and corolla white to pinkish in color. Flowering and fruiting occurs from September - June. Fruits are red color, pea size berries, having many seeds inside. the leaves and fruits are used as medicine. Fruits contain antioxidant, antimicrobial and antifungal properties (Dabech et al., 2013). Ripped berries are edible and used in traditional medicine.

Nitraria retusa. *Nitrariaretusa* (Forssk.) Asch. is a native perennial halophyte species that belong to the botanical family Nitrariaceae. It is distributed in Algeria, Tunicia, Saudi Arabia and Kuwait. In Arabic it is called as Ghardag. It is a drought and salt tolerant species which grows along shallow and hummocks on saline grounds near the coastal areas. It has tiny, white to green, fragrant flowers It produces fleshy red fruits during summer (Boulos, 2009). Fruits of *Nitraria* are one seeded fleshy drupes having hemispherical shape and attractive red color. The fruits are sweet, edible and highly nutritious.

Calotropis procera. *Calotropis procera* (Ait.) R. Br. locally known as Sodom apple belongs to the botanical family Asclepiadaceae is an evergreen, erect shrub with a woody stem. In Arabic it is called as Ushar. It commonly grows on waste lands and grows abundantly in the semi-arid and arid zones. It has simple stems with only a few branches, which are light grey-green in color and covered in a fissured, corky bark. The fairly large, grey-green leaves grow in opposite pairs along the stems and are smooth, with a pointed tip and heart-shaped base. The large, waxy, white flowers have deep purple spots or blotches at the base of each of the five petals, and are grouped in umbels. It produces a simple, fleshy fruit in a grey-green inflated pod, containing numerous flat, brown seeds with tufts of long, white silky hair (floss) at one end. It exudes a milky white sap (latex) when the plant is cut or broken, which although toxic is widely used in many traditional medicines.

The fruits of the above mentioned native shrubs were harvested during the month of June and brought to the laboratory for seed extraction. *Lycium* and *Nitraria* fruits were washed in water, air dried at room temperature and kept inside a plastic fruit box, and maintained in the refrigerator until the beginning of the seed extraction process. *Calotropis* fruits were collected before the cracking of the capsule while the seeds and floss are intact.

After collecting all the fruits of *Lycium* and *Nitraria*, the cleaned fruits were hand crushed in a clean glass vessel. After crushing, the liquid mass containing seeds and fruit skin were filtered in a clean glass jar using sterile cheesecloth. After separating the fruit juice, the seeds and fruit skin debris were again transferred in a jar containing water and stirred until the seeds settle down inside the container. The skin debris floated on the surface were removed and the seeds were washed four times with fresh water. After washing the seeds were oven dried at 35˚ C for 24 h and crushed the dry seed crumb using hand for separating individual seeds and transferred to sterile storage boxes and stored in the refrigerator. The fruit juice was bottled and refrigerated.

The *Calotropis* fruits were opened using a surgical blade and the entire seeds were removed without separating the floss. The floss were kept inside a container and kept for air drying at room temperature for 24 h and the dried floss were stored inside a closed container until used. The seeds were extracted from the floss and the collected seeds were air dried at room temperature or oven dried at 30˚ C for 24 h and stored for seedling production.

Results and Discussion

The fruit harvest from *Lysium* and *Nitraria* bushes was very difficult due to the presence of thorns and much easier from *Calotropis*. The fruits of *Nitraria* were one seeded while the *Lycium* fruits were with multiple seeds. Average weight of *Nitraria* fruit was 1.3 gram and each bush produced about 2.5 Kg fruits. The unripened mature fruits were separated before the seed extraction and kept 3 days at the room temperature for ripening. After crushing the seeds and skin were floated on the fruit juice. The juice was separated using filtration through cheesecloth and one Kg of fruits yielded about 450 ml of fruit juice. The seed and skin were mixed in fresh water and the seeds were collected and dried. Each seed weight was 0.5 g and after drying the weight was about 0.25 g. *Lycium* fruits were little smaller than the *Nitraria* fruits. One Kg of *Lycium* fruits contained about 2000 fruits. Each bush produced about 1.5 Kg fruits. One Kg of fruits yielded about 300 ml of fruit juice and 250 grams of seeds. The seeds were very small like egg fruits. After filtering the fruit Juice, the fruit skin and seeds mix were transferred to water for 5 min to separate the seeds from the skin. The seeds settled down and the skin with water were separated from the seeds. About 250 g seeds were obtained from one Kg fruits and after drying the seed weight was 200 g. The fruit juice was the by-product of *Lycium* and *Nitraria* seed extraction process (Figure 1). *Nitraria* fruit juice is highly nutritious having key nutrients such as carbohydrates, macro and micro minerals, and vitamins. It is also a natural source of antioxidants (Hegazy et al., 2013). Many wildlife forms feed on the fruits and leaves of this plant. The fruit juice has antioxidants, antimutagenic and antimicrobial activities (Boubaker et al., 2010, 2012; Mariem et al., 2014). It is used for the treatment of hypertension (Salem et al., 2011) and is used in traditional medicine by local inhabitants for having hypoglycemic effects. *Lycium* fruit juice is also nutritious and used in traditional medicines for curing several ailments. Published research report on Lycium confirmed the antimicrobial, Hepatoprotective and antioxidant nature of fruit extract (Dabech et al., 2013; Gaweesh et al., 2015).

The *Calotropis* fruit collection for seed extraction was easier than the other two native shrub species studied. The fruits of this plant are called as sodom apple even though it is not a fleshy fruit. Actually it is a capsule which split open during maturity to disperse the seeds. each seed has a silky white floss which carry the seeds for away from the mother plant. Each fruit weighed about 15 g including the seeds and floss. Total number of seeds in each capsule was up to 300 and each seed weighed about 0.03 g. The mature fruits need to be harvested before cracking of the capsule in order to collect the seeds from the floss easily. Each fruit had 3 g floss and each floss weighed about 0.01 g. One fully grown plant produced 100 fruits, 30,000 seeds and 300 g floss. This plant has many industrial applications (Varshney and Bhoi, 1987) and recently an protease enzyme named Procerain B isolated (Singh et al., 2011) that could be used for the cheese industry. The floss of the this plant is the by-product of seed extraction. The floss is used in insulators, textiles and stuffing. The milky latex of this plant is used in traditional medicine.

Figure 1. Native fruits of Kuwait. Fig. 1. *Lyciumshawii* fruiting; Fig.2. *L. shawii* harvested fruits; Fig.3. *L. shawii*cleaned fruit for seed extraction; Fig. 4. *Nitrariaretusa* harvested fruits; Fig. 5. *N. retusa* cleaned fruits for seed extraction; Fig. 6. *N. retusa* fruit juice; Fig. 7. *Calotropisprocera* mature fruit; Fig. 8. *C. procera*seed with floss; Fig.9. *C. procera* floss separated from the seeds.

From this research study, we conclude that economically important by-products could be obtained from native plants during the seed extraction. Conversion of these by-products obtained during the fruit processing for the seed extraction in to value added industrial products could be beneficial for the society and bring more economic returns to the native seed producers. In addition conversion of these native seed by products in to high value industrial products will attract more people in the field of native seed collection which will support and speed up the desert rehabilitation activities.

Acknowledgement

The authors thank KFAS for financial support and KISR management for support and encouragement.

References

Boulos L (2009). Flora of Egypt. Four volumes. Cairo: Al-Hadara Publishing, Egypt.

Boubaker, J., I. Skandrani and I. Bouhlel. 2010. Mutagenic, antimutagenic and antioxidant potency of leaf extracts from Nitrariaretusa. Food and Chemical Toxicology, 48: 2283–2290.

Boubaker, J., M. Ben Sghaier, S. Ines, K. Ghedira, and L. Chekir-Ghedira. 2012. Isorhamnetin 3-O-robinobioside from Nitrariaretusa leaves enhance antioxidant and antigenotoxic activity in human chronic myelogenous leukemia cell line K562. BMC Complementary and Alternative Medicine, 12: 135.

Dahech, I., Farah, W., Trigui, M., Hssouna, A. B., Belghith, H., Belghith, K. S. and F. B. Abdallah. 2013. Antioxidant and antimicrobial activities of *Lycium shawii* fruits extract. International Journal of Biological Macromolecules, 60: 328-333.

Gaweesh, A., Sengab, A. E. N. B., El-Hefnawy, H. M., Osman, S. M. and A. M. Abdou. 2015. Phytoconstituents, Cytotoxic, Antioxidant and Hepatoprotective Activities of the Aerial Parts of Lycium shawii R. Growing in Egypt. Med. Aromat. Plants, 4: 180.

Hegazy, A. K., Al-Rowaily, S. L., Faisal1, M., Alatar1, A. A., El-Bana, M. I. and A. M. Assaeed. 2013. Nutritive value and antioxidant activity of some edible wild fruits in the Middle East. Journal of Medicinal Plant Research, 7:938-946.

Mariem, C.,Sameh,M.,Nadhem, S., Soumaya, Z., Najiba, Z. and E. G. Raoudha. 2014. Antioxidant and antimicrobial properties of the extracts from *Nitrariaretusa* fruits and their applications to meat product preservation.Ind. Crops Prod., 55: 295–303.

Salem, J. H., Chevalot, A., Harscoat-Schiavo, C., Paris, C. and C. Humeau. 2011. Biological activities of flavonoids from Nitrariaretusa (Forssk.) Asch. and their acylated derivatives. Food Chem. 124:486-494.

Singh, A. N. and V. K. Dubey. 2011. Exploring Applications of Procerain B, a Novel Protease from *Calotropis procera*, and Characterization by N-Terminal Sequencing as well as Peptide Mass Fingerprinting. Applied Biochemistry and Biotechnology, 164: 573-580.

Varshney, A. C. and K. L. Bhoi. 1987. Some possible industrial properties of Calotropis procera (Aak) floss fibre. Biological wastes, 22: 157-161.

Effect of Storage on Germination of Kuwait's Native Seeds

Omar Al-Saeed, Amwaj Al-Roomi, Naryana Bhat and Arvind Baht
Kuwait Institute for Scientific Research, Kuwait

e-mail:osaeed@kisr.edu.kw

Abstract

Information regarding the hardiness of seeds produced Kuwait's native plants is scarce and therefore must be addressed in order to increase knowledge on the adaptability of such species. Germination method was basic using petri dishes and 90mm filter paper. Germination time period for all seeds was one month. Seeds stored for approximately 10 years under toom temperature from a total of 10 plant species were grown under 12 hour photo period under temperature conditions between 15/20°C and 100 seeds grown under 20/30°C. Significant successful germination under light conditions was achieved by two species Zygophyllum qatarense with 58% total average germination followed by Gypsophila capillaris with 57% total average germination, however all other species were below 40% germination success rate with the lowest being Brassica tenuifolia with 7% average germination. Under continuous dark conditions the highest germination rate was achieved by Gynandriris sisyrinchium with a total of 43% which was significantly higherthanseeds from the same species germinated under light conditions which averaged 27%. Only Gynandriris s. seeds germinated better under dark conditions, all other species seeds germinated significantly higher under light conditions. The results indicated that some native seeds are indeed hardy with respect to storage time periods, and that mostly, light conditions were more suitable for germination in almost all assessed species. The outcome of the experiment leads to more questions with regard to hardiness of niative seeds which will need to be addressed in the future, such as what temperatures are most suitable for seed storage as well as assessing a greater range of seeds from a more diverse collection of seeds from native species.

Key words: Hardiness; Native plants; Seeds, adaptability; Viability; Storage; Germination; Photoperiod; Temperature; Diversity

Introduction

Seed adaptability is crucial for the success of plants, most plant populations arise from successful seed estabilishment and germination. The biological mechanisms that enable plant species to withstand surrounding conditions are both physiological and structural, which include phenotypical, reproductive, inter and intra cellular processes (Bewley and Black, 1994). Different plant species varry in their capability to survive, under given environmental conditions and stresses. Dormancy, can be considered as the state of which viable seeds remain inactive under ideal conditions (Bewley and Black, 1994). Germination can be defined as, the period of events that occur due to the initial uptake of water by inactive seeds, until newly formed visible plant structures have emerged from the seed casing (Bewley and Black, 1994). It is crucial to understand seed germination and the componnents that effect seed germination in order to conserve and protect native plant communities. There is limited information on germination of kuwaits native seeds specifically and the effects of abiotic components such as light, temperature and time on native seed germination.

Dessert environments are characterized by poor nutrient and water availability, high temperatures, and more saline sandy soils with poor capablity to retain water (Omar *et al.*, 2007, Omar *et al*., 2008, Suleiman *et al.*, 2010). Kuwait consists mostly of a desert environment with low annual rain fall and high temperatures during the summers up to 50° C (Al helal *et al.*, 1989). Although seeds contain enough nutrients for the embryo to grow and establish into an autotrophic organism, even under favourable conditions, plants may not be successful during early or later stages of development, due to seasonal patterns (Bewley and Black, 1994; Vleeshouwers *et al.*, 1995; Lange, 1996). For example in desert environments winters can provide, enough rain, and low enough temperatures for seeds to grow, however, if germinated at the end of the winter season, seeds may germinate and grow but will face unfavourable harsh summer conditions, which would lead to failure to develop or survive. Thus, dormancy of seeds is a necessary adaptaion for the success of plant communities. Therefore, this study was conducted to test the viability of native seeds, after a 10 year time period of dormancy, and the ability to germinate under controlled light and temperature lab environments.

Materials and Methods

The study was conducted in Kuwait using varying locations. Seeds of *echium rauwolfii, helianthemum lippii, plantago boissieri, vaccaria hispanica, Salvia spinosa, gypsophila capillaris, cyperus conglomeratus, zygophyllum qatarense, gynandriris sisyrinchium, Brassica tenuifolia,*were collected from Sulaibiya research station, Liyah research station, Julaia and different areas from Abdaly and Wafra. Seeds were collected from different plants of the same species to provide genetic disversity. Seeds were air dried and surrounding plant matter was removed by hand. The seeds were stored for approximately 10 years under room temperature 20-25°C in brown paper bags. Seeds were germinated during April and May 2017.

A total of 40 petri dishes were used with one 90mm Whatman filter paper within each petri dish for each species. 25 seeds were placed in each petri dish with a total of 8 petri dishes for each species totalling 200 seeds. After seeds were placed in the petri dishes, 5-10ml distilled water was added for each petri dish. All seeds were germinated in one growth chamber under 12 hour photo period under temperature conditions between 15/20°. To emulate a dark environment throughout the experiment, 4 replicate petri dishes for each species were covered with aluminium foil (2 layers) until no light was penetrating the petri dishes, and 4 replicate petri dishes for each species were not covered. Growth chamber lights were used. Germination period was one month. Seed counts of seeds germinated under light conditions were recorded daily, and germinated seeds were removed. Seeds within the covered petri dishes were removed and recorded only after one month had passed.

Data. Total average germination of each 4 replicates was calculated into the mean percent (mean germinated seeds/total seedsx100). Mean was calculated seperately for average light and average dark germination percentages for each species.

Results and Discussion

Abiotic factors such as light and temperature are crucial components associated with the germination of seeds in most plant species (Bewley, 1997). Surrounding temperature and water availability are the most vital components for successful germination of kuwaits native seeds (Koornneef et al., 2002). However as shown in Table 1 in some plant species light is also a contributing factor with regard to the successful germination of seeds.

According to the resutlts light greatly affected germination in *Zygophyllum qatarense,* 58% germinated under 12 hour photoperiod conditions and only 10 % in continuous dark conditions. *Gypsophila capillaris* also germinated more successfully under light conditions with 57% germination under light and 13% germination under dark conditions. Only Gynandriris sisyrinchium seeds favoured total dark environments with 43% germination under continuous dark conditions and 27% under 12 hour photoperiod. Vaccaria hispanica, Salvia spinosa echium rauwolfii plantago boissieri, Cyperus conglomeratus, Brassica tenuifolia and Helianthemum leppii all germinated more successfully under light conditions.   In most cases germination successes was more than double under light conditions. This shows that Kuwaits native seeds favour light conditions in order to germinate, which disagrees with the statement that continuous dark conditions are ideal for seed germination. Light has been shown previously to promote seed germination in model plants such as *Arabidopsis* (Penfield *et al.*, 2005) which agrees with the results of the conducted experiment.

Table 1. Germination count of selected species in dark and light environments

Plant Species	**Total light**	**Total dark**	**Average % (Dark)**	**Average % (Light)**	**Total Germinated**
Zygophyllm qatarense	58	10	10	58	68
Gypsophila capillaris.	57	13	13	57	70
Vaccaria hispanica	34	9	9	34	43
Salvia spinosa	32	12	12	32	44
Echium rauwolfii	28	7	7	28	35
Gynandriris sisyrinchium	27	43	43	27	70
Plantago boissieri	22	8	8	22	30
Cyperus conglomeratus	13	3	3	13	16
Brassica tenuifolia	7	3	3	7	10
Helianthemum lippii	11	6	6	11	17

Overall the most successfully germinated seeds were *Zygophyllum qatarense* (68%) and *Gypsophila capillaris* (70%) and *Gynandriris sysrinchium* (70%). All other recorded species germinated much lower overall in both dark and light condition. *Cyperus conglomeratus* (16%) and *Brassica tenuifolia* (10%) were recorded with the lowest total germination. The results showed that native plant seeds do indeed vary in their capability to remain dormant over long time periods, since different species varried greatly in their success to germinate (Table 1 and).

Conclusions

From the results it is evident that seeds indeed respond to surrounding light conditions. Seeds of *Gypsophila capillaris*, *Zygophyllum qatarense* and *Gynandriris sysrinchium* were shown to be more hardy in sustaining viability over longer periods of time, which shows variation in the adaptation of plant species seeds. Variation in the ability to germinate under both light and dark coniditions was also shown by the results of the experiment. Recommendations for further investigation of the dormancy and germination of native seeds would include, increasing the diversity of seeds from similar landscapes, testing the effects of water quantities on seed germination, a more detailed and accurate investigation on the effects of time on seed viability, and also the effects of temperature on native seeds.

References

Al-Helal, A. A., Al-Farraj, M. M., El-Desoki, R. A., & Al-Habashi, I. (1989). Germination response of Cassia senna L. seeds to sodium salts and temperature. *Journal of the University of Kuwait(science). Kuwait*, *16*(2), 281-288.

Bewley, J. D. (1997). Seed germination and dormancy. *The plant cell*, *9*(7), 1055.

Bewley, J.D., and Black, M. (1 994). Seeds: Physiology of Development and Germination. (New York: Plenum Press).

Koornneef, M., Bentsink, L., & Hilhorst, H. (2002). Seed dormancy and germination. *Current opinion in plant biology*, *5*(1), 33-36.

Omar, S. A. S. and N. R. Bhat. 2008. Alteration of the *Rhanterium epapposum* plant community in Kuwait and restoration measures. *International Journal of Environmental Studies* **65**: 139-155.

Omar, S. A. S.; Y. Al-Mutawa; and S. Zaman. 2007. *Vegetation of Kuwait*. Kuwait Institute for Scientific Research. P. 47-89.

Penfield, S., Josse, E. M., Kannangara, R., Gilday, A. D., Halliday, K. J., & Graham, I. A. (2005). Cold and light control seed germination through the bHLH transcription factor SPATULA. *Current Biology*, *15*(22), 1998-2006.

Smith, A. 2000. Root-induced solubilization of phosphate in the rhizosphere of lowland rice. *New Phytology* **129**: 325-336.

Suleiman, M. K.; N. R. Bhat; M. S. Abdal; S. Zaman; S. Jacob; and R. Thomas. 2010. Propagation studies in *Farsetia aegyptia* Turra. *International Journal of Biodiversity and Conservation* **2**: 30-32.

Vleeshouwers, L.M., Bouwmeester, H.J., and Karssen, C.M (1995). Redefining seed dormancy: An attempt to integrate physiology and ecology. J. Ecol. 83, 1031-1037.

A Comparative Study of Seed Germination in Some Perennial Native Species in Kuwait

L. Almulla
Environment and Life Sciences Research Center, Kuwait institute for Scientific Research, Kuwait
e-mail: lalmulla@kisr.edu.kw

Abstract

Kuwait's native plants are highly adapted to the local environmental conditions characterized by hot and dry winds, periodic droughts, mild winter temperatures including frost, frequent dust storms and high soil salinity. Under such harsh environmental conditions, these plants have evolved mechanisms to withstand drought, extreme temperature conditions, high salinity, pests, and that allowed them to be used in greenery projects not only in the desert but also in urban areas. Furthermore, there is a need to prevent the loss of Kuwait's desert flora by conserving them and utilizing them sustainably in restoration projects. The aim of this study was to test seed germination methods and enhancing growth of some selected native plants, such as *Anvillea garcinii* and *Convolvulus oxyphyllus*. The germination in seeds with dormancy or those that are difficult germinate under natural conditions, were found to be improved with certain pre-sowing treatments such as temperature, acid scarification, hormonal treatment, etc. The results of these studies will be discussed in this presentation to show how these findings can serve as guidelines for future efforts to propagate native plants for use in rehabilitation and landscape projects in the country.

Keywords: Rehabilitation, restoration, urban greenery, biodiversity conservation, arid climate.

Introduction

Biodiversity conservation has become one of the challenging priorities for many countries, including the state of Kuwait, to combat species extinction. Several native perennial plant species in Kuwait and Arabian Peninsula are threatened due to various environmental factors or endangered, or even difficult to propagate. Although the introduced ornamental plants are essential in providing diversity in urban and suburban landscape projects, a majority of them require excessive irrigation water and maintenance, to cope with the harsh summer weather conditions. Therefore, the use of a proper blend of exotic and native plant species could lead to the establishment of sustainable greenery in urban and suburban areas.

The arid ecosystems of the world can support plants that have minimum water requirements and are drought-and salt-tolerant, to be used in afforestation, landscaping, and gardening projects, as drought has always been a normal recurrent event in arid and semiarid lands (Le Houérou, 1996). Native plants in gardens can potentially aid in conservation by contributing to the genetic diversity and buffer small and otherwise isolated populations from extinction (Whelan et al., 2006). In addition to their contribution to the integrity of the environment, plants are invaluable sources of useful genes for the genetic improvement of crop plants (Abo El-Nil, 1997). Shrubs specifically are significant desert rangeland vegetation and have the potential to be utilized in the urban landscape (Abo El-Nil et al., 1993). The use of native plants in urban landscape/ greenery will also reduce fertilizer, pesticide, and insecticide use, thereby, minimizing threats to the local environment.

Due to the limited and rapidly declining natural water resources in Kuwait and the expensive desalinated water, the present practice of using water-thirsty exotic ornamental plants for greenery development is not practical and sustainable in the long-term. Since native plants are better adapted to the local environment, they can endure long spells of drought, withstand high soil salinity levels, and provide a more natural effect to landscape projects; their use in landscape projects will both conserve natural resources and produce sustainable greenery. While continuation of evaluation of additional native plants is essential to increase diversity of plant resources for greenery projects, there is an urgent need to mass multiply the proven native plants for their large-scale use. Hence, this study was aimed at standardization of effective mass multiplication techniques of C*onvolvolus oxyphyllus* and *Anvillea garcinii* using seeds.

Materials and Methods

Germination Study. The seeds of the two native plants *Convolvolus oxyphyllus* and *Anvillea garcinii* were selected for seed germination study. The seeds used for germination experiments were either collected from Kuwait desert or procured from KISR seed bank. Each treatment was replicated five times and 10 seeds were used for each replication. The seeds were incubated at 22°C, under three lights, 12-h light, at 40% humidity.

Anvillea garcinii also called as Arabian oxeye, is a woody perennial desert shrub. The yellow, disc-shaped flowers of the *Anvillea garcinii* grow singly on thick stalks producing the seeds. The seeds of *Anvillea garcinii* were exposed to dry heat (50^{o}C) for 10 or 20 d with or without treatment with 0, 250, 500 ppm GA_3. Control seeds were not subjected to any pretreatment.

Convolvulus oxyphyllus, is a perennial shrub which can grow up to 60- to 70-cm height. In addition to the dry heat treatments (as explained in *Anvillea garcinii*), another experiment which included scarification was also conducted. The seeds were scarified by subjecting them to either acid (immersing the seeds in concentrated sulphuric acid for 20, 30, 40, 50, 60, 70, 80, 90 and 100 min) or mechanical scarification prior to their incubation. After the acid scarification, the seeds were washed thoroughly in running water and sown on the filter paper in the Petri dish. The effects of mechanical scarification and acid scarification were studied and compared with untreated seeds (control).

Data Collection and Analysis. Observations on germination percentage, height, number of leaves, as well as root and shoot biomasses were recorded at the termination of the experiment.

The data on germination percentage in various treatments were arcsine-transformed before performing analysis of variance (ANOVA) (McDonald, 2009). The data on germination, rooting percentage, and biomass were analyzed using ANOVA procedure and Duncan's Multiple Range Test to ascertain the significant differences among treatments using IBM® SPSS® software of version 22 (Little and Hill, 1978). For germination studies, the data were analyzed by two-way ANOVA, with duration of exposure to dry heat and various concentration of GA_3 as the main factors. Non transformed data of germination and rooting percentage are presented in tables. For acid scarification treatments, one-way ANOVA was performed on arcsine transformed data using SPSS software.

Results and Discussions

Germination Study

Anvillea garcinii. The germination details are presented in Table 1. Pretreatment with heat for 20 d, followed by soaking in 500 GA_3, resulted in the highest germination percentage (82%) and number of vigorous seedlings compared to control (Table 1). It also produced taller seedlings with good shoot and root biomass.

Main effect: There was significant main effect due to heat on germination percentage [$F (2, 45) =10.484$, $p<0.001$], final height [$F (2, 27) =19.409$, $p<0.001$] and the number of leaves [$F (2, 27) =37.750$, $p<0.001$] . An increase in all of the aforementioned parameters was noticed when the heat duration was increased from 0 to 20 d.

There was significant main effect of GA_3 concentration on final plant height [$F (2, 27) =7.682$, $p<0.001$] and number of leaves [$F (2, 27) =4.750$, $p<0.001$]. There was significant increase in final height of the plant when the GA_3 concentration was increased from 0 to 500 ppm.

Interaction effect: There was significant interaction between duration of heat and GA_3 concentration on number of leaves [$F (4, 27) =7.250$, $p<0.001$].

Convolvulus oxyphyllus. Pretreatment with heat, GA_3, or their combination did not produce any desirable results in the germination of *Convolvulus oxyphyllus* (Data not included). However, acid scarification for 100 min and mechanical scarification were effective in enhancing the germination percentage (Table 2). There was significant difference in number of leaves and root biomass of plants raised from various pretreatments. Lower duration of acid exposure (20 min) produced plants with more number of leaves.

Table 1. Effect of Dry Heat (50^0C) and GA_3 on Germination of *Anvillea garcinii*

1a. Effect of Dry Heat (50^0C) and GA_3

Dry Heat	GA_3	Germination (%)	Final Height (cm)	No. of Leaves	Dry Weight Basis (g)	
					Root Biomass	Shoot Biomass
10	0	44	2.0	3.7b	0.00033	0.0009
10	250	44	2.0	3.0bc	0.00083	0.0009
10	500	34	2.1	2.7bc	0.00017	0.0004
20	0	64	2.2	5.3a	0.00080	0.0108
20	250	58	2.2	5.7a	0.00047	0.0032
20	500	82	2.5	6.0a	0.00193	0.0057
0	250	52	2.1	5.3a	0.00180	0.0041
0	500	34	2.1	3.0bc	0.00033	0.0031
0	0	40	2.0	2.3c	0.00027	0.0017
Significance Dry		**	**	**	NS	*
Significance GA_3		NS	**	**	NS	NS
Dry heat + GA_3		NS	NS	**	NS	NS

1b. Effect of Heat

Dry Heat	Germination Percentage	Final Height (cm)	No. of Leaves	Dry Weight Basis (g)	
				Root Biomass	Shoot Biomass
10	40 b	2.04 b	3.11 b	0.0004	0.0007 b
20	69 a	2.29 a	5.67 a	0.0011	0.0066 a
No heat	41 b	2.08 b	3.56 b	0.0008	0.0030 b
Significance	**	**	**	NS	*

1c. Effect of GA_3

GA3	Germination Percentage	Final Height (cm)	No. of Leaves	Dry Weight Basis (g)	
				Root Biomass	Shoot Biomass
250	51	2.09 b	4.67 a	0.0010	0.0027
500	50	2.23 a	3.89 b	0.0008	0.0031
0	49	2.09 b	3.78 b	0.0005	0.0045
Significance	NS	**	*	NS	NS

Table 2: Germination and the Root and Shoot Biomass *Convolvulus oxyphyllus* Seedlings under Different Treatments

Treatment	Germination (%)	No. of leaves	Final height (cm)	Dry Weight Basis (g)	
				Root Biomass	Shoot Biomass
Acid Treatment 20 min	36d	7.0 a	2.9	0.0001 b	0.0031
Acid Treatment 30 min	36d	5.7 b	3.0	0.0016 b	0.0031
Acid Treatment 40 min	34d	6.7 a	2.7	0.0041 a	0.0058
Acid Treatment 50 min	46d	4.0 c	2.7	0.0001 b	0.0004
Acid Treatment 60 min	66bc	4.0 c	2.3	0.0006 b	0.0033
Acid Treatment 70 min	68b	4.0 c	2.3	0.0002 b	0.0018
Acid Treatment 80 min	70b	4.0 c	2.1	0.0003 b	0.0060
Acid Treatment 90 min	72b	4.0 c	2.3	0.0001 b	0.0006

Acid Treatment 100 min	88a	4.0 c	2.6	0.0001 b	0.0040
Mechanical Scarification	98a	4.3 c	2.5	0.0001 b	0.0017
Control	0e				
Significance	**	**	NS	*	NS

Identical letters within a single column indicate differences that are not significant at $p \leq 0.05$; NS: Non-Significant ** = Significant at $P \leq 0.01$ * = Significant at $P \leq 0.05$

Conclusions

Germination of the seed is influenced by various external and internal factors. *S*eed must not be in a state of dormancy, and the environmental requirements for germination of that seed must be met before germination can occur (*Bell, 1999)***.** In *Anvillea garcinii*, exposure to dry heat for 20 d could enhance the germination percentage, final height, number of leaves eventually to produce superior quality seedlings. Dry heat treatment followed by 500 GA_3 could enhance the germination percentage to produce taller, vigorous seedlings. As yet, no previous research findings to compare the results of the germination studies on this species have been generated.

In *Convolvulus oxyphyllus***,** it is interesting to note that the germination percentage increased with increase in the duration of acid treatment up to 100 min. These results indicated the presence of physical dormancy in the form of hard seed coat in this species.

Acknowledgments

The authors extend acknowledgement and appreciation for Kuwait Institute for Scientific Research, for funding and supporting this project.

References

Abo El-Nil, M.; L. Al-Sabah; H. Al-Menaie; S. Bastaki; and S. Zaman. 1993. Mass propagation of stress tolerant desert plants by tissue culture. Phase l. Kuwait Institute for Scientific Research, Report No KISR4300, Kuwait, pp 83-86.

Abo El-Ni,l M. 1997. Tissue culture of native plants in the developing countries. III International Symposium on in Vitro Culture and Horticultural Breeding, *ISHS Acta Horticulturae*. **447**: 507-514

Bell D.T. 1999. The process of germination in Australian species. *Australian Journal of Botany* **47**: 475-517.

Le Houérou, H.N. 1996. Climate change, drought and desertification. *Journal of Arid Environments* **(34)** (2): 133 – 185.

Little, T. M. and F. J. Hill. 1978. *Agricultural Experimentation: Design and Analysis*. United States of America: John Wiley and Sons, Inc.

McDonald J.H. 2009. *Handbook of Biological Statistics* 2nd Ed., Baltimore, Maryland: Sparky House Publishing.

Whelan, R.J; D.G. Roberts; P.R. England; and D.J. Ayre. 2006. The potential for genetic contamination vs. augmentation by native plants on urban gardens. *Biological Conservation* **128(**4):493-500.

Understanding the Seed Germination and Adaptation Strategy of Selected Arabian Desert Plant and its Implications for Vegetation Restoration

Arvind Bhatt, Narayan R. Bhat, Majda K. Suleiman, Thomas M. Thomas and Siju Mathew
Kuwait Institute for Scientific Research, Kuwait
e-mail: abhatt@kisr.edu.kw

Abstract

Plant growth and establishment are naturally very slow processes especially under desert environment due to low water availability, high soil temperatures, high salinity levels and low nutrient availability. These factors restrict the plant growth and survival by controlling seed germination and seedling establishment. Therefore, ecosystem restoration and combating desertification under such hostile conditions can be a daunting task. However, the success of rehabilitation of damaged desert ecosystems can be greatly enhanced by using native species, which are well adapted to the local environmental conditions. Under desert condition, plants use various adaptative and germination strategy to cope with extreme environmental condition such as production of hetromorphic seeds from a single species which has been related with combination of divergent germination strategies (opportunistic vs cautious strategies). Similarly production of mucilaginous seeds has been reported to have multiple functions in seed maturation, dispersal and germination. Whereas, maintaining the aerial seed in desert condition have an adaptive significance by protecting them from biotic and abiotic factors. All these strategy have been related to their long term persistent under desert condition.

Introduction

Vegetation rehabilitation is one of the most efficient ways for ecosystems restoration and combating desertification (Li et al., 2004). However, extreme climatic condition such as high temperature, low unpredictable rainfall, high evaporation rates and higher soil salinity are the main factors that creates the challenging condition for the plants under desert condition (Abu Sukar et al., 2007; Khan and Weber, 2006). Therefore, using native plants, which are well adapted to their local environmental and edaphic condition could be one of the main approaches that can be used for desert restoration restoration/rehabilitation (Abella et al., 2012). The native plants play an important ecological as well as economical role in desert environments by stabilizing the sand dunes and preventing soil (Phondani et al., 2016). However, availability of seeds and planting material is one of the main constraints that affect their uses by restoration biologist. Besides this, complete information about the propagation is restricted to only few desert species especially in Gulf countries make it more difficult to used them for restoration purposes (Shahin and Salem, 2014; Bhatt et al., 2016a; Bhatt and Santo, 2017a).

Seed germination and seedling establishment are the most critical stage in plants life cycle because they plays an important role in determining the long-term persistence of populations and species and also provide important insight for conservation and management (Baskin and Baskin, 2014; Hoyle et al., 2015). Therefore, seeds should remain dormant when the environmental conditions are unfavorable for seedling establishment. Temperature, light and soil salinity are the main environmental factors which play an important role in regulating the seed germination of desert species (El- Keblawy et al., 2011; El- Keblawy and Bhatt, 2015; Bhatt and

García, 2016; Bhatt et al., 2016b; Bhatt et al., 2017). Therefore, success of plant species in desert environment is primarily dependent on optimal conditions for germination and recruitment by influencing the seedling survival (Evans and Etherington, 1990; Masuda and Washitani, 1992; El- Keblawy, 2004). Because conditions for seedling establishment may not be favorable for all species immediately after seeds dispersal or even during the subsequent growing season. Therefore, seed germination timing dictates a seedlings seasonal exposure and thus has strong fitness consequences (Simons and Johnston, 2000; Donohue, 2005). In Arabian Desert, the best season for seed germination and seedling establishment is in winter (October to march) because of low temperature and higher chances of rainfall during that time of the year. However, actual time for germination and seedling establishment for both annuals and perennials in desert condition might depend on the nature of season, which may vary from year to year (Watts et al., 2011).

In order to survive in desert, various desert species develop various germination strategies that are adaptations to extreme environments (Gutterman, 1993). Heteromorphic seeds from a single species growing in desert climate may be combination of divergent germination strategies (opportunistic vs cautious strategies) to maintain their long-term persistence under such climate (Venable, 1985; Gutterman, 2002). Seeds of many species produce mucilage (known as myxospermy), once they imbibe the water and have multiple functions in seed maturation, dispersal and germination (Gutterman and Shem-Tov, 1997; Huang et al., 2000; Thapliyal et al., 2008; Yang et al., 2010). Under desert condition, seeds are exposed to various genotoxic agents such as UV irradiation, high temperature and water deficit. Therefore, in order to maintain their genome integrity, they have evolved special DNA repair mechanisms by producing mucilaginous seeds as part of their long-term survival strategy (Huang et al., 2008). Maintaining the aerial seed in desert condition has been reported to have an adaptive significance in the unpredictable harsh desert environmental conditions (van Oudtshoorn and van Rooyen, 1999), which ensure the long occupancy of plants at favorable habitats (Ma et al., 2010). Besides this, seeds stored in aerial seed bank are protected from biotic (predation) and abiotic factors (heat) before their release from the mother plant (Gutterman and Ginott, 1994). For some desert plants, seed release is triggered by environmental cues (such as seasonal rain and wind) coinciding with the occurrence of favorable conditions for seed germination and seedling establishment (Gutterman and Ginott, 1994; Aguado et al., 2012).

Heteromorphism

Seed heteromorphism, defined as the production of different types of seeds that's vary in morph, size, shape or testa colour by a single species which appears in many species of different family such as Asteraceae, Brassicaceae, Chenopodiaceae and Poaceae (Imbert, 2002; Matilla et al., 2005). Production of hetromorphic seeds has been correlated with variation in dispersal, dormancy germination, longevity behavior, ability to persist in soil seed bank, and seedling growth strategies (Mandák and Pysek, 2005; Lu et al., 2010; Bhatt and Santo, 2017b). Therefore, production of heteromorphic diaspores is one of the most effective strategies for adaptation and increasing the reproductive success of desert plants, under unpredictable climatic conditions (Harper, 1977).

Previous studies demonstrated that both morphological (seed mass and colour) and physiological (germination and dormancy) differences exist in different coloured seed (Table 1). Therefore, productions of heromorphic seed have been related to the combination of different adaptive strategies for their successful survival in harsh desert environmental condition. For example, one morph could germinate quickly and produces a higher number of seedlings (high risk taking strategy) in early growing seasons. Whereas, other morph with low and slow germination make take a ‘low-risk’ strategy (Venable, 1985; Bhatt et al., 2017). Indeed, heavier seed has been found to show higher germination percentages, higher salinity tolerance and better germination recovery as compared to the small seeds in most of the previous studies. However, further studies are necessary to investigate seedling growth performance of the different morphs in real field conditions. Therefore, presently we are testing the impact of different colours (yellow and red) seeds of *Calligonum comosum* in germination and seedling survival under field condition.

Table 1. Effect of Different Parameter Studied in Selected Heteromorphic Species from Arabian Desert

Species	Seed weight	Effect of presence/ absence of dispersal unit	Illumination and temperature condition	Salinity tolerance	Germination recovery	Reference
Halopyrum mucronatum	+	-	+	+	-	Khan and Ungar (2001)
Salicornia europaea	+	-	+	+	-	Ungar (1979)
Salsola rubescens	+	+	+	+	+	El- Keblawy et al. 2013
Atriplex canescens	+	-	+	+	+	Bhatt and Santo 2016
Haloxylon salicornicum	+	+	+	+	+	Bhatt et al. 2016 b
Salsola vermiculata	+	+	+	+	+	Bhatt et al. 2017 c

Mucilage Production by Seeds

Production of mucilage in response to water imbibition by seeds (myxodiaspory) has been related to the adaptation of desert species because presence of mucilage has been reported to play role in seed dispersal, seeds protection from predation, facilitation of seed hydration, regulating the germination and early seedling growth by moisture retaining of seeds and increasing the seed-soil particle contact (Gutterman and Shem-Tov, 1996; van Oudtshoorn and van Rooyen, 1999; Kreitschitz et al., 2009: Yang et al., 2012). However, it inhibits germination in some species by acting as a physical barrier that controls water and oxygen diffusion to the inner seed tissues (Huang and Gutterman, 1999; Bhatt et al., 2016c). During dormancy in arid environments, mucilage can harvest sufficient dew for seed cells to repair DNA damage, and thus maintain genetic integrity for longer periods (Yang et al., 2011). However, specific role of seed mucilage varies with species and their environmental context (Western, 2012; Yang et al., 2012).

Presence of seed mucilage either enhanced or reduced the germination percentages which indicate that specific role of seed mucilage varies with species (Yang et al., 2012; Veiga-Barbosa and Pérez-García, 2014; Bhatt et al., 2016 c). It is believed that presence of mucilage in some species enhance the germination by facilitating the seed hydration by moisture retaining and also increasing the seed-soil particle contact (Yang et al., 2012; North et al., 2014) (Table 2). However, in some species, it inhibits germination by acting as a physical barrier that controls water and oxygen diffusion to the inner seed tissues (Huang and Gutterman, 1999; Bhatt et al., 2016c). However, little is known about the ecological role of seed mucilage in seedling emergence especially in Arabian desert species. Therefore, recently we initiated the study on *Farsetia aegyptia* and *Savignya parviflora* to understand the role of mucilage in seedling emergence. The findings will provide insights into the ecophysiological mechanisms by which mucilage regulates germination and seedling growth and contributes to our understanding of the ecological significance of mucilage in the success of plants in unpredictable desert environments.

Table 2. Role of Mucilage on Seed Germination in Selected Species from Arabian Desert

Species	**Presence of Mucilage on Germination**		**Reference**
	Enhanced	**reduce**	
Salvia aegyptiaca	-	-	Gorai et al. 2011
Plantago albicans	+	-	Veiga-Barbosa and Pérez-García 2014
Lepidium sativum	+	-	Sodaeizade et al. 2015
Lavandula subnuda	-	+	Bhatt et al. 2016 c
Lepidium aucheri	+	-	Bhatt et al. 2016 c
Boerhavia elegans	-	+	Bhatt et al. 2016 c
Plantago ciliate	-	+	Bhatt et al. 2016 c
Plantago amplexicaulis	+	-	Bhatt et al. 2016 c

Aerial Seed Bank

Aerial seed bank is a common phenomenon in many species which are distributed in unpredictable, harsh deserts of the arid and semi-arid regions (Kamenetsky and Gutterman, 1994; (van Oudtshoorn and van Rooyen, 1999). Aerial seed bank may have various benefits such as (i) maximize seed availability when conditions are favourable for germination and seedling establishment, (ii) ensure that seeds are available after a fire, even if none was produced the previous year, (ii) result in high seedling/juvenile densities, which could reduce the growth of competitors and thus increase chances of survival and (iv) indirectly cause seeds to fall on the optimum substrate (seed bed) for germination and seedlings. In desert plants, aerial seed bank plays important roles in the protection of seeds against granivores, anchorage against surface run-off, bet-hedging risks by spreading seed dispersal in time, and retaining seeds in a favorable microhabitat (Ellner and Shmida, 1981). Moreover, canopy seed storage ensures minimal seed death especially in desert where soil salinity is quite high and might cause the seed death if they stay on the ground. Therefore, the major role for aerial seed bank is to protect seeds from unfavorable conditions in the soil and release them when conditions are favorable for germination and seedling growth (Lamont et al., 1991). Recent studies have found that seeds released from aerial seed bank have little dormancy and germinate immediately after dispersal (Aguado et al., 2012). Consequently, aerial seed bank would play the same role as the soil seed bank; both protect seeds from unfavorable conditions and release them when optimal field conditions for seed germination and seedling emergence are met (Thanos, 2004). The environmental factors associated with seed in the soil seed bank differ from those in the aerial seed bank. Because, seeds in a soil seed bank are either buried at different depths or stored on the soil surface. Seeds stored on the soil surface are exposed to light and face diurnal fluctuations in the temperatures. Conversely, seeds buried in the soil are stored in darkness and face less diurnal temperature fluctuations. In the aerial seed bank, however, seeds are exposed to light and experience less fluctuation in diurnal temperatures.

These studies provides a new insight for understanding the functional significance and adaptive strategies of aerial seed banks storage in population maintenance of desert plants, showing as the phenomenon of serotiny and its effects are species-specific (Table 3). Therefore, results obtained from these studies could have a practical implications for conservation and restoration biologists for the propagation and cultivation of these species, as early collection of fresh mature seeds and their *ex situ* conservation in seed banks, facilitate constant supply of high quality seeds. However, further studies are required to test the germination of selected desert species those which have the ability to form aerial seed bank during different time duration in order to know how it affect the germination, dormancy, seed mortality and consequently the seedling survival. Which will provide further insight whether aerial seed bank is an adaptive strategy for germination and seedling survival of desert plant.

Table 3. Effect of Aerial Stored Seed on Germination of Selected Species from Arabian Desert

Species	Germination		Reference
	Stored at room temperature	Stored in aerial seed bank	
Halocnmum strobilaceum	High	Low	El-Keblawy et al. 2015
Halopeplis perfoliata	Low	High	El-Keblawy et al. 2015
Anastatica hierochunitica	High	High	Bhatt et al. 2016 d
Blepharis ciliaris	High	Low	Bhatt et al. 2016 d
Scrophularia deserti	High	Low	Bhatt et al. 2016 d

In conclusion, understanding the adaptation and germination strategy of desert plant is essential for using them in desert restoration. The details knowledge about plant species and their adaptation and germination strategy would be helpful in conservation, restoration and revegetation processes. Incorporating the mixture of species which produce heteromorphic seeds, mucilegenous seeds and species those have ability to form aerial seed bank would be essential for planning and restoration of long term restoration program.

References

Abella, S. R.; D. J. Craig; S. Smith; and A. C. Newton. 2012. Identifying native vegetation for reducing exotic species during the restoration of desert ecosystems. *Restoration Ecology* **20**: 781-787.

Abu Sukar, H. K.; F. H. Almerri; and A. A. Almurekki. 2007. Agro-hydro-meteorological data book for the State of Qatar. *Doha, Qatar*.

Aguado, M.; M. J. Vicente; J. Miralles; J. A. Franco; and J. J. Martínez-Sánchez. 2012. Aerial seed bank and dispersal traits in *Anthemis chrysantha* (Asteraceae), a critically endangered species. *Flora* **207**: 275-282.

Baskin, C. C.; and J. M Baskin. 2014. *Seeds: ecology, biogeography, and evolution of seed dormancy and germination*. Academic Press: San Diego, CA.

Bhatt, A.; and F. Pérez-García. 2016. Seed dormancy of *Ochradenus baccatus* (Resedaceae), a shrubby species from Arabian Desert regions. *Revista de Biología Tropical* **64**: 965-974.

Bhatt, A.; F. Pérez-García; M. Mercedes Carón; and D. Gallacher. 2016a. Germination response of *Salsola schweinfurthii* (Chenopodiaceae) to salinity and winged perianth removal. *Seed Science and Technology* **44**: 428-434.

Bhatt, A.; S. S. Phartyal; and A. Nicholas. 2016b. Ecological role of distinct fruit-wing perianth color in synchronization of seed germination in *Haloxylon salicornicum*. *Plant Species Biology* doi: 10.1111/1442-1984.12133.

Bhatt, A.; P. C. Phondani; S. S. Phartyal; A. Santo; and D. Gallacher. 2016d. Influence of aerial seed banks on germination response in three desert plant species. *Journal of Plant Ecology* rtw113.

Bhatt, A.; P. C. Phondani; S.S. Phartyal; and D. Gallacher. 2017. Role of perianth colour in germination and salinity tolerance of *Salsola vermiculata* in Arabian deserts. *Nordic Journal of Botany* doi: 10.1111/njb.01502.

Bhatt, A.; and A. Santo. 2016. Germination and recovery of heteromorphic seeds of *Atriplex canescens*. *Plant Ecology* 1-11.

Bhatt, A.; and A. Santo. 2017b. Does perianth colour affect the seed germination of two desert shrubs under different storage periods and conditions?. *Nordic Journal of Botany* doi: 10.1111/njb.01593.

Bhatt, A.; and A. Santo. 2017a. Effects of photoperiod, thermoperiod and salt stress on *Gymnocarpos decandrus* seeds: potential implications in restoration ecology activities. *Botany* doi: 10.1139/cjb-2017-0086.

Bhatt, A.; A. Santo; and D. Gallacher. 2016 c. Seed mucilage effect on water uptake and germination in five species from the hyper-arid Arabian desert. *Journal of Arid Environments* **128**: 73-79.

Donohue, K. 2005. Seeds and seasons: interpreting germination timing in the field. *Seed Science Research* **15**: 175-187.

El-Keblawy, A. 2004. Salinity effects on seed germination of the common desert range grass, *Panicum turgidum*. *Seed Science and Technology* **32**: 873-878.

El-Keblawy, A.; E. Al Neyadi; S. S. Rao; and A. H. Al-Marzouqi. 2011. Interactive effects of salinity, light and temperature on seed germination of sand dunes glycophyte *Cyprus conglomeratus* growing in the United Arab Emirates deserts. *Seed Science and Technology* **39**: 364-376.

El-Keblawy, A.; A. Bhatt; and S. Gairola. 2015. Storage on maternal plants affects light and temperature requirements during germination in two small seeded halophytes in the Arabian deserts. *Pakistan Journal of Botany* **47**: 1701-1708.

El-Keblawy, A.; and A. Bhatt. 2015. Aerial seed bank affects germination in two small-seeded halophytes in Arab Gulf desert. *Journal of Arid Environments* **117**: 10-17.

El-Keblawy, A.; A. Bhatt; and S. Gairola. 2013. Perianth colour affects germination behaviour in wind-pollinated *Salsola rubescens* in Arabian deserts. *Botany* **92**: 69-75.

Ellner, S.; and A. Shmida. 1981. Why are adaptations for long-range seed dispersal rare in desert plants. *Oecologia* **51**: 133-144.

Evans, C. E.; and J. R. Etherington. 1990. The effect of soil water potential on seed germination of some British plants. *New Phytologist* **115**: 539-548.

Gorai, M.; H. Gasmi; and M. Neffati. 2011. Factors influencing seed germination of medicinal plant *Salvia aegyptiaca* L.(Lamiaceae). *Saudi Journal of Biological Sciences* **18**: 255-260.

Gutterman, Y. 1993. *Seed Germination in Desert Plants*. Adaptations of Desert Organisms. Springer: Berlin.

Gutterman, Y. 2002. *Survival strategies of annual desert plants*. Springer: Berlin.

Gutterman,Y.; and S. Ginott. 1994. Long-term protected seed bank in dry inflorescences of *Asteriscus pygmaeus*; achene dispersal mechanism and germination. *Journal of Arid Environments* **26**: 149-163.

Gutterman, Y.; and S. Shem-Tov. 1996. Structure and function of the mucilaginous seed coats of *Plantago coronopus* inhabiting the Negev Desert of Israel. *Israel Journal of Plant Sciences* **44**: 125-133.

Gutterman, Y.; and S. Shem-Tov. 1997. The efficiency of the strategy of mucilaginous seeds of some common annuals of the Negev adhering to the soil crust to delay collection by ants. *Israel Journal of Plant Sciences* **45**: 317–327.

Harper, J. L. 1977. *Population biology of plants.* London, Academic Press

Hoyle, G. L.; K. J. Steadman; R. B. Good; E. J. McIntosh; L. M. Galea; and A. B. Nicotra. 2015. Seed germination strategies: an evolutionary trajectory independent of vegetative functional traits. *Front. Plant. Sci*. *6.* doi: 10.3389/fpls.2015.00731.

Huang, Z.; I. Boubriak; D. J. Osborne; M. Dong; and Y. Gutterman. 2008. Possible role of pectin-containing mucilage and dew in repairing embryo DNA of seeds adapted to desert conditions. *Annals of Botany* **101**: 277-283.

Huang, Z.; and Y. Gutterman. 1999. Water absorption by mucilaginous achenes of Artemisia monosperma: floating and germination as affected by salt concentrations. *Israel Journal of Plant Sciences* **47**: 27-34.

Huang, Z.; Y. Gutterman; and Z. Hu. 2000. Structure and function of mucilaginous achenes of *Artemisia monosperma* inhabiting the Negev Desert of Israel. *Israel Journal of Plant Sciences* **48**: 255–266.

Imbert, E. 2002. Ecological consequences and ontogeny of seed heteromorphism. *Perspectives in Plant Ecology, Evolution and Systematics* **5**: 13-36.

Kamenetsky, R.; and Y. Gutterman. 1994. Life cycles and delay of seed dispersal in some geophytes inhabiting the Negev Desert highlands of Israel. *Journal of Arid Environments* **27**: 337-345.

Khan, M. A.; and I. A. Ungar. 2001. Alleviation of salinity stress and the response to temperature in two seed morphs of *Halopyrum mucronatum* (Poaceae). *Australian Journal of Botany* **49**: 777-783.

Khan, M. A.; and D. J. Weber (Eds.) 2006. *Ecophysiology of high salinity tolerant plants* (Vol. 40). Springer Science & Business Media.

Kreitschitz, A.; Z. Tadele; and E. M. Gola. 2009. Slime cells on the surface of Eragrostis seeds maintain a level of moisture around the grain to enhance germination. *Seed Science Research* **19**: 27-35.

Lamont, B. B.; D. C. Le Maitre; R. M. Cowling; and N. J. Enright. 199). Canopy seed storage in woody plants. *The Botanical Review* **57**: 277-317.

Li, X. R.; H. L. Xiao; J. G. Zhang; and X. P. Wang. 2004. Long-Term Ecosystem Effects of Sand-Binding Vegetation in the Tengger Desert, Northern China. *Restoration Ecology* **12**: 376-390.

Lu, J.; D. Tan; J. M. Baskin; and C. C. Baskin. 2010) Fruit and seed heteromorphism in the cold desert annual ephemeral *Diptychocarpus strictus* (Brassicaceae) and possible adaptive significance. *Annals of Botany* **105**: 999-1014.

Mandák, B.; and P. Pysk. 2005. How does seed heteromorphism influence the life history stages of *Atriplex sagittata* (Chenopodiaceae)?. *Flora* **200**: 516-526.

Masuda, M.; and I. Washitani. 1992. Differentiation of spring emerging and autumn emerging ecotypes in *Galium spurium* L. var. echinospermon. *Oecologia* **89**: 42-46.

Matilla, A.; M. Gallardo; and M. I. Puga-Hermida. 2005. Structural, physiological and molecular aspects of heterogeneity in seeds: a review. *Seed Science Research* **15**: 63-76.

North, H. M.; A. Berger; S. Saez-Aguayo; and M. C. Ralet. 2014. Understanding polysaccharide production and properties using seed coat mutants: future perspectives for the exploitation of natural variants. *Annals of Botany* **114**: 1251-1263.

Phondani, P. C.; A. Bhatt; E. Elsarrag; Y. M. Alhorr; and A. El-Keblawy. 2016. Criteria and indicator approach of global sustainability assessment system for sustainable landscaping using native plants in Qatar. *Ecological Indicators* **69**: 381-389.

Shahin, S. M.; and M. A. M. Salem. 2014. The lifeline rescuers of tomorrow's landscaping in the United Arab Emirates (UAE): All the hope rests on the indigenous plants. Proceedings of the 2nd International Conference on Modern Trends in Science, Engineering & Technology (ICMTSET). Zelus International, Dubai.

Simons, A. M.; and M. O. Johnston. 2000. Variation in seed traits of *Lobelia inflata* (Campanulaceae): sources and fitness consequences. *American Journal of Botany* **87**: 124-132.

Sodaeizade, H.; A. A. Mosleh; and H. Tajamolian. 2015. The effects of mucilage on improving of drought tolerance of *Lepidium sativum* l. In seed germination and early seedling growth 1017-1027.

Thanos, C. A. 2004. Bradychory—the coining of a new term. In: Arianoutsou, M.; and V. P. Papanastasis (Eds.). Proceedings 10th MEDECOS conference, Rhodes, Greece. Millpress, Rotterdam, pp. 1–6.

Thapliyal, R. C.; S. S. Phartyal; J. M. Baskin; and C. C. Baskin. 2008. Role of mucilage in germination of *Dillenia indica* (Dilleniaceae) seeds. *Australian Journal of Botany* **56**: 583–589.

Ungar, I. A. 1979. Seed Dimorphism in *Salicornia europaea* L. *Botanical Gazette* **140**: 102-108.

van Oudtshoorn, K. V. R.; and M. W van Rooyen. 1999. Restriction of Dispersal Due to Reduction of Dispersal Structures. In Dispersal Biology of Desert Plants. Springer Berlin Heidelberg, pp. 93-119.

Veiga-Barbosa, L.; and F. Pérez-García. 2014. Germination of mucilaginous seeds of *Plantago albicans* (Plantaginaceae): effects of temperature, light, pre-sowing treatments, osmotic stress and salinity. *Australian Journal of Botany* **62**: 141-149.

Venable, D. L. 1985. The evolutionary ecology of seed heteromorphism. *The American Naturalist* **126**: 577-595.

Watt, M. S.; M. Bloomberg; and W. E. Finch-Savage. 2011. Development of a hydrothermal time model that accurately characterizes how thermo-inhibition regulates seed germination. *Plant, Cell and Environment* **34**:870-876.

Western, T. L. 2012. The sticky tale of seed coat mucilages: production, genetics, and role in seed germination and dispersal. *Seed Science Research* **22**: 1–25.

Yang, X.; J. M. Baskin; C. C. Baskin; and Z. Huang. 2012. More than just a coating: ecological importance, taxonomic occurrence and phylogenetic relationships of seed coat mucilage. *Perspectives in Plant Ecology, Evolution and Systematics* **14**: 434-442.

Yang, X.; M. Dong; and Z. Huang. 2010. Role of mucilage in the germination of *Artemisia sphaerocephala* (Asteraceae) achenes exposed to osmotic stress and salinity. *Plant Physiology and Biochemistry* **48**: 131-135.

Yang, X.; W. Zhang; M. Dong; I. Boubriak; and Z. Huang. 2011. The achene mucilage hydrated in desert dew assists seed cells in maintaining DNA integrity: adaptive strategy of desert plant *Artemisia sphaerocephala*. *PLoS One*, 6: e24346.

Effects of Using Infra-Red Reflective Covering Material on Greenhouse Environment under Kuwait's Environment for Plant Production

H. E. Al-Mansour, N. R. Bhat, B. M. Thomas and M. S. Albaho
Environment and Life Sciences Research Center
Kuwait institute for Scientific Research, Kuwait
e-mail: hman@kisr.edu.kw

Abstract

Agriculture sector is one of the most important sectors to attain food security in Kuwait. Due to the harsh growing environmental conditions during summer season, greenhouses are the only sustainable option for producing fresh crops in Kuwait. Achieving desired cooling efficiency inside the greenhouse is real challenge, especially during summer months, when the ambient temperature reaches 50^0C. The Infrared reflective materials used as covering in greenhouses to prevent the entry of infrared (IR) and near infrared (NIR) rays has been found to be effective in reducing the temperature inside the greenhouse thereby creating favorable microclimate for plant growth. The objective of this study was to evaluate the effect of these covering materials and compare them with the normal covering material on the greenhouse indoor temperature. Soilless cultivation of tomato (*Lycopersicon esculentum* cv. Fuego) and cucumber (*Cucumis sativus* cv. Basha) was used for evaluation. Results show that during winter months, IR levels were 65-75% lower in greenhouses that were covered with IR reflective materials than those in the control (normal covering) greenhouses. Consequently, the indoor temperatures were nearly 10^0C lower in IR greenhouses compared to that in normal greenhouses. This was reflected in the growth performance of tomato plants, where plants in normal greenhouses grew taller, contained more leaves and higher chlorophyll contents compared to those in the IR glasshouses. They also produced more number of fruits, bigger fruits and higher fruit yield than those in the IR glasshouses. Similar effects were observed in the case of cucumber also.

Keywords: Protected environment, greenhouse infrared material, crop production, greenhouse structure.

Introduction

Food security and the high demand on the crop production are increasing. The difficulty of open filed crop production in Kuwait harsh weather, the affect by unfavorable weather conditions, as well as the limitation of land and water resources available for agriculture are significant factors limited the availability of the needed local crops. Therefore, several countries across the world wild have shifted to use protected environment agriculture to reduce the effects of suboptimal growing conditions on plant growth and thus, have significantly developed and expanded their food production capacities, and significantly enhanced the sustainability of farming processes and production (Al-Nasser and Bhat, 1998; Bhat et al., 2002; Gupta and Chandra, 2002; Sethi and Sharma, 2007; Kumar et al., 2009; Abdel-Ghany et al., 2012; DePascale and Maggio, 2005; Gulrez et al., 2013).

The improvement and development of a sustainable, economically viable protected environment food production system is a priority area of research within Kuwait. Protected environment agriculture (PEA or PA), the most sustainable option under harsh climatic conditions, is under immense pressure to enhance productivity and the efficiency of water use. The two elements of protected environment agriculture namely are a suitable structure to create optimum growing environment and the efficient growing techniques. Whereas a significant improvement has been made to develop efficient growing techniques for large number of vegetables producing in the greenhouse, developments, improvements, and upgrade of greenhouse structure are straightaway needed to enhance the protected environment food production system both efficient and fully sustainable (Bhat et al., 2009a; Albaho et al., 2012).

Different material used as greenhouse coving material. Polyethylene films are widely used in Kuwait to cover the greenhouse structures. This covering material is highly inefficient in achieving the desired cooling, produces much less crop yield, and requires greater maintenance, and frequent replacement, especially under the harsh climate. Hence, the global greenhouse industry switched to glass several years ago. In fact, under Kuwait's climate with extremely high temperatures, greater proportion of UV radiation in the sunlight, dry winds and frequent sand storms, polyethylene films degrade rapidly, most of the time, within a year, thereby severely affecting the cooling efficiency. In view of these facts, KISR is promoting the use of more durable covering materials like polycarbonate and glass as a covering materials for sustainable protected environment (PE) food production and this has been well been received by leading local producers.

Because of extremely high ambient light intensity with substantially high near infrared (NIR) fraction, there is enormous heat-gain inside the greenhouse, especially during the summer months (Bhat et al., 2009b; Fig. 1). NIR is the region closest in wavelength to the radiation detectable by human eye (0.7 – 1.0 µm). Under such conditions, the widely used evaporative cooling system in Kuwait, despite consuming huge quantities of water (approximately 25 l/ m^2/ d, which is almost 10 times the normal requirement), is not able to create optimal indoor conditions for the year-round production of most crops (Bhat et al., 2009b) Thus, the main challenge in greenhouse cooling under Kuwait’s climatic conditions is to completely eliminate or at least substantially reduce the entry of heat-producing NIR radiation into these structures. The use of infrared (IR) reflective or absorbing covering materials has been shown to reduce the heat load inside the greenhouse by as much as 47%, which results in 5 to 10^0C lower indoor temperatures than conventional covering materials and significantly enhance crop productivity and product quality (Sonneveld and Bochov, 2013; Abdel-Ghany et al., 2012; Liu, 2013). The IR radiation, which is popularly known as heat radiations, is typically defined as values between 700 – 800 nm. The challenge is to block IR radiations, without affecting the photosynthetically active radiation (PAR), which designates the spectral band of solar radiation ranging from 400 to 700 nm.

This paper intended to evaluate the IR reflective covering materials, such as glass and polycarbonate as a covering material for greenhouses under Kuwait’s environment. Six greenhouses were involved in this study, three with IR reflective glass and three with normal glass. Green house diminutions are shown in figure 2. The IR reflective material used in this project is the Li-F co-doped FTO prototype (LFTO) developed by ITRI. This material was produced by depositing high conductivity and transparent tin oxide films on the glass at temperatures ranging between 300^oC and 500^oC using the temperature controlled ultrasonic spray pyrolysis techniques. The characteristics of LFTO at 380^oC are with a sheet resistance value of 50Ω/±1%, visible light transmittance of 85%, high chemical and thermal stability, and resistance volatility within 5%.

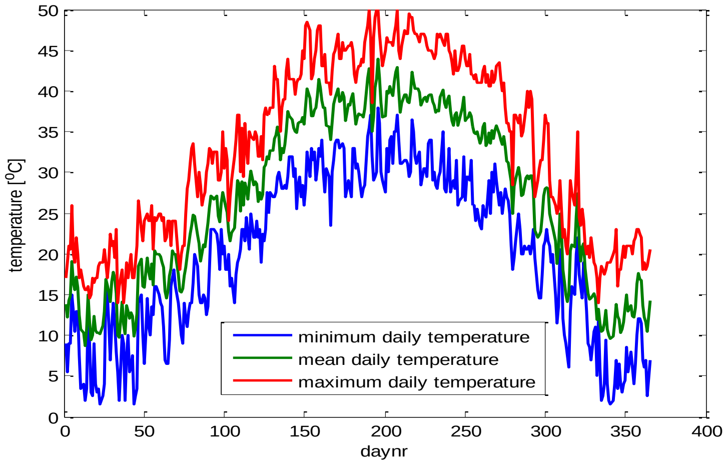


Figure 1. Daily maximum, mean and minimum temperature in Kuwait.

Specifications/ Sketch of the Experimental Greenhouses for Testing IR Reflective Glass

1. Size of the greenhouse – 4.8 x 5.6 m. Side walls are 4 m and the ridge is 5 m.

3. The main frame: Hollow sections 4 mm thick GI; secondary posts made up of 'U' sections - 75 mm wide and 4 mm tick GI; For fixing glass - aluminium hollow pipes (25 mm wide) glass panels will be provided with glass beading and hooks.

4. Size of glass panel 0.8 X 1.0 m

5. Cover - the tempered glass or laminated glass; Glass thickness: two layers of 5mm glass (12.23 x 2 Kg/m^2).

5. Exchange of air in the greenhouse need to be once every 45 seconds to get effective cooling therefore a fan capable of 135 cubic meter of air per minute for each unit.

6. Slope should be close to 20 to 26 degree to obtain the most available light transmission.

7. Requirement for glass for three 4.8 x 5.6 m houses = 156 x 3 = 468 pieces of 0.8 x 1.0 m size (372 m^2)

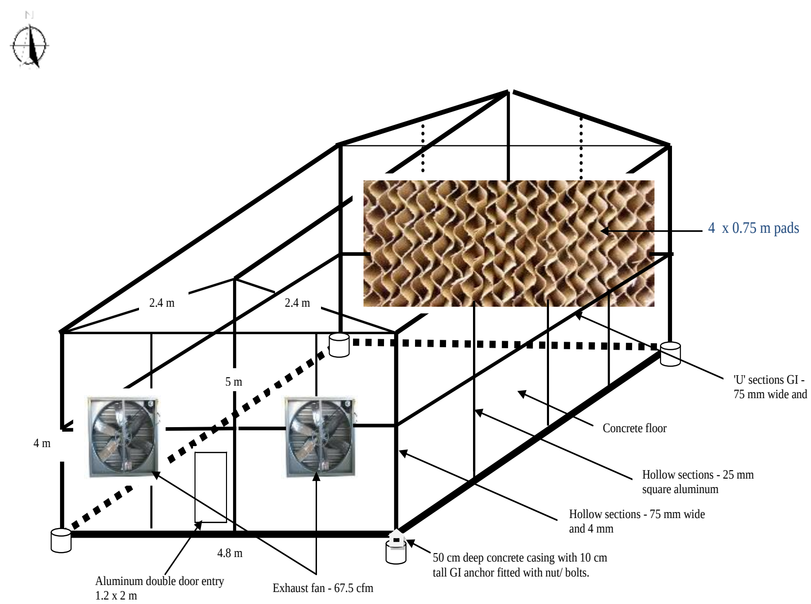


Figure 2. Conceptual design developed for experimental greenhouses.

Materials and Methods

Temperature evaluation, indoor environmental conditions were monitored and recorded in each experimental greenhouse for the proper evaluation of the performance of the prototype IR reflective glass under Kuwait's environment. IR radiation inside the greenhouses was measured using Linshang's IR Power Meter. Indoor temperature was monitored and recorded in the same intervals.

Plant production test, the main objective of this test was to evaluation the performance of experimental greenhouses based on plant growth, productivity, and produce quality during the production cycle. This test involved two experiments production of two vegetable crops tomato and cucumber.

First Experiments, five gallon polyethylene pots, uniformly filled with the above soilless substrate, and were planted with one healthy, uniform seedling having three to four fully developed leaves. The seedlings used in the study were raised in soilless substrates. The seedlings were placed in experimental glasshouses in a double row system with 50 cm between rows and 40 cm between plants within the row. Recommended cultural practices for greenhouse crop production and uniform preventative plant-protection measures were followed during the course of the study.

The two experimental treatments (IR reflective and normal glass as cover), was replicated three times in a randomized complete block design. Each experimental unit (treatment) consisted of 100 plants and data were recorded on 18 randomly selected plants in each treatment. The data recorded included chemical and physical analysis of the growing media at the start of study, periodic plant height, number of leaves, chlorophyll index, days to first flowering and first harvest, number of fruits and fruit yield, and crop duration (days from transplanting to final harvest). The data were subjected to analysis of variance (ANOVA) using R procedures and significant treatment means were separated using the Duncan's multiple range test.

The second experiment with cucumber (*Cucumis sativus* L.) as test crop was initiated in January 2016. Seeds of F_1 hybrid 'Basha' were sown in January 2016, and the healthy seedlings with two true leaves were transplanted into 25 litre containers filled with the above stated soilless substrate in February 2016. The seedlings were placed in the experimental greenhouses in a double row system 50 cm apart, at 40 cm plant to plant spacing. The crop was raised under standard cultivation practices for greenhouse cucumber production, under measured quantity of irrigation, on soil moisture depletion basis; and the data on irrigation from the day of transplanting till the end of the study is monitored. Uniform preventative plant-protection measures were followed during the course of the study.

The two experimental treatments (IR reflective and normal glass as greenhouse cover), was replicated three times in a randomized complete block design. Each experimental unit (treatment) was planted with 80 plants and data were recorded on 18 randomly selected plants in each treatment. The data recorded included periodic plant height, number of leaves, chlorophyll index, days to first flowering and first harvest, number of fruits and fruit yield, and crop duration. The data were analyzed using 'R' statistics.

Results and Discussion

Infrared (IR) transmission inside the IR reflective and normal greenhouses was recorded four times a day at two hours interval (8.00, 10.00, 12.00 and 14.00 hrs.) for a period of five months (November 2015 to March 2016). IR transmission was measured at three critical locations inside the greenhouse viz. center of the greenhouse, near the cooling pad and near the exhaust fan to ascertain the effect of shading if any. The average values for the three recorded locations inside greenhouse shown in Table 1-4. Review of average IR transmission data revealed that the IR reflective glass under evaluation effectively prevented/ reduced IR transmission into the greenhouses at any point of time observed during the study. IR radiation measured at the center of the IR reflective glass greenhouse recorded an upward trend until 12.00 noon, followed by a drop at 14.00 hrs, during the entire course of study. Reduction in the NIR radiation in the IR reflective glasshouses can be attributed to the IR reflective property of the test material.

Table 1. Average of Infrared (IR) Transmission Recorded at the of Experimental Greenhouses at 8.00 hrs

Treatments	Average of IR Transmission									
	01–15 Nov, 2015	16–30 Nov, 2015	01-15 Dec, 2015	16-31 Dec, 2015	01-15 Jan, 2016	16-31 Jan, 2016	01-15 Feb, 2016	16-29 Feb, 2016	01-15 Mar, 2016	15-31 Mar, 2016
IR Reflective Glass	6.52	5.43	3.53	4.80	4.10	3.32	3.69	4.46	6.23	6.90
IR Non-reflective Glass	39.05	45.09	32.51	35.69	38.68	23.90	22.42	28.21	34.36	42.84
% IR Reduction	79.86	86.06	88.46	83.42	86.96	84.73	82.57	83.60	81.42	82.42

Table 2. Average of Infrared (IR) Transmission Recorded at the of Experimental Greenhouses at 10.00 hrs

Treatments	Average of IR Transmission									
	01–15 Nov, 2015	16–30 Nov, 2015	01-15 Dec, 2015	16-31 Dec, 2015	01-15 Jan, 2016	16-31 Jan, 2016	01-15 Feb, 2016	16-29 Feb, 2016	01-15 Mar, 2016	15-31 Mar, 2016
IR Reflective Glass	17.00	19.96	15.25	18.13	19.78	20.26	26.70	33.72	28.90	29.94
IR Non-reflective Glass	98.38	119.58	98.72	116.39	130.64	115.78	150.77	174.77	154.15	155.73
% IR Reduction	80.98	80.44	81.34	81.16	81.75	80.54	79.10	77.58	80.16	75.80

Table 3. Average of Infrared (IR) Transmission Recorded at the of Experimental Greenhouses at 12.00 hrs

Treatments	Average of IR Transmission									
	01–15 Nov,	16–30 Nov,	01-15 Dec,	16-31 Dec,	01-15 Jan,	16-31 Jan,	01-15 Feb,	16-29 Feb,	01-15 Mar,	15-31 Mar,

	2015	2015	2015	2015	2016	2016	2016	2016	2016	2016
IR Reflective Glass	15.81	23.81	18.82	24.25	28.87	28.35	31.32	31.04	32.24	19.49
IR Non-reflective Glass	112.07	114.42	133.55	152.38	178.71	168.97	195.05	205.77	235.88	156.32
% IR Reduction	80.00	75.28	82.18	81.06	81.40	81.73	82.67	82.69	83.50	84.09

Table 4. Average of Infrared (IR) Transmission Recorded at the of Experimental Greenhouses at 14.00 hrs

Treatments	Average of IR Transmission									
	01–15 Nov, 2015	16–30 Nov, 2015	01-15 Dec, 2015	16-31 Dec, 2015	01-15 Jan, 2016	16-31 Jan, 2016	01-15 Feb, 2016	16-29 Feb, 2016	01-15 Mar, 2016	15-31 Mar, 2016
IR Reflective Glass	12.85	5.39	5.10	7.07	6.70	7.86	8.58	13.11	14.09	14.25
IR Non-reflective Glass	74.70	69.89	80.32	103.59	115.92	102.51	128.51	125.56	134.76	127.97
% IR Reduction	76.94	86.84	86.96	83.78	87.14	84.91	85.76	79.34	78.41	81.35

Fortnightly average of ambient temperature recorded at two hour intervals (8.00, 10.00., 12.00. and 14.00 hrs.), for a period of five months (November 2015 to March 2016), during the course of study is presented in Tables 5 -8. Perusal of the temperature records revealed that the IR reflective glass greenhouses recorded lower ambient temperature throughout the course of study, the effect being more pronounced at higher atmospheric temperatures. Temperature reduction (%) was more effective during noon hours (12.00 and 14.00 hrs.), and was at the minimum during the early hours (8.00 hrs.), during the entire course of study. However, the magnitude of IR radiation reduction in IR reflective glass greenhouse was not proportionately translated to temperature reduction during the experimentation; which demands further investigations to ascertain the correlation between IR radiation and buildup temperature in protective environment.

Table 5. Average of Ambient Temperature Recorded in Experimental Greenhouses at 8.00 hrs.

Treatments	Average of Ambient Temperature									
	01–15 Nov, 2015	16–30 Nov, 2015	01-15 Dec, 2015	16-31 Dec, 2015	01-15 Jan, 2016	16-31 Jan, 2016	01-15 Feb, 2016	16-29 Feb, 2016	01-15 Mar, 2016	15-31 Mar, 2016
IR Reflective Glass	23.74	20.81	20.33	15.36	15.89	15.89	15.80	18.41	21.83	22.30
IR Non-reflective Glass	24.07	21.44	22.21	16.42	16.81	15.85	16.50	19.74	23.07	22.44
% IR Reduction	1.37	2.94	8.46	6.46	5.47	-0.25	4.24	6.74	5.37	0.62

Table 6. Average of Ambient Temperature Recorded in Experimental Greenhouses at 10.00 hrs

Treatments	Average of Ambient Temperature									
	01–15 Nov, 2015	16–30 Nov, 2015	01-15 Dec, 2015	16-31 Dec, 2015	01-15 Jan, 2016	16-31 Jan, 2016	01-15 Feb, 2016	16-29 Feb, 2016	01-15 Mar, 2016	15-31 Mar, 2016
IR Reflective Glass	24.85	24.22	20.42	19.48	20.93	20.79	20.33	23.33	26.23	28.78

IR Non-reflective Glass	25.19	25.30	22.24	22.67	24.04	23.08	22.89	25.54	28.60	29.74
% IR Reduction	1.35	4.27	8.18	14.07	12.94	9.92	11.18	8.65	8.29	3.23

Table 7. Average of Ambient Temperature Recorded in Experimental Greenhouses at 12.00 hrs

Treatments	Average of Ambient Temperature									
	01–15 Nov, 2015	16–30 Nov, 2015	01-15 Dec, 2015	16-31 Dec, 2015	01-15 Jan, 2016	16-31 Jan, 2016	01-15 Feb, 2016	16-29 Feb, 2016	01-15 Mar, 2016	15-31 Mar, 2016
IR Reflective Glass	27.11	26.85	23.94	20.91	22.21	23.48	22.41	25.71	28.17	26.43
IR Non-reflective Glass	27.44	28.67	26.45	25.45	26.42	27.10	25.89	29.83	32.46	29.62
% IR Reduction	1.20	6.35	9.49	17.84	15.93	13.36	13.44	13.81	13.22	10.77

Table 8. Average of Ambient Temperature Recorded in Experimental Greenhouses at 14.00 hrs

Treatments	Average of Ambient Temperature									
	01–15 Nov, 2015	16–30 Nov, 2015	01-15 Dec, 2015	16-31 Dec, 2015	01-15 Jan, 2016	16-31 Jan, 2016	01-15 Feb, 2016	16-29 Feb, 2016	01-15 Mar, 2016	15-31 Mar, 2016
IR Reflective Glass	26.19	26.15	23.03	21.07	23.04	23.54	23.60	27.60	27.42	27.56
IR Non-reflective Glass	26.30	27.26	26.09	24.97	27.78	27.63	28.27	31.33	31.35	31.40
% IR Reduction	0.42	4.07	11.73	15.62	17.06	14.80	16.52	11.91	12.54	12.23

Experiment 1: Tomato (*Lycopersicon esculentum* cv. Fuego)

Vegetative growth parameters of tomato observed during the course of experimentation is presented in Tables 9 - 14. The study revealed that cherry tomato grown in the IR reflective glasshouse remained stunted and vegetatively, were inferior to those grown in normal glasshouse. The plants grown under IR reflective glass greenhouse treatment though recorded a steady increment in height; were smaller than those in the normal glasshouse throughout the duration of the study (Table 9). At 120 days after planting (DAP), the average height of tomato plants in the IR reflective glass was 223.92 cm as against 232.28 cm recorded in the normal glass treatment.

Table 9. Average Plant Height acquisition in Tomato (*Lycopersicon esculentum* cv. Fuego)

Treatment	Average Plant Height (cm)								
	0 DAP	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
IR Reflective Glass	14.5	38.3	79.4	95.7	122.3	140.8	162.6	196.1	223.9
Non-IR Reflective Glass	14.5	42.2	92.2	110.5	137.2	153.0	172.0	203.6	232.3

Significance[y]	NS	**	**	**	**	**	**	**	*

[x] DAP = Days after planting, [y] ** = Significant at P ≤ 0.01, * = Significant at P 0.01 - 0.05, NS = Not significant.

The trend for leaf production was similar to that observed for plant height with plants in the IR reflective glass treatment tomatoes recording lower number of leaves compared to the normal glass treatment (Table 10). The maximum number of leaves (31.22/ plant) was produced in the IR Non-reflective glass treatment whereas on an average 29.97 leaves produced per plant in IR reflective glass treatment tomatoes at 120 DAP.

Table 10. Average Number of Leaves in Tomato (*Lycopersicon esculentum* cv. Fuego)

Treatment	Average Number of Leaves								
	0 DAP	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
IR Reflective Glass	3.95	7.12	11.68	9.95	12.43	17.05	21.87	26.40	29.97
Non-IR Reflective Glass	3.90	7.78	12.65	12.30	14.62	18.67	24.33	26.83	31.22
Significance[y]	NS	*	**	**	**	**	**	NS	NS

[x] DAP = Days after planting, [y] ** = Significant at P ≤ 0.01, * = Significant at P 0.01 - 0.05, NS = Not significant.

The average chlorophyll index (SPAD value) recorded at 15 day intervals is presented in Table 11. Plants in the IR reflective glass treatment recorded lower chlorophyll indices until the initial 60 DAP than those in the IR Non-reflective treatment. From 75 to 105 DAP, plants in the IR reflective glass treatment excelled their counterparts for this parameter. Both the IR reflective glass and IR Non-reflective glass treatments recorded maximum SPAD values of 60.53 and 65.04, respectively, at 120 DAP.

Table 11. Average Chlorophyll Index (SPAD value) in Tomato (*Lycopersicon esculentum* cv. Fuego)

Treatment	Average Chlorophyll Index								
	0 DAP	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP	120 DAP
IR Reflective Glass	15.87	43.38	42.83	47.00	50.94	57.77	58.51	58.24	60.53
Non-IR Reflective Glass	15.77	44.59	46.88	50.39	54.27	55.46	55.55	56.77	65.04
Significance[y]	NS	NS	**	**	**	*	*	NS	**

[x] DAP = Days after planting, [y] ** = Significant at P ≤ 0.01, * = Significant at P 0.01 - 0.05, NS = Not significant.

Tomatoes grown in the IR Non-reflective glass greenhouse attained harvestable maturity at 93 DAP, whereas the plants under IR reflective glass treatment recorded its first harvest at 108 DAP (Table 12). IR Non-reflective glass treatment tomatoes recorded an average of 2.34 fruits/ plant as against an average of 1.0 fruit per plant in IR reflective glass treatment tomatoes during the first harvest. Irrespective of the treatments, number of fruits per harvest recorded a steady increase during the course experimentation. However, the normal glass treatment tomatoes recorded significantly higher number of fruits (110.08) as against an average of 65.63 fruits per plant in the IR reflective glass treatment tomatoes (Table 12).

Table 12. Average Number of Fruits in Tomato (*Lycopersicon esculentum* cv. Fuego)

Treatment	Number of fruits/Plant						
	2 Feb, 2016	15 Feb, 2016	21 Feb, 2016	28 Feb, 2016	7 Mar, 2016	13 Mar, 2016	21 Mar 2016
IR Reflective Glass		1	2.84	3.98	10.14	16.90	30.77
IR Non-reflective Glass	2.34	5.24	6.26	9.28	20.92	23.43	42.62

It is evident from Table 13 that the tomatoes under IR reflective glass treatment trailed by 13 days for the first harvest, and produced smaller (14.2 g vs. 30.26 g) fruits than those in the IR Non-reflective glass treatment during the maiden harvest. Fruit yield per harvest, irrespective of the treatments; recorded a steady increase during the course experimentation. The IR non-reflective glass treatment tomatoes recorded significantly higher total fruit yield (606.50 g) against an average yield of 326.02 g fruits per plant in tomatoes from the IR reflective glass treatment (Table 14).

Table 13. Fruit Yield (g) in Tomato (*Lycopersicon esculentum* cv. Fuego)

Treatment	Yield (gm)/plant						
	2 Feb, 2016	15 Feb, 2016	21 Feb, 2016	28 Feb, 2016	7 Mar, 2016	13 Mar, 2016	21 Mar 2016
IR Reflective Glass		14.2	20.95	20.89	52.96	82.87	134.16
IR Non-reflective Glass	30.26	38.79	41.64	50.29	120.53	118.44	206.55

Table 14. Average Number of Fruits and Fruit Yield (g) per plant in Tomato (Lycopersicon esculentum cv. Fuego)

Treatments	Average Number of Fruits	Average Fruit Yield (gm)
IR Reflective Glass	65.63	326.02

Non-IR Reflective Glass	110.08	606.50
Significance[y]	**	*

[x] DAP = Days after planting, [y] ** = Significant at P ≤ 0.01, * = Significant at P 0.01 - 0.05, NS = Not significant.

Experiment 2: Cucumber (*Cucumis sativus* cv. Basha)

The study revealed that cucumbers grown in the IR reflective glasshouse remained inferior in vegetative growth attributes (Tables 15 – 16) to those grown in the normal glasshouse. Cucumbers grown in IR reflective glass greenhouse recorded significantly lower plant height than the cucumbers grown in normal glasshouse throughout the duration of the study (Table 15). Average height difference was recorded maximum at 40 DAP (42.01 cm).

Table 15. Average Plant Height acquisition in Cucumber (*Cucumis sativus* cv. Basha)

Treatment	Average Plant Height (cm)							
	0 DAP[x]	10 DAP[x]	20 DAP[x]	30 DAP[x]	40 DAP[x]	50 DAP[x]	60 DAP[x]	70 DAP[x]
IR Reflective Glass	5.62	7.57	18.37	46.08	89.77	119.12	163.12	245.38
Non-IR Reflective Glass	5.97	8.5	32.93	65.83	131.78	159.8	204.13	256.7
Significance[y]	NS	**	**	**	**	**	**	*

[x] DAP = Days after planting, [y] ** = Significant at P ≤ 0.01, * = Significant at P 0.01 - 0.05, NS = Not significant.

Table 16. Average Number of Leaves in Cucumber (*Cucumis sativus* cv. Basha)

Treatment	Average Number of Leaves							
	0 DAP[x]	10 DAP[x]	20 DAP[x]	30 DAP[x]	40 DAP[x]	50 DAP[x]	60 DAP[x]	70 DAP[x]
IR Reflective Glass	2.05	2.25	5.35	9.68	14.65	17.92	22.18	30.93
Non-IR Reflective Glass	2.12	2.63	6.32	12.15	19	22.02	25.62	32.37
Significance[y]	NS	**	**	**	**	**	**	NS

[x] DAP = Days after planting, [y] ** = Significant at P ≤ 0.01, NS = Not significant.

Leaf production in cucumbers exhibited a similar trend to that observed for plant height, with plants in the IR reflective glass treatment recording significantly lower number of leaves compared to the normal glass treatment (Table 16). However, the IR reflective greenhouse grown cucumbers attained comparable number of leaves to the non-IR reflective greenhouse cucumbers at 70 DAP. The maximum number of leaves (32.37/ plant) was produced in the IR Non-reflective glass treatment against an average of 30.93 leaves produced per plant in IR reflective glass treatment cucumbers at 70 DAP. No significant effect of the treatments was observed in chlorophyll index recorded in experimental cucumbers. Both the IR reflective and Non IR reflective greenhouse grown cucumbers recorded maximum chlorophyll index at 20 DAP (Table 17).

Table 17. Average Chlorophyll Index (SPAD value) in Cucumber (*Cucumis sativus* cv. Basha)

Treatment	Average Chlorophyll Index (SPAD value)			
	10 DAP[x]	20 DAP[x]	40 DAP[x]	60 DAP[x]
IR Reflective Glass	25.63	41.85	40.35	40.12
Non-IR Reflective Glass	25.59	44.79	40.31	40.49
Significance[y]	NS	NS	NS	NS

[x] DAP = Days after planting, [y] NS = Not significant.

Relative earliness in fruit initiation and attainment of harvestable maturity was observed in cucumbers in the IR Non-reflective glass greenhouse. Cucumbers in IR non reflective glass treatment recorded significantly higher number of fruits (33.12) as against an average of 27.3 fruits per plant in the IR reflective greenhouse grown cucumbers. Similar trend was observed in average per plant cucumber production (Table 18). IR reflective greenhouse grown cucumbers yielded 2132 g/ plant whereas the IR non-reflective greenhouse excelled with an average yield of 33.12 g/ plant.

Table 18. Average Number of Fruits and Fruit Yield (g) in Cucumber (*Cucumis sativus* cv. Basha)

Treatments	Average Number of Fruits	Average Fruit Yield (gm)
IR Reflective Glass	27.3	2132
Non-IR Reflective Glass	33.12	2808
Significance[y]	**	**

[x] DAP = Days after planting, [y] ** = Significant at $P \leq 0.01$.

Conclusions

It is clear from the data that IR reflective material effectively blocked the entry of infrared (IR) and near infrared (NIR) radiations from sunlight and lowered the indoor temperatures throughout the testing period. Consequently, plants growing in IR glasshouses had lower ET and total water requirement. Because of lower indoor temperatures, it is expected that the cooling requirement and hence, the volume of water required for cooling will be substantially lower in IR glasshouses compared to that in normal glasshouses. However, these observations should be verified by evaluating the performance during the summer months and determining the extent of reduction in IR and NIR radiations and the resulting reduction in indoor temperature and cooling load and, improvement in cooling efficiency. Both tomato and cucumber plants in normal greenhouses grew taller, contained more leaves and higher chlorophyll compared to those in the IR glasshouses. Consequently, plants in the normal greenhouses produced more number of fruits, bigger fruits and higher fruit yield than those in the IR glasshouse. The water-use-efficiency of tomato and cucumber grown during winter months was also higher in normal glass house than that in the IR glasshouse.

Recommendations

During the winter, the ambient temperatures are lower and the light intensity during most part of the study period was less than half of the normal values. Therefore, these findings are specific to the winter only and there is need to repeat these studies during the summer months when the ambient light intensity and temperature.

References

Abdel-Ghany, A. M.; I. M. Al-Helal; S. M. Alzahrani; A. A. Alsadon; I. M. Ali; and R. M. Elleithy. 2012. Covering materials incorporating radiation-preventing techniques to meet greenhouse cooling challenges in arid regions: A review. *The Scientific World Journal*. Article ID906360, pp. 11.

Albaho, M.; N. R. Bhat; B. Thomas; S. Isath Ali; and P. George. 2012. Evaluation and demonstration of growbags technique for protected cultivation. Kuwait Institute for Scientific Research, Report No. KISR10725, Kuwait.

Al-Nasser, A., and N. R. Bhat. 1998. Protected agriculture in the State of Kuwait. In *Protected Agriculture in the Arabian Peninsula*. Edited by A. T. Moustafa, A. T., A. Al-Mohammadi, A. Abou-Hadid and J. M. Peacock. Syria: International Center for Agricultural Research in the Dry Areas (ICARDA), pp. 17- 26.

Bhat, N. R.; M. Albaho; H. Al-Mazidi; S. Omar; R. K. Suri; and G. Maheshwari. 2002. Integrated research program for the development of sustainable protected agriculture in Kuwait. Kuwait Institute for Scientific Research, Proposal, Kuwait.

Bhat, N. R.; M. Albaho; M. K. Suleiman; S. Al-Dosery; B. Thomas; S. Isath Ali; P. George; R. Bellen; and M. Al-Zalzaleh. 2009a. Optimization and pilot scale organic greenhouse vegetable production. Kuwait Institute for Scientific Research, Report No. KISR9724, Kuwait.

Bhat, N. R.; M. Albaho; G. Maheshwari; Y. Al-Hadban; and S. Bou-Hamad. 2009b. Establishment of self-reliant, sustainable protected environment food production system in Kuwait. Kuwait Institute for Scientific Research, Proposal, Kuwait.

DePascale S., and A. Maggio. 2005. Sustainable protected cultivation at a Mediterranean climate. Perspectives and challenges. Acta Horticulturae **691**: 29-42.

Gulrez, S. K. H.; A. M. Abdel-Ghany; I. M. Al-Helal; S. M. Alzahrani; A. A. Alsadon. 2013. Evaluation of PE films having NIR reflective additives for greenhouse applications in arid regions. *Advances in Materials Science and Engineering*. Article ID 575081.Volume No, pp 8.

Gupta M, and P Chandra. 2002. Effect of greenhouse design parameters on conservation of energy for greenhouse environmental control. *Energy* **27**:777-794.

Kumar, K. S; K. N.Tiwari; and M. Jha. 2009. Design and technology for greenhouse cooling in tropical and subtropical regions: A review. *Energy and Buildings* **41**: 1269–1275.

L. Jia-Ming. 2013. Smart greenhouse related research in ITRI. Presented at Kuwait Institute for Scientific Research, May 28, 2013.

Sethi, V. P., and S. K. Sharma. 2007. Experimental and economic study of a greenhouse thermal control system using aquifer water. *Energy Conversion and Management* **48 (1)**: 306 –319.

Sonneveld P., and Iwan van Bochov, 2013. Climate control of a greenhouse with concentrating solar power. International Conference on Agricultural Engineering February 24 to 26, Muscat, Oman.

Section 4

Seed Farming

Native Seed production and processing under very dry conditions: seed farming as a tool to improve seed availability

Cándido Gálvez-Ramírez
Semillas Silvestres, S.L., C/Aulaga nº 24, Córdoba (P.C. 14012), Spain
e-mail: candido@semillassilvestres.com

Abstract

Restoration of dryland ecosystems is a global challenge due to the special difficulty of implementing classical technics of land restoration. Under these conditions, large-scale plant introduction achieved through seedling planting technology shows limited success due to dry stress after plantations. Therefore, for the goal of achieving large-scale restoration projects, seed-based restoration with native seed acquires greater importance.

The approaches of seed farming on Europe, USA and Australia regarding seed farming are different, although they share a big concern about native seed productions. Some recommendations for seed farming under the very restricted conditions of drylands are inspired on traditional farming. Seed collection from wild populations for large-scale restoration implies taking into account ethical and practical constraints, as well as the higher production cost due to the difficulty of total mechanization. Wild populations can provide certain quantities of native seeds on dryland areas. Some traits common on this flora, like easily seeds dispersion, narrow collection windows, mixed maturity small crops or high interannual variability in seed production can deeply constrain the seed availability. Therefore, to overcome this shortfall in seed supply for the large-scale native seed production, seed farming seems to be a proper tool in a global scenery where the climate change imposes new constraints to the production of genetically appropriate seeds. Native species seed production needs to use innovative crop equipment imaginatively to overcome some key step of the processes increasing effectiveness and performance. Stockpiling big amounts of seeds under appropriate conditions require a multi-criteria decision analysis and information relative to seed longevity and seed storage behavior.

Keywords: drylands, seed production, harvesting, processing

Introduction

Drylands are found in most of the world´s biomes and climatic zones, cover about 41 percent of the Earth´s land surface and are home to 2 billion people. According to FAO, the majority of this people depend on forest, other wooded lands, grasslands and trees on farms for income and to meet basic needs.

The United Nations Environment Management Group (2011) defines drylands as lands where the ratio of annual precipitation and mean annual evapotranspiration, the aridity index (AI) is less than 0.65. Therefore, the drylands are characterized by drastic water deficit, hot temperatures, and dry and poor soils. Drylands are widely affected by desertification, biodiversity loss, poverty and food insecurity. Such problems are exacerbated by climate change and rains unpredictability, apart from disrupted transhumance, migration, social conflicts, weak governance and inadequate policies.

The importance of drylands for the provision of goods and environmental services is generally undervalued and are currently suffering from degradation because of sustainability practices and poor management.

Under these circumstances, an ecological restoration can be recognized as a priority action for habitat conservation. However, achieving this goal, currently demanded on a large scale, is a great challenge.

The achievement of global terrestrial restoration requires large-scale wild species planting, and regardless of which large-scale plant reintroduction, it is to be achieved through planting seedling, or through the direct sowing of seed to site the availability and effective of use of seeds is fundamental to success (Nevill et al. 2016). Recently, several works highlight the importance of quantity and quality of seeds on restoration, and of providing sufficient quantities of genetically appropriate seeds requires a change of mentality (Broadhurst et al. 2015).

Under the very constrained abiotic limitations and hazardous conditions of drylands, the genetic quality of the material (plant or seed) is crucial to enhance the success. The aim of traditional outline is to gather *local, yet ample* and unmodified by erroneous seed production methods. This could allow to avoid the risk of non-adaptation and the implementation of the principles of the *home site advantages*. Furthermore, the goal of producing an appropriate material in terms of amount and origin can be addressed as the bottleneck in many ecological projects, even more taking into account the ethical and social aspects of the issue. On this issue, it is possible to point out several approaches. Visser and Reheul (2001) inspired from conventional forage breeding practice advise to use selected material with consistently superior vigor on drylands with native steppic species (*Stipa lagascae*). Intraspecific hybridization (polycrossing) followed by *in situ* natural adaptation (Jones, 2003) may help to develop seed better suited to future condition. Meanwhile the approach based on the principle of *the right seed in the right place at the right time* (Olfield and Olwell, 2015) are assumed currently by the "native seed community" to use a wider understanding of the genetic suitability (Nevill et al., 2016; Jøgersen et al., 2016).

Historically, seeds used in restorations have been sourced from nearby wild populations. Although using wild populations as a seed source may be a good practice where local ecotypes are desired and populations are accessible, it could be unfeasible or inadvisable due several reasons.

Wild collection has its limits both because it is very time consuming and costly to arrive at populations, and because removing the seeds from the population can negatively impact the demography, genetics and future conservation of the donor and restored population. A practical solution to overcome this issue and produce seeds from wild species is through seed farming, the cultivation of wild plants to produce seed crops and seed supply for use on restoration (Nevill et al. 2016; Broadhurst et al. 2015).

To this new approach of agricultural native seed production, plants of individual species are harvested separately and propagated to increase the amount of seed available in the next generations. This production approach must at least preserve a high genetic diversity of species during the collection and propagation, and limit the number of generation in propagation. Contrary to what happens with the direct harvest from donor-sites with result of a mixture of plant and seeds, specie-specific multiplication aims high germination rate, purity of harvested seeds and must be supported on a system of documentation and control of the flow of goods. All this process must be assured by an independent certificate (Reiger et al. 2014).

But native seed production through seed farming means not only a simple seed multiplication. To address this challenge on local or regional scale several biological and technical problems must be solved. Tischew et al. (2011) point out seven main tasks for a successful restoration program regarding the seed production: identifying species-specific seed zones, genetically adapted materials, formulating strategies to track plant materials, developing seed technology, understanding pollinator requirements, identifying cultural practices and developing effective strategies for reestablishing native plant communities.

The implementation of these techniques and production approach, under the very restricted conditions on dryland areas, is a big challenge not only by the biotic limitations during the production or in the use on the restorations, but also due to the very big areas and scarcity of resources. Aware of the challenge, we proposal some advices and general rules of special importance to produce and process native seed under very dry conditions.

Seed Farming: A Worldwide Matter in Three Continents

During the 1930s the Dust Bowl or Dirty Thirties severe dust storms damaged the ecology and agriculture of American and Canadian prairies deeply, due to a severe drought and the farming methods applied the previous years. Then the soil erosion affected 400,000 km^2 and forced tens of thousands of families to abandon their farms.

After this big disaster, the needs of using native plants to address the ecological restoration of large areas aroused. Since those moments many resources were used to increment the number of species and amount of seeds to achieve this goal. Later, a critical shortage of native plant materials available for seedling following the extensive wildfires of 1999 and 2000 lead U.S. Congress to direct the Bureau of Land Management and U.S. Forest Service to facilitate development of a long-term program to provide a state and economical supply of native plant materials for restoration and rehabilitation efforts on public lands.

More recently big fires in sagebrush in four Western States and recurrent hurricanes and flooding made necessary a basic stabilization, rehabilitation or other restoration treatments. To achieve this goal the National Seed Strategy (2015-2020) was developed by a partnership of 12 Federal agencies and 300 non-Federal cooperators in the U.S. Implementation aims to ensure the availability of genetically appropriate native seeds. Among its main goals are research to improve seed production through seed farming through the establishment of a nationwide network of native seed collectors, farmers and growers.

Although the native seed production in Europe was regulated in 2010, many proposal, strategies and cooperation between policy makers, researchers and stakeholders point out the European native seed production as an emergent business. The European Union 2020 Biodiversity Strategy targets to restore at least 15% of degraded ecosystems by 2020. Highlights the significance of the native seed sectors as well as the need to improve the large-scale production and availability (De Vitis et al. 2017).

The Directive 2010/60/UE (European Commission) provided for certain derogation for marketing of fodder plant seed mixtures to use in the preservation of the natural environment, defines the “preservation mixtures” and let their production by natural collection or seed farming.

Before and after this regulation, several European research projects (NASSTEC, SALVERE, SOS-PRADERAS) have addressed the native seed production as one of the main weaknesses for the restoration goal to a large scale. To achieve this continental goal several countries have developed national strategies so far. The current situation regarding the development of native seed production is very diverse. Meanwhile some countries have been able to set up a multipartner network and a certification system is working currently (i.e. Germany, France, Austria, Norway…); others are on the way. All of them base its production on seed farming by ethical, technical and economic reasons. Today, native seed farming could represent a new productive sector in the Mediterranean countries where farmers look for alternative crops for their non-profits current production

On the other hand, the big challenge regarding the ecological restoration in Australian seed farming is a developing matter so far. Some recent actions are addressing to a higher pressure on seed collectors. Many organizations are now undertaking seed collection on a vast scale that could end up in a loss of biodiversity. Therefore, the Biodiversity Conservation Act 2016 passed through the Parliament last year. The new most significant changes are related to liability and penalties to wild seed collectors without distinction either as to whether the seed is collected from Crown or private land (Grose 2017).

The Australian Network for plant Conservation Inc. (ANPC) and the Revegetation Industry Association of Western Australia (RIAWA) are currently involved in a strategy to identify what are the key issues and gaps in the Australian nation and regional seed supply and how learn from the American scene with the aim to assist in creating a true native seed industry. Definitely seed farming will be an issue to explore to overcome some of their current gaps and weaknesses.

The Plant Material Selection

Seed production in natural ecosystems must ensure the regeneration of locally adapted plant species, which provide habitat for wildlife, stabilize soils, control surface-water flow, and contribute to ecological integrity and resilience (Oldfield and Olwell, 2015). To achieve these goals the plant material origin is fundamental.

Typically, to planning and managing a restoration the list of chosen species is shaped by the current environmental conditions and species assemblies at the site, the future goals for the function or ecological trajectory of the site/system and practical factors of budget and availability of suitable plant material (Ladouceur et al., 2017). Therefore, for seed production and supply is fundamental to develop a species selection based on traits to its suitability for restoration, using the combined attribute values for generating and supply. This selecting method could include a DEXi multi-attribute decision making program to guarantee the best species selection. Frischie and al. (under review) give an example of selecting methodology for dicots promoting seed farming to be used as cover crop in olive groves in the South of Spain under dry conditions.

Another important issue to point out regarding the plant material is the seed provenance. Seeds of local provenance are widely recommended for restoration projects because of some reasons, that included avoiding genetic contamination of local population, increasing restoration success through better seedling establishment, survival and growth of locally adapted plant material and avoid outbreeding depression.

To the achievement of minimal loss of biodiversity and local adaptation during the multiplication process, different strategies can be applied.

One strategy has an eco-regional approach, where topography, climatic or edaphic data for zones of ecologically similarity are drawn and the zones encompass geographic areas with similar ecological conditions, such geology, climate, vegetation, soils and hydrogeology (Mahalowich and McArthur 2004). Another strategy is based on an adaptive focus. On this approach, the best adapted plant populations are used as seed source and to quantify adaptive potential of the populations seed of different origin are tested on common garden experiments

(Bischoff et al. 2006). And the third approach is to use genetic tolls in definition plant genetic structure and rules for intraspecific biodiversity conservation in restoration practices and preserve genetic integrity of local. It involves a goal of maintaining the natural spatial genetic structure of the species, as well as preserving genetic diversity to ensure long-term population survival and reproduction (McKay et al. 2005; Malaval et al. 2010).

Jøgersen et al. (2016) point out that it is not mutually exclusive and may well be combined to cover several aspects of revegetation. Following the recommendation of Malaval et al. (2010) to retain the highest possible genetic variability in seed mixture, next simple rules should be assumed:

- A minimum of three populations should be collected for each species and in each seed zone.
- Each of the three populations should be collected in constraining ecological conditions as defined by exposure, natural habitat and bedrock.
- The collection of samples for each of three or more populations should be evenly distributed throughout the zone.
- To avoid genetic drift during seed production, newly collected seeds in situ populations should be regularly introduced into the seed pool.
- To reduce the potential for accidental selection during seed multiplication, harvest the entire planted population.

When there aren´t enough seed to start the new crop, soil seed bank can be used to collect seeds or produce plants under controlled conditions. Despite the presence of seeds on soil is influenced by several factors like dormancy, predation and longevity among others, soil seed bank can show important density of seeds. Depending on the habitats (Baskin and Baskin 2014) number of seed per square meter range between 1 and 24,491 in desserts, or higher in arable lands soil seed banks. These figures support a new use of soil seed bank like a seed collection from which start the crop with an adequate number of seeds and genetic variability.

The Seed Farming Technology in Drylands

The Crop Management

The UNEP´s classification system subdivides drylands on the basis of AI into hyper-arid lands, arid lands, semiarid lands and dry sub-humid lands. All of them show a common pattern regarding to the agriculture possibilities and seed productions.

Under these conditions of moderate to severe moisture stress during a substantial part of the year, seed farming requires special cultural techniques and crop and farming systems adapted for successful and stable agricultural production. Therefore, the dryland cropping occurs in areas where the average water supply to the crop limits yield to less than 40 percent of full (not water-limited) potential. The growing days range from 1 to 59 in arid regions or from 60 to 119 growing days in semi-arid regions.

On one side, on the contrary to agricultural species, dryland natives show a germination pattern very variable (Basking and Basking, 2014). Soil characteristics, temperatures and rainfall make the germination happens only under certain conditions. The presence of morphological, morphophysiological, physiological, physical or combinational dormancy are widespread on the dryland flora. So, to achieve the establishment of the crop an appropriated knowledge of dormancy and the treatments to break is need. At least the optimum germination temperatures, data for light-dark requirements, amount of rainfall and type of dormancy must be studied and understood before trying to set up a new crop.

Creswell and Martin (1998) give some useful advices for dryland farming that can be equally implemented for native seed farming with the advantage of the good adaptation of our species to the dry environment.

Water is one of the main problems of agriculture in arid regions. Native plants are very well adapted to xeric conditions and they have developed different strategies to overcome water stress. Therophytes have a short life cycle that can germinate, grow, and produce seeds during a short period of available moisture. Perennials (herbaceous or shrubs) can develop long root systems which have the ability to gather water over a wide area. A correct election of sowing date for the therophytes and a wide plantation grade will be critical to the success on these species.

On the other hand, rainfall in arid regions may be of little use for crop plants because the amount is too small to penetrate the soil sufficiently. Thus, a correct management of soil to avoid any water loss should be used. On this sense arrangement of sowing lines in contour lines, micro dikes, or other bioengineering techniques can have a relevant role to avoid any water loss during the few rains.

The major effects of heat and wind are to increase the rate of evaporation of water, and thus to increase the effects of aridity. Wind may also cause mechanical damage to crops. Both are combating to changing the microclimate. The effects of winds can be reduced by windbreaks (lines of trees perpendicular to the direction of prevailing winds). However, a windbreak may also rob crops of light, water and nutrients. So, its advantages must be weighed against the disadvantages in any particular environment. Windbreaks can also be constructed of non-living materials, which are likely to be expensive.

Heat is received principally from the sun and can be reduced by shading. But, shading also reduces the yields of plants. A light shade such as that below a coconut planting or a protective screen or lathwork can be useful in reducing heat and retaining moisture, with only a minimum loss of yield.

The election of the site for the seed farming regarding the soil priorities is very important. Although soils of the arid tropics are highly variable, as they are in any climate, it is possible to make some generalizations about such soils. The evaluation of the potential fertility and rock origin is important to allow a reasonable plant development and in consequence the yield feasible.

Because of low rainfall in desert soils minerals derived from breakdown of rocks are not leached from the soil. In some cases where the soil is periodically flooded or irrigated, the soil might be saline as well. Such soils support few crops.

The multiplication plot sites must be as clean as possible from weeds. It is critical to select a place with a not very active soil seed bank and it is discouraged to use place where the livestock have been stabled. This leaves a huge amount of seeds on the ground that must be eliminated.

The superior competitive ability of exotic annuals makes necessary to eliminate or reduce these weeds prior to seeding. Although the most effective control of exotic annual grasses has been achieved with pre-emergent, one or several stale seed beds can help to reduce costs and time of soil preparation. After the sowing, selective herbicides in combination with manual weeding must be used to avoid a weed invasion in the crops.

The presence of weeds not only limits the establishment of the crop and a yield reduction but also can produce seed batches contamination, increase the costs and decrease its quality.

The Harvesting

One of the advantages of seed farming over the wild collection is the possibility of harvest mechanization. Although small multiplication plots are harvested by hand normally, different tools can help to do it faster and easier. In medium or large size plots mechanization will help to do an efficient, fast and cheap harvest. However, some species with a very long dispersal period could be harvested by hand even in large plots gathering time to time only the ripen fruits before the dehisces or shattering.

There is not only one harvesting method for all species and conditions. Manual and mechanical harvesting shows advantages depending on the plant biotypes and can be recommended to harvest on wild population or crop production. In the next table this information is summarized.

Table 1. Advantages and Use Recommendations of Native Seed Harvest Methods Depending on the Biotypes.

Harvest Methods	Biotypes	Advantages	Use Recommendations
Manual from wild	Annuals Perennials Shrubs	• Produce clean seeds in proper maturity • Collection everywhere • Easy to have many accessions	• Small quantities for foundation seeds • When is needed a high intraspecific variability
Mechanical from farm	Annuals Perennials	• High yield in short time • Small cost per unit • Process standardization	• Large yield programs • Limits on workforce • Availability of farm facilities
Mechanical from wild	Shrubs	• Collection from wild population since the beginning • Avoid crop implantation costs • Different equipment available	• An ecosystems seed-mix is recommended • Species of slow growth • Large demand in short time

To optimize the yield for agriculturally produced native seeds, specially under dry conditions, harvesting seeds has to consider the diverse traits of the different species.

Varieties with longer blooming periods, longer maturity and those which continue to ripe after harvest, can be harvested by hand or by a sweeper machine several times. However, almost all grasses, and many forbs are suitable for direct threshing with a combine harvester. On this method special attention must be paid to avoid biomass harvested overheating due to the high humidity degree and temperatures during the harvesting season usually.

An alternative method for species dispersed by wind is suction. Many arid land species show adaptation to wind dispersion through a down. It could help to gather seeds from the ripe fruit or even from the soil covered with a fabric using a suction equipment.

Nevertheless, despite these useful methods to increase the yield, a decision system to choose the best species not only by ecosystems recovery reasons but under the yield and seed quality point of view is needed. This work has been already developed successfully in the south of Spain to select forbs and grasses for ground covers in olive groves. In this region, many forbs and grasses have the potential to enhance biodiversity and provide ecosystem services yet remain understudied and underutilized for ecological restoration. Therefore, we evaluated 27 Mediterranean dicots (Figure 1) and 10 grasses for four main characteristics important to seed farming: establishment, growth way, phenology and yield. The results provide seed producers with useful recommendations for sowing rate, row spacing and harvest time for each species.

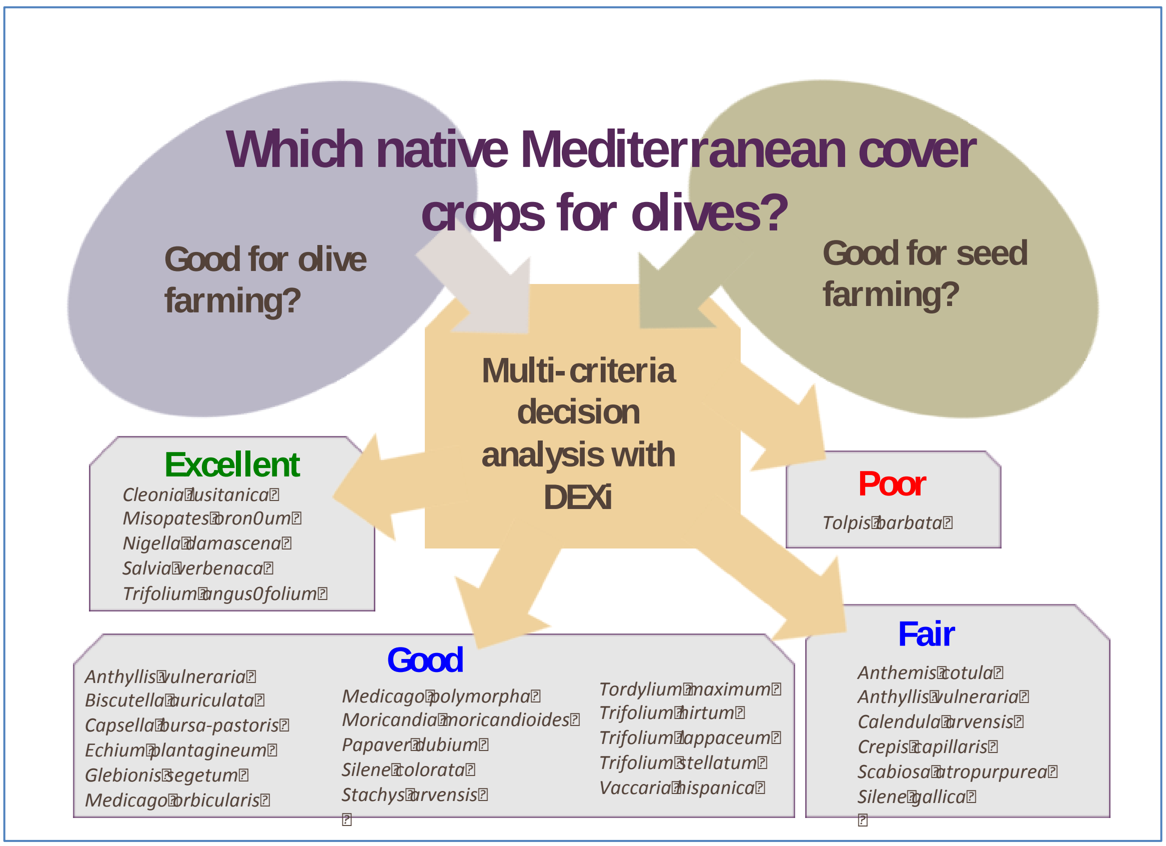


Figure 1. Multi-criteria decision analysis results on Mediterranean cover crops for olive taking into account seed farming and ground cover usability on field.

Characterization of seed farming traits for these native forbs and grasses provides a starting point to stimulate the native seed production sector. Now 30 species are under seed farming multiplication and several thousands of hectares of ground cover with Mediterranean woody crops are planted every year. These results demonstrate that an appropriated species selection is crucial to implement large scale multiplication programs with native species due to the diversity of wild species and their non-domesticated status. Trial evaluations can help determine which species and which cultural practices are most suitable for seed farming.

Native Seed Processing

Drying, cleaning and storing the collected material is an important issue everywhere. Harvested seeds must be processed with care to avoid any damage during each one of the phases. Big lots must be processed as soon as possible to avoid seed heating due to the high water content on seeds or on impurities. However, a very fast drying could cause damage on some seeds. Therefore, a climate-controlled dry room before cleaning is advisable.

Seed farming needs medium size equipment adapted to our proper scale. Nowadays, several equipment makers offer good but expensive solutions/tools, however agricultural seed processing equipment can be easily adapted to our needs with great results and low cost.

Something to highlight related to the seed storage talking about native seeds is having enough information related to storage behavior and seed longevity. Many technical information offered from the researcher institutions for native seed storage is related to seed banks. The implementation of these methods for seed farming production makes no sense usually due to the scale change in terms of amount and time.

Some easy rules like storage seeds on dry conditions better than on cool conditions, track the germination and a good yield planning to adapt the production to real needs, can help to give sense to the seed storage.

Conclusions

Restoration of dryland ecosystems is a global challenge due to the special difficulty of implementing classical technics of land restoration. Under these conditions native seed acquires greater importance for the goal of achieving large seed-based restoration projects.

Wild seed collections imply taking into account ethical and practical constraints, as well as the higher cost, and some traits common on dryland flora, can constrain deeply the seed availability. To overcome this shortfall on a large-scale native seed production, seed farming seems to be a proper tool to produce genetically appropriate seeds.

Native species seed production needs to use innovative crop equipment imaginatively to overcome some key step of the processes increasing effectiveness and performance.

Stockpiling big amounts of seeds under appropriate conditions requires multi-criteria decision analysis, and information relative to seed longevity and storage behavior must overcome the lack of knowledge on many wild species.

References

Baskin, C.C. and J.M. Baskin. 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. *Academic Press.*

Bischoff, A; L. Cremieux; M. Smilauerova; C. S. Lawson; S.R. Mortimer; J. Dolezal and V Lanta. 2006. Detecting local adaptation in wide-spread grassland species – the importance of scale and local plant community. Jou*rnal of Ecology* **94**:1130-1142.

Broadhurst, L.; Holpley, T.; L. Li and M. Begley. 2015. Using seed production areas to meet restoration targets and secure genetic diversity. *Australian Plant Conservation: Journal of the Australian Network for Plant Conservation* **3**:7-8.

Creswell, R. and F.W. Martin. 1998. Dryland farming: crops and Techniques on arid regions. *ECHO Technical Note*. http://www.echonet.org

De Vitis, M.; H. Abbandonato, K.W. Dixon; G. Laverack; C. Bonomi and S. Pedrini. 2017. European Native Seed Industry: Characterization and Perspectives in Grassland Restoration. *Sustainability*. 9, 1682; doi:10.3390/su91016282

European Commission. 2010. Commission Directive 2010/60/EU of 30 August 2010 providing for certain derogations for marketing of fodder plant seed mixtures intended for use in the preservation of the natural environment. *Off. J. Eur. Union* 2010, **228**, 10–14.

Frischie, S.; C. Gálvez-Ramírez; G. Squire and B. Jiménez-Alfaro. Trait-based species selection tool for promoting dicots seed farming of native cover crops in olive orchards. Agriculture, Ecosystems and Environment (under review).

Grose, D. 2017. Seed collector take note. Revegetation Industry Association of Western Australia Inc. Newsletter. June: 4-5.

Jones, T.A. 2003. The restoration gene pool concept: Beyond the native versus non-native debate. Restoration Ecology **11**:281-290.

Jøgersen, MH.; A. Elameen; N. Hofman; S. Klemsdal; S. Malaval and S. Fjellheim. 2016. What´s the meaning of local? Using molecular markers to define seed transfer zones for ecological restoration in Norway. Evolutionary Applications **9**: 673-684

Ladouceur, E.; B. Jiménez-Alfaro; M. Marin; M. De Vitis; H. Abbandonato; P.P.M., Iannetta, C. Bonomi and H.W. Pritchard. 2017. Native Seed Supply and the Restoration Species Pool. *Conserv. Lett*. 0, 1–9. doi:10.1111/conl.12381

Mahalovich, M.F. and E. D. McArthur. 2004. Sagebrush (*Artemissia* spp.) seeds and plant transfer guidelines. *Native Plant Journal* **5**:141-148

Malaval, S.; B. Lauga, C. Regnault-Roger and G. Largier. 2010. Combined definition of seed transfer guidelines for ecological restoration in the French Pyrenees. *Applied Vegetation Science* **13**:113-124.

McKay, J.L.; C.E. Christian; S. Harrison and K.L. Rice. 2005. How Local is Local? – a review of practical and conceptual issue in the genetics of restoration. *Restoration Ecology* **13**:432-440.

Nevill, P.G.; S. Tomlinson; C.P. Elliot; E.K. Espeland; K.W. Dixon and D.J. Merrit. 2016. Seed production areas for the global restoration challenge. Ecology and Evolution. Commentary. DOI: 10.10002/ece3.2455.

Oldfield, S. and P. Olwell. 2015. The Right Seed in the Right Place at the Right Time. *BioScience*. **9**: 955-956.

Rieger, E; B. Feucht and M. Wieden. 2014. *Agricultural propagation of native seeds and development of a certification procedure in Germany*. (in Kiehl, K.; A. Krimer, N. Shaw and S. Tischew. *Guidelines for native seed production and grassland restoration*. Cambridge Scholars Publishing). P. 101-117.

Tischew, S.; B. Youtie; A. Kirmer; and N. Shaw. 2011. Farming for restoration: Building bridges for native seeds. *Ecological Restoration* **29**: 219-222.

Visser, M. and D. Reheul. 2001. Restoring depleted Tunisian dryland with native steppic species: where should we source the seeds? Genetic Resources and Crop Evolution **48**:567-578.

Immature Seed Culture Technique an Alternative Method for the Mass Production of Native Shrubs

C. Sudhersan, S. Jibi, L. Al-Sabah and S. Al-Melhem
Kuwait Institute for Scientific Research, Kuwait.
Email: schellan@kisr.edu.kw

Abstract

Native perennial shrubs are more important in the desert re-vegetation or greenery program. Currently, the required large quantities of native shrub seedlings are not available in the nurseries for the abovementioned purpose. The reasons are: unavailability of large number of viable seeds, lack of knowledge about native seed biology and native seed germination. Immature seed culture technique will be an alternative method for the mass production of healthy native shrub plantlets. Through this technique, it is possible to produce healthy rooted plantlets and understand about the factors that controls in seed germination of different native plant species. A study was undertaken in the KISR plant tissue culture laboratory to develop *in vitro* culture technique for the mass production of selected native shrub species using immature seeds as explants. Immature seeds of *Rhanterium epapposum* (Arfaj), *Calotropis procera* (Ushar), *Calligonum polygonoides* (Artah), *Farsetia aegyptia* (Al-Lbanah), *Lycium shawii* (Awsaj), *Nitraria retusa* (Ghardaq) and *Ochradenus baccatus* (Gurdi) were collected from the plants maintained in the KISR station for research and innovation (KSRI) at Kabd. All the immature seeds collected from the above mentioned seven species of perennial native shrubs were cleaned and surface sterilized using 1% sodium hypochlorite solution and planted in culture medium prepared using different concentrations of Murashige and Skoog (MS) basal salts. All the cultures were maintained in temperature and light controlled growth rooms under 16/8 h light/dark or under total darkness. Immature seed germination and seedling growth and development were observed and recorded. Results from this study provided valuable information for the mass production of these perennial native shrubs.

Keywords: Native shrubs, immature seeds, in vitro culture, MS media.

Introduction

Native plants are key components of the global biological biodiversity, and are highly adapted to the local environmental and climatic conditions. They provide a beautiful and healthy environment, in addition to preserving the ecological balance, and provide shelter to many other organisms that depend upon them for their survival. Many native plants have the potential for use as animal fodder, soil protector from erosion, and in phyto-remediation and ornamental landscaping. Native plants require less care and maintenance for their survival than the introduced exotics. Kuwait's desert ecosystem has been damaged by several factors including natural calamities and anthropological activities (Sudhersan et al., 2003; Omar and Bhat, 2008). Efforts on natural rehabilitation has failed due to the harsh environmental and climatic conditions. Therefore, the desert ecosystem has to be developed through artificial means. The desert rehabilitation program requires large quantities of native plants especially perennial shrubs and trees within a short duration for the restoration of Kuwait's damaged desert ecosystem.

Due to the harsh environmental conditions of Kuwait, it is difficult to produce large quantities of native plants through natural means of plant propagation. Native plant seed collection and nursery establishment are difficult and highly expensive under Kuwait's climatic conditions. Native plants produced through vegetative cuttings failed to establish well in the desert in many of the past experiments due to weak root system. Micropropagation is the alternative to produce large number of rooted plants in short period. However, micropropagation method produces only clonal plants that is generally not preferred for the rehabilitation program. Therefore, micropropagation integrated with immature seed culture technology has been attempted for the large-scale native plant production. The major objectives of this research study were to develop an easy protocol for plant production through immature seed culture and to shorten the time taken for the seedling production from the pollination to seed harvest, and also to overcome the embryo abortion due to high temperature during the seed maturation stage. In the present study, immature seeds of *Rhanterium epapposum* (Arfaj), *Calotropis procera* (Ushar), *Calligonum polygonoides* (Artah), *Farsetia aegyptia* (Al-Lbanah), *Lycium shawii* (Awsaj), *Nitraria retusa* (Ghardaq) and *Ochradenus baccatus* (Gurdi) were collected from the natural wild populations during 2017 and used for the experimentation. The details of the study are reported herein.

Material and Methods

Immature seeds of *Rhanterium epapposum*, *Calotropis procera*, *Calligonum polygonoides*, *Farsetia aegyptia*, *Lycium shawii*, *Nitraria retusa* and *Ochradenus baccatus* were collected from KSRI and Kuwait desert during May 2017. All the immature seeds collected from the desert field were brought to the KISR Plant tissue culture laboratory for the *in vitro* culture.

Immature seeds of all the above mentioned native plant species were prewashed with soapy water and surface-sterilized with 50% commercial Chlorox with a drop of Tween 20 for 30 min and ultrasonic vibration for 1 min. After rinsing four times with sterile distilled water, the immature seeds were used as explants for the study.

MS macro and micro nutrients (Murashige and Skoog, 1962) was used as the basal culture media for all the seven native plant species. For the immature seed culture, 5 different culture media namely NMC, NM1, NM2, NM3, NM4 were prepared using different concentrations of MS basal salts and sucrose. MS culture media containing organic additives like Thiamine HCl, Phyridoxine HCl, Nicotinic acid, Calcium panthothenate and Glycine at 2.5 mg/l, and Myo inositol and Glutamine at 100 mg/l was used for further multiplication of the seedlings raised from the immature seeds. MS basal media with organic additives and different concentrations of cytokinins was used for the multiplication experiments using stem nodal segments as explants. .

Experiments were carried out to develop protocol for the micropropagation of each selected native plant species using different concentrations of Benzyl adenine (BA). All culture media were gelled with 1.5 g/l Gelrite. The pH of the media was adjusted to 5.6 prior to autoclaving. All culture media were autoclaved at 121˚ C for 15 min and stored in a media storage room until utilized. Immature seeds, stem nodal and shoot tip explants collected from the immature seedlings of all the seven species were placed on the surface of the sterilized culture initiation media and kept in temperature and light controlled culture rooms. To study the effect of light on immature seed germination, 50 % of the cultures were maintained in dark and 50 % maintained under 12 h light. Each of the experiments contained 40 explants per treatment, and the experiments were repeated twice. All cultures were renewed by subculturing every 15 d, and the contaminated cultures were discarded. Observations were made once every 10 d and the morphogenetic responses of the explants were recorded.

Rooted plantlets were washed in running tap water to remove the nutrient media that causes fugal attack in the root system during the acclimatization procedure. Cleaned rooted plantlets were treated with 0.5% Benlate solution and planted in sterile soil mix in small (5 cm dia pots) plastic pots. The soil mix prepared by mixing sand, peat moss, and humus at a 1:1:1 ratio was autoclaved at 121˚ C for 45 min prior to planting the rooted plantlets. All the rooted plantlets transferred to the soil mix were acclimatized for 20 d at controlled temperature prior to greenhouse acclimatization. Plantlets were acclimatized to the greenhouse environmental conditions before the field experimentation by gradual exposure to low humidity.

Results and Discussion

Immature Seed Culture. Germination from immature seeds of native plants is a simpler alternative to *in vitro* culture of immature zygotic embryos. Native seeds extracted as early as 10 days after pollination were successfully cultured provided they were transferred aseptically and without injury in culture medium containing appropriate sucrose concentration. Among the 7 species studied, except *Calligonum polygonoides* all the other 6 species germinated within 7 days duration in culture media NM4 containing 10 g/l sucrose. Immature seeds of *Lycium shawii* did not germinate under light condition. Under total darkness, the immature seeds germinated within 7 days. The germinated seedlings transferred to light immediately after germination developed into healthy seedlings. The cultures maintained in the dark continuously became long, weak and yellowish in color in the beginning and died later on. All the weak plants moved before dying condition to light turned healthy and green after a week time. *Rhanterium* seeds germinated when the seeds are placed vertically facing the root pole down in the media. Seeds immersed inside the culture media did not germinate. Seeds placed horizontally in the culture media surface germinated but, failed to establish into a seedling. All the seeds planted vertically were established into seedlings and successfully acclimatized to the normal conditions (Figure 1; a-f and Figure 2; a-b). No morphological or physiological changes in regenerated plants were detected in the *in vitro* immature seed culture method. Immature seeds of *Calligonum* germinated after 90 days. This may be due to the physiological dormancy and this species requires modified culture media using different growth hormones to speed up the germination process. All other species, responded to the culture media NM4 and the immature seed germination was 100%. In the case of *Nitraria*, the seeds removed from the seed coat germinated faster than the normal immature seeds. Seed production and natural seed germination under Kuwait climatic condition is very poor in certain perennial native plant species. Seeds of many native plants require pre-treatment for the germination (Sulaiman et al., 2009; Zaman et al., 2010). All the above mentioned 7 native perennial species produce large number of seeds annually. However, seedling production is very low for the desert rehabilitation. Immature seed culture technology will be an additional tool for large-scale plant production in short duration.

Micropropagation, The shoot tip and stem nodal segment explants of the selected native plant seedlings raised from immature seed culture technique were well established in MS culture medium containing 0.1 mg/l BA within 30 days duration. *Lycium shawii* and *Ochradenus buccatus* explants elongated into rooted plantlets after 45 d even without any growth regulators in the culture media. However, all other species required growth regulators for growth and multiplication, and rooting. Among all the 7 species studied, *Rhanterium epapposum* required a special culture media for the growth and multiplication while all other species responded to a common culture media. Most of the cultures of *Rhanterium* showed callusing of leaves and stem even in media without cytokinin. This is due to the presence of endogenic growth hormones in the tissue explants. Nodal segments and shoot tip explants isolated from the *in vitro* grown plantlets of *Nitraria* when cultured in MS culture media containing 3 mg/l BA showed high morphogenetic potential in plantlet regeneration and multiplication while *Lycium* and *Ochradenus* responded negatively. Literature study indicated that information on native plant micropropagation is scanty when compared to the other crops and ornamental plants. The micropropagation method developed for *Lycium* and *Ochradenus* (Sudhersan et al., 2003) was modified and more simplified for the large-scale production of these two species through the present study. Immature seed culture technique was standardized for all the 7 species studied. However, micropropagation of *Rhanterium*, *Calligonum*, *Calotropis* and *Farsetia* need further experimentation for the optimization of culture media and protocol for large-scale micropropagation.

Figure 1. *Rhanterium eppaposum* Oliv. a. Immature seeds; b. Immature seed germination *in vitro*; c. Cotyledon stage seedling; d. Plantlet (10 d old); e. Plantlet (15 d old); f. Rooted plantlet.

a b

Figure 2. *Rhanterium epapposum* Oliv. a. In vitro seedling ready to soil transfer; b. acclimatized plant.

Acknowledgment

The financial support (PR17-12SL-04) from KFAS and full support and encouragement from the KISR's management are hereby acknowledged.

References

Evans, D. A., W. R. Sharp, P. V. Ammirato, and Y. Yamada, 1983. Handbook of Plant Cell Culture. New York, USA: Macmillan Publishers, 970 pp.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plantarum 15:473-497.

Omar, S. A. S. and N. R. Bhat, 2008. Alteration of the *Rhanterium epapposum* plant community in Kuwait and restoration measures. Intl. J. Environ. Studies, 65:139-155.

Sudhersan, C., M. AboEl-Nil, and J. Hussain, 2003. Tissue culture technology for the conservation and propagation of certain native plants. Journal of Arid Environments 54: 133-147.

Suliman, M. K., Bhat, N. R.,Abdal, M. S., Zaman, S., Jacob, S. and R. R. Thomas. 2009. Germination Studies in Rhanterium epapposum Oliv. World Applied Sciences Journal, 7: 468-471.

Zaman, S., Padmesh, S. and H. Tawfiq. 2010. Germination Ecology of Rhanterium epapposum Oliv. American Journal of applied Sciences, 7: 1321-1326.

Responses of *Peganum harmala* to Different Types and Levels of Irrigation for Restoration of Desert Landscapes in Kuwait

Mohammad A. Islam, Sheena Jacob, Narayana R. Bhat, Majda K. Suleiman and Rini R. Thomas
Kuwait Institute for Scientific Research, Kuwait
*Email: aislam@kisr.edu.kw

Abstract

Arid climatic conditions and anthropogenic disturbances are the most critical factors that have contributed towards a very poor natural regeneration of native species in Kuwait's desert ecosystem. To augment the presence of native species in the desert environment, numerous research projects on different aspects of artificial regeneration have been undertaken by Kuwait Institute for Scientific Research in recent years. *Peganum harmala* is a perennial native shrub of Kuwait which grows mainly during summer months. The lush green nature and beautiful flowers of *Peganum harmala* make it a highly potential plant for landscaping as well. In this study, *Peganum harmala* was mass propagated through seeds and subjected to three irrigation levels (75%, 50%, and 100% of estimated crop evapotranspiration) and three irrigation systems (drip, subsurface at 15cm depth, deep surface at 30cm depth) to determine the optimum cultural practices. Untreated seeds, which had almost 100% germination rate, were used for propagation. Survival of the *Peganum harmala* seedlings increased by 84% when irrigated at 50% level compared to 100% irrigation through drip system. Irrigation type significantly increased *Peganum harmala* seedling height increment. Plants irrigated at 50% level through drip irrigation exhibited the highest increment in height. Meanwhile, maximum increment in canopy cover was observed in plants irrigated at 75% lever through drip irrigation. Ground cover was also influenced by irrigation type and highest ground cover was recorded for drip irrigated plants. Such differential responses to irrigation could be important as they influence species productivity and could be used for future restoration projects involving similar native species in Kuwait.

Key words: Mass propagation, irrigation regime, revegetation, arid landscape.

Introduction

Kuwait is characterized by harsh weather conditions, and its landscape is dominated by desert ecosystems. It suffers from severe land degradation, which is exacerbated by the inherent fragility of soils and loss of native vegetation cover in Kuwait's deserts (Al-Awadhi et al., 2003). Native plants are the key elements of local and global biological diversity. Therefore, conservation and regeneration of native plants can play an important role in the rehabilitation of Kuwait's deserts. Native species usually thrive under prevailing climatic conditions of Kuwait. However, inherently poor soil quality and unpredictable seasonal rainfall often hamper their establishment and natural regeneration process. Hence, it is necessary to augment their presence in the landscape through artificial regeneration, which would require sound and tested protocols for seed collection, storage, germination, and transplanting of seedlings of native species. Additionally, species specific information is needed on their water requirements after transplanting. It has been shown that irrigation is imperative for the survival and growth of plants in greenery and restoration projects, particularly in arid conditions like Kuwait. The scarcity and high cost of water in Kuwait demand a careful determination of the optimal amount of water applied to discern their economical and environmental impact. High levels of irrigation are often applied in order to maximize plant growth and yields. However, excessive irrigations may result in high infiltration especially in sandy soils.

Although *Peganum harmala* L. is native shrub species of Kuwait, it is less abundant throughout the desert landscape. It has been reported to occur in Umm Al-Aish satellite station (Omar et al. 2009). Considering the importance of its conservation, efforts were made to study the effects of different irrigation types and the amount of irrigation on survival and morphological growth of *Peganum harmala* in Kuwait's desert environment.

Materials and Methods

Untreated seeds of *Peganum harmala*, which had almost 100% germination rate in controlled environment growth chambers, were used for propagation. Seedlings of *Peganum harmala* were hardened in greenhouse prior to transplanting in the field in February-March 2015. The selected site was cleaned and planting holes with 30 cm diameter were dug using hydraulic auger and tractor. *Peganum harmala* seedlings were planted at 2.0 x 2.0 m spacing. The irrigation system with water meters was installed with drip, subsurface at 15 cm depth and deep at 30 cm depth irrigation. Additionally, three irrigation levels (75, 50, and 100%) of estimated crop evapotranspiration [ETc] under three irrigation systems (drip, subsurface, and deep surface) were administered. Drippers with a discharge rate of 4 L/ h at 1.0 atmospheric pressure were used to supply a predetermined volume of water based on ETc. ETc and ETo (Reference evapotranspiration) were calculated using the FAO-Penman-Monteith method, as described in Allen et al. (1994a, b). A complete randomized block design was used with three replications of each treatment. Each treatment (plot) had 15 plants. Seedling survival and initial plant morphological parameters such as plant height and stem diameter were measured immediately after transplanting to the field and after completion of one growing season from which percent growth rate was determined. Data on survival, percent growth rate of plant height and percent growth rate of stem diameter were collected, compiled, and analyzed using SPSS software version 22.0 (IBM Corporations). Data was analyzed using two-way analysis with type and amount of irrigation as factors.

Results and Discussion

Although amount of irrigation did not significantly affect survival percentage, it showed an interesting trend in response to the amount of irrigation seedlings received. Seedlings that received (irrespective of irrigation type) 100% irrigation had a survival of 47% after first growing season compared to 65% survival in seedlings that received 50% irrigation. Seedlings that received 75% irrigation had 50% survival after the first growing season. Similarly, irrigation type also had no significant effect on seedling survival. The survival rates of *Peganum hermala* seedlings were 57%, 50% and 55% under drip, deep and sub-surface irrigation, respectively. Several abiotic and biotic factors may influence the survival and growth of plant species in restoration projects. In semiarid regions with Mediterranean-type climates, the availability of water has been considered the main abiotic factor limiting regeneration and restoration success (Rey-Benayas et al., 2002; Siles et al., 2010, Becerra et al., 2011). However, the efficiency and effectiveness of the irrigation systems depends on many factors including soil type, plant species, soil structure and soil fertility, weed competition, and site microclimate. Hence, understanding what techniques can enhance the survival and growth in dry ecosystems, and also reduce the need irrigation, is crucial for planning the restoration of desert lands.

We also measured responses in growth parameters under different irrigation types with varying amount of irrigation. The effect of irrigation type and amount of irrigation was evident in the growth parameters measured in *Peganum hermala* seedlings. For example, type of irrigation had significant ($p<0.001$) effect on percent height growth of *Peganum harmala* (Fig. 1). Seedlings irrigated with drip irrigation exhibited significantly higher height increment than those were irrigated with deep irrigation. There were no significant differences in height increment between drip and sub-surface irrigated seedlings. However, type of irrigation and amount of irrigation did not have a significant ($p<0.07$) interaction effect on percent height growth of *Peganum harmala* (Figure 1).

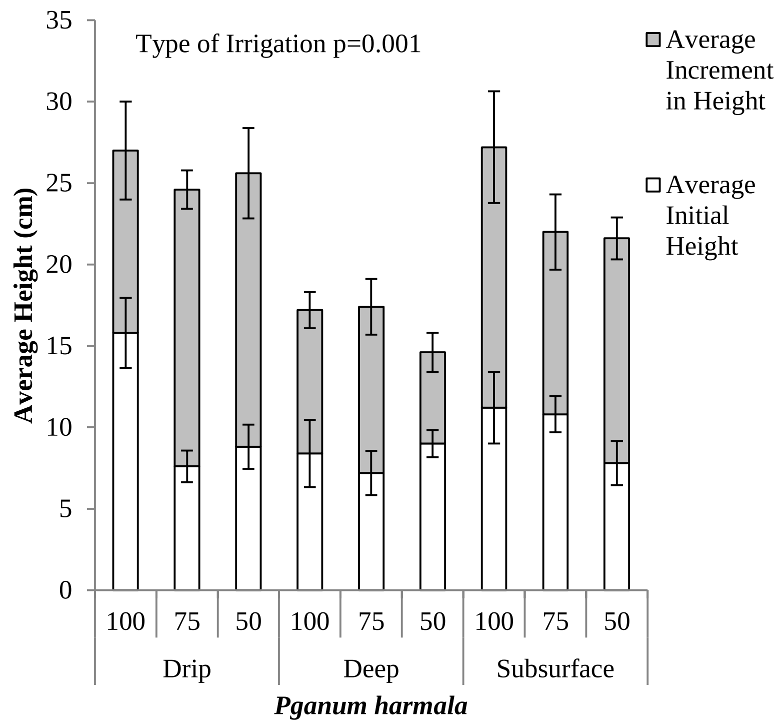


Figure 1. Effect of irrigation amount and irrigation types on height increment of *Peganum hermala* seedlings.

Similarly, irrigation type had significant ($p<0.020$) effect on percent stem diameter growth (Figure 2). Seedlings irrigated with drip irrigation showed significantly higher stem diameter growth than deep irrigated seedlings. However, there were no significant differences in the increment of stem diameter between drip and sub-surface irrigated seedlings (Figure 2).

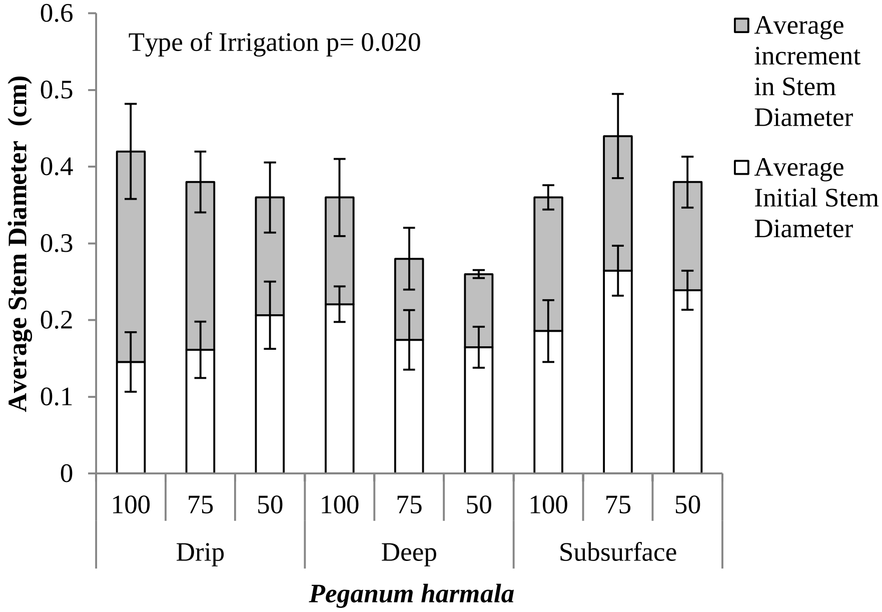


Figure 2. Effect of irrigation amount and irrigation types on stem diameter increment of *Peganum hermala* seedlings.

Both irrigation type (p<0.0001) and irrigation amount (p<0.0001) had significant effect on the canopy cover increment of *Peganum hermala* seedlings after the first growing season (Figure 3). There were also significant (p<0.038) irrigation type and irrigation amount interaction effect on canopy cover increment (Figure 3). Drip irrigated seedlings showed significantly higher canopy cover increment than those irrigated with deep irrigated seedlings. Moreover, canopy cover increased under sub-surface irrigated seedlings as amount of water decreased. Similar trend was observed under drip irrigated seedlings but not as distinctive and pronounced as was observed under sub-surface irrigated seedlings. It is worth mentioning here that *Peganum hermala* seedlings that were irrigated to field saturation had the highest canopy size compared to other native species such as *Lycium shawii, Nitraria retusa, Ochradenus baccatus, and Rhanterium epapposum* (Almulla et al., 2017).

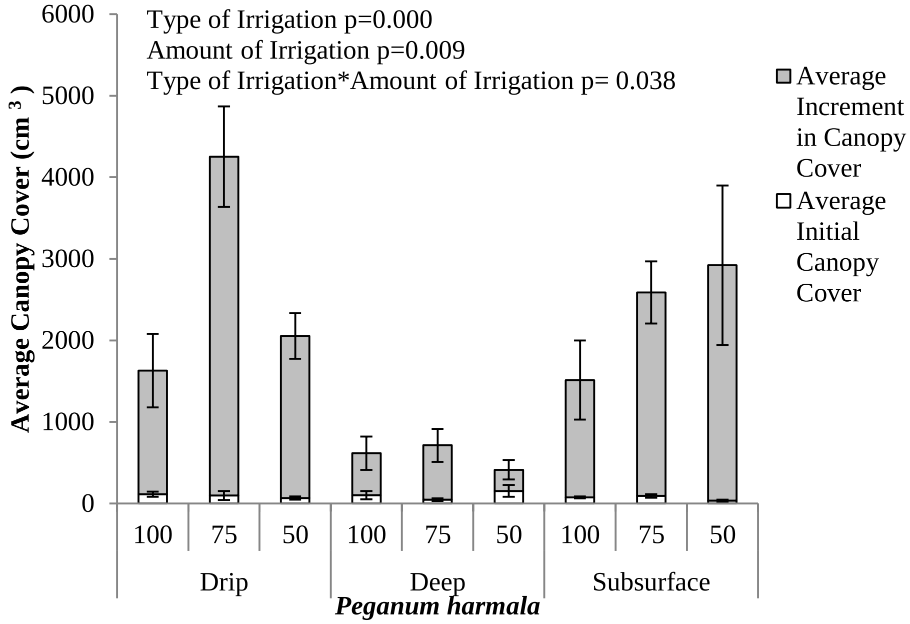


Figure 3. Effect of irrigation amount and irrigation types on canopy cover increment of *Peganum hermala* seedlings.

These observed growth responses could be important as they influence the species' productivity (Jacobs et al., 2005) and could be used for future restoration projects undertaken in Kuwait. It was observed that the irrigation type had a significant effect on height, crown diameter, and stem diameter increment. The results of this study are consistent with a previous study where irrigation was shown to increase the survival and growth of native species in a degraded ecosystem of central Chile (Becerra et al., 2013). However, the our observation are in contrast to the one observed by Suleiman et al. (2011) where non-water stressed *Peganum hermala* showed maximum height and canopy cover.

The significant increment in height and canopy cover in *Peganum harmala* could be attributed to their ability of greater light interception and carbon gain (Van Volkenburgh, 1999), which will ultimately, at a larger scale, contribute toward ecosystem productivity. When irrigation types were taken into consideration, it can be concluded that the seedlings of *Peganum harmala* showed superior growth under drip irrigation when irrigated at 50% level. It is plausible that providing drip irrigation at 100% level could be counterproductive to achieve higher growth. Given that sandy soil predominates the experimental site, supplying water at 100% level could lead to a higher infiltration loss. Moreover, watering one-year-old seedlings with deep irrigation could have also resulted in higher infiltration loss as most of the seedlings of *Peganum harmala* did not develop a deep enough root system to exploit the water supplied by the deep irrigation system. From our observation, it can be concluded that drip irrigation was most effective on the survival and height growth of *Peganum hermala*. Carbon gain and biomass allocation strategy in relation to water availability of a species are strong indicators of the species' long-term growth performance. Therefore, these factors should be critically evaluated when selecting native species for future restoration projects.

Acknowledgements

The authors would like to thank Kuwait Institute for Scientific Research for providing funds, logistical support and encouragement during the implementation of this project.

References

Al-Awadhi, J. M.; R. Misak; and S.A.S. Omar. 2003. The causes and consequences of desertification in Kuwait: A case study of land degradation. *Bulletin of Engineering Geology and the Environmen* **62**: 107–115.

AlMulla, L; M.K. Suleiman; N.R. Bhat; S. Jacob; M.A. Islam; and R.R. Thomas. 2017. Propagation Studies on Selected Keystone Plant Species for Use in Restoration Projects in Kuwait. *International Journal of Agricultural Science* **2:** 27-31.

Allen, R.G.; M. Smith; and L.S. Pereiara. 1994a. An update for the definition of reference evapotranspiration. *International Council of Irrigation and Drainage Bulletin* **43**: 1-34.

Allen, R. G.; M. Smith; and L.S. Pereiara. 1994b. An update for the calculation of reference evapotranspiration. *International Council of Irrigation and Drainage Bulletin* **43**: 35-92.

Becerra, P.; V. González; C. Smith-Ramírez; and J. Armesto. 2011. Spatio-temporal variation in the effect of the herbaceous layer on seedling survival of woody species in a semiarid ecosystem. *Journal of Vegetation Science* **22**: 847-855.

Becerra, P. I.; G. Cruz; S. Ríos; and G. Castelli. 2013. Importance of irrigation and plant size in the establishment success of different native species in a degraded ecosystem of central Chile. *Bosque* **34**(1): 103-111.

Jacobs, D. F.; F.K. Salifu; and J.R. Seifert. 2005. Relative contribution of initial root and shoot morphology in predicting field performance of hardwood seedlings. *New Forests* **30**:235–251.

Omar, S.A.S.; Y. Al-Mutawa; and S. Zaman. 2009. Vegetation of Kuwait. Kuwait Institute for Scientific Research. pp:79.

Rey Benayas, J.M.; A. López-Pintor; C. García; N. de la Cámara; R. Strasser; and A. Gómez. 2002. Early establishment of planted Retama sphaerocarpa seedlings under different levels of light, water and weed competition. *Plant Ecology* **159**: 201-209.

Siles, G.; P.J. Rey; J.M. Alcántara; J.M. Bastida; and J.L. Herreros. 2010. Effects of soil enrichment, watering and seedling age on establishment of Mediterranean woody species. *Acta Oecologica* **36**: 357-364.

Suleiman, M.K.; N.R. Bhat; S. Jacob; R.R. Thomas; and G. D'Cruz. 2011. Performance of selected native plants under deficit irrigation. *World Journal of Agricultural Sciences* **7(1)**: 19-25.

Van Volkenburgh, E. 1999. Leaf expansion – An integrating plant behaviour. *Plant, Cell and Environment*. **22**: 1463–1473.

Section 5

Seed-based Restoration of Dryland Ecosystems

Utilization of Desert Rodents in Dispersing Native Seeds: Potential Role in Restoring Damaged Dry Lands

Matrah A. Al-Mutairi
Kuwait Institute for Scientific Research, Kuwait
e-mail: mmutairy@kisr.edu.kw

Abstract

Desert rodents play crucial role in consuming and dispersing native seeds in different habitats but in dry range lands, this can be critical since seeds are limited and weather conditions are harsh. The advantage for rodents feeing on native seeds is that it can help in dispersing seeds by caching and storing them in burrows underground which may support the growth of these seeds into seedlings and full plants. This study was designed to investigate the consumption rate of the main rodent lesser jerboa in the arid ecosystems of Kuwait for native seeds; three experiments were performed using the seeds of native annual and perennial plants. In a cafeteria-style choice experiment, lesser jerboas were found to feed on the seeds of the perennial *Haloxylon salicornicum* (46%), followed by the annuals Savignya parviflora (21%) and *Brassica tournefortii* (17%), and the perennial *Rhanterium epapposum* (16%). However, consumption of seeds from the two perennial bushes under laboratory conditions was not significantly different, perhaps because seeds of the less preferred species have a lower protein content, so that more of them (by weight) were consumed. Microscopic analysis of the contents of wild-caught lesser jerboa digestive tracts showed that the seeds of annual plants were present at high frequencies. The mean (± standard deviation) number of seed fragments in the digestive tracts of 21 lesser jerboas identified as the annual Savignya parviflora was 3.0 ± 2.0, for perennial *Rhanterium epapposum* it was 2.1 ± 1.0 and for annual Brassica tournefortii 0.8 ± 1.0. *Haloxylon salicornicum* was not included as it does not grow in the same study site as the other three plants types. The findings support that lesser jerboa feeds on seeds of both annual and perennial plants and it might be used as a seed disperser for vegetation of damaged ecosystems.

Keywords: lesser Jerboa, Annual, Perennial, Consumption, Utilization.

Introduction

Understanding the relationships between the fauna and flora of ecosystems is crucial to understanding the links and processes that shape ecosystems. Arid ecosystems are of special importance because they are under threat of desertification; this result in habitat loss for many plants and animals adapted to arid conditions. The vegetation of Kuwait is of particular scientific interest because the mean annual rainfall is high compared to that in most deserts (almost 120 mm). As argued by Shmida (1985) and Shmida and Wilson (1985), it can therefore be regarded as representing a transitional stage between semi-desert and desert vegetation. Such transitional areas are highly sensitive to human-induced change and therefore provide an effective index of human interference (Schlesinger et al. 1990).

Plants are vital in arid ecosystems; they provide food and shelter for animals, and stabilize soil, thus reducing erosion. Arid and semi-arid regions are characterized by mostly annual plants which complete their life-cycle within the short wet season (Zohary 1973). In the arid lands of Arabia, drought and high temperatures place severe pressures on plant growth from late spring to early autumn. These conditions generally favour short-lived life forms such as annual plants, because their seeds spend most of the environmentally harsh period in dormancy, thus allowing these plants to survive (Baskin and Baskin 1998). About 70% of the plants known from Kuwait are annuals and this proportion is substantially higher in desert areas (Boulos and Al-Dosari 1994).

The rationale for studying the diet of many animal species, including rodents, is that food has an important effect on breeding and the population dynamics of rodents, and they in turn play an important role in ecosystems dynamics through seed predation and dispersal. Seeds represent an important source of food for most rodents in arid ecosystems. There is a shortage of available water, and the high fat content in seeds provides a significant source of metabolic water. In addition, seeds are slow to digest, high in energy, and have higher concentrations of nutrients than any other part of the plant. Rodents favour the seeds of perennial plants, but only if other seed types are abundant (Graetz 1981); most desert rodents are granivorous. Ants, on the other hand, exert large predation pressure on seeds. Due to their large numbers, they can harvest seeds very quickly and efficiently. The majority of seeds harvested by ants are either fed to their brood or stored in their nest (Graetz 1981). In Kuwait, there are only two species of ant that consume seeds alongside other animal and plant parts (Delima et al. 2002). Termites also consume seeds, but their number and distribution is very limited in Kuwait, so their effect is negligible (Zaman et al. 2005).

Desert animals, such as rodents, ants and birds, may also affect the composition and distribution of populations of plants by selecting, fetching and conveying seeds (Gutierrez and Whitford 1987). Seed dispersal by rodents plays a critical role in the seeding and distribution of plants (Hulme 1998). Rodents may forage for seeds all year round (uninterrupted by migration, dormancy or diet switching). As explained by Longland and Bateman (1998), rodents are capable of detecting seeds buried at different digging them up, transporting them, and of burying seeds at different locations and depths. Seed germination is generally affected by light, temperature and rainfall, and depends on the depth in the soil to which they are buried (Xuehua et al. 2008). Desert rodents' seed hoarding behavior plays a key role in placing a variety of seeds at different microhabitats, which helps in germination. Rodents therefore have both beneficial and harmful effects on seeds within a landscape. Seeds are subject to predation before, during, and after dispersal (Janzen 1971). Non-hoarding rodents may be detrimental to seed survival and germination, by acting as seed predators (Heske et al. 1993; Howe and Brown 1999). Others with hoarding instincts can be detrimental or beneficial to seed survival, depending on the levels of hoarding and immediate predation (Kerley et al. 1997; Vander Wall and Longland 2004; Lobo et al. 2009). Internationally, small mammal species are recognized as partly responsible for maintaining plant communities, by preventing the establishment and dominance of woody plant species, and by thus preventing the transition of grassland areas into woodlands (Weltzin et al. 1997). Rodents and ants have been shown to decrease seed reserves of desert annual plant communities (Reichman 1979; Abramsky 1983). Large seeds are most affected by rodent predation; plants could therefore avoid or reduce predation by producing small seeds in large quantities rather than fewer large seeds (Archer and Pyke 1991).

In summary, rodents are key ecosystem engineers which affect plant community patterns by burrowing, hoarding, predating and dispersing seeds, consuming plants, urinating and defecating, and by shaping plant evolution (Martin 2003; Yoshihara et al. 2009). Ecosystem engineers may assist in conservation and management projects (Boogert et al. 2006; Byers et al. 2006). Organisms tend to have more positive ecosystem effects in more harsh environments, through the amelioration of physical stress (Crain 2008). Thus, rodent ecosystem engineers could be important facilitators of restoration in degraded. The lesser jerboa feeds on seeds, among other plant parts such as roots, sprouts, grains and cultivated vegetables (Nowak 1999), and stores seeds at its burrow. The aim of this study was to examine the seed choices of the lesser jerboa, which has a potential to be used as an ecosystem engineer for the rehabilitation of degraded lands in the State of Kuwait. Seed consumptions were tested in the field, in the laboratory, and by analysis of the diet of wild-caught individuals, to determine to what extent seeds of perennial plants are consumed by the lesser jerboa, and to what extent perennial plants used in ecosystem rehabilitation can support the lesser jerboa. Perennial plants are widely used to restore degraded habitats in Kuwait due to their ability to stabilize sand and provide shelter and food for different wild animals. I used lesser jerboa from different parts of Kuwait to determine their consumption for the seeds of perennial plants, and to what extent perennial plants used in ecosystem rehabilitation can support the lesser jerboa..

Materials and Methods

Different lesser jerboas were used for each of the three experiments detailed below; no individual was used in more than one experimental design. Animals were collected from different parts of Kuwaiti arid lands except for the third experimental set where digestive tract contents were analyzed. These were all collected from the protected Kabd Research Station where both annual and perennial tested plants are present.

Native seed choice in the feeding arena: preference trials were conducted in arenas, to determine whether the lesser jerboa have different consumption rates for the seeds of plant species growing naturally in the arid lands of Kuwait, in the habitats where lesser jerboas are found. 12 lesser jerboas were spotlighted (similar approach as in Scott et al. 2005) and captured in various parts of the Kuwaiti desert between March and May 2015. Animals were placed in feeding arenas with four standard glass Petri dishes. Each of these dishes was 5 cm apart from the next and contained one type of four species of seeds, in a ‘cafeteria style’ choice experiment. All experiments were carried under natural photoperiod and ambient temperature. Each animal was brought in a cage that was placed at one end of the arena opposite to the placed Petri dishes.

It was then released and allowed to explore the four Petri dishes. When the animal starts to feed on a seed type, then, this seed type was recorded as the first feeding choice of the animal.

The feeding arena consisted of an outdoor enclosure 2 meters in diameter, containing clean sand and covered on top by a mesh barrier that allowed easy observation and ventilation. The enclosure was big enough to allow free movement, and animals were able to explore before choosing. The trials took place immediately after sunset, when lesser jerboas start to forage for food in the wild. Animals were kept for four days to perform these tests. During this period, they were kept at the Animal House Unit at the Biological Sciences department, Faculty of Science, Kuwait University. Animals were mostly fed by commercial rodent's pellets and fresh lettuce leaves when not in feeding trials. No seed remains were collected at this experiment as it proved almost impossible to collect seeds and seed parts from the sand. Therefore, the net weights of seed types consumed were carried in a different experimental procedure. Seeds were collected from the study sites and frozen until needed. Three species from the Kabd intact area *(R. epapposum, Brassica tournefortii and Savignya parviflora*), and one from outside this area (*H. salicornicum*), were chosen because they are abundant and easily identified and handled. Rodent tracks were seen next to these plants, and their seeds were found at the entrance of some rodents' burrows as well as underneath bushes. *R.epapposum*, a perennial bush, is the dominant plant species in the Kabd area and is widely used for restoration processes. H. salicornicum, another perennial bush, dominates in the north of Kuwait and was collected in November 2010 from the Rawdatayn area and frozen. H. salicornicum is also a sand stabilizer and widely used for restoration projects.

Native seed consumption in the laboratory: 28 lesser jerboas were caught in the western and northern Kuwaiti desert, including at the Kabd research station located in the Kabd area: ten were released immediately at the capture point because they were either juvenile, pregnant or lactating. Animals were spotted on night transects carried out from slow-moving vehicles with powerful spotlights, and then caught by net. After capture, individuals were weighed to the nearest 0.5 g using a Pesola light-line spring scale, 1 kg. Animals were sexed and aged (adult or juvenile) and their breeding condition determined. Males were classified as scrotal or non-scrotal, females as perforate, non-perforate, pregnant or lactating (enlarged nipples). The 18 individuals retained for the experiment were transported to the Animal House at Kuwait University, Biological Sciences Department, held in individual metal cages with solid plastic floors containing rodent bedding, and given a diet of rodent pellets and lettuce leaves to provide moisture. Animals were allowed three days to settle before the start of feeding trials. They were kept in a dedicated room in the animal house without artificial lighting, to mimic the natural photoperiod cycle experienced by lesser jerboas, which are nocturnal and spend the day in burrows. The room had a controlled temperature of 20 - 25°C. Before the experiment, bedding and all other food were removed from the cages to facilitate seed collection, and each lesser jerboa was served with 2.0 g of seeds from either *R. epapposum* or H. salicornicum on a Petri dish. The animal was left to feed for 24 hours, and then the remaining seeds (whole, partially eaten, or husked) were removed, separated from any faeces, and weighed to calculate the net weight of seeds consumed. This was repeated on the following day using the other seed species. Each of the 14 animals which survived for the entire experiment completed four trials for each seed species.

Microscopic analysis of digestive tracts for native seeds: reference slides were made from the seeds of three plant species collected from Kabd (*R. epapposum, B. tournefortii and S. parviflora*) which were crushed and preserved in ethanol. These species were chosen because they were abundant and easy to identify and collect, and because their seeds are easy to identify under light microscopy. Reference slides were prepared by straining the seed suspension (mesh size 0.40 mm x 0.40 mm; see Best et al. 1993); a drop of the strained suspension was placed on a clean slide, covered by a cover slip, and examined under light microscopy, paying particular attention to the size, shape and colour of the seed fragments.

Twenty-one lesser jerboas were collected from the KISR research station at Kabd in April, May and June 2011 when most plants shed seeds, killed by placing in a tightly closed container with high volumes of ether. Once confirmed dead, the animal was dissected on the same night of capture before any change to the contents of its digestive tract could occur. The entire digestive tract from the oesophagus to the anus was removed, and the stomach, small intestine, and large intestine separated, washed and weighed. The caecum and faeces were discarded, as they did not provide useful dietary information; the faeces contained empty seed capsules without any colour or details. The stomach contents and the crushed intestinal contents (pellets) were preserved in 10 ml of 80% ethanol.

After five to seven days the samples were examined under light microscopy. A subsample was passed through a sieve with a mesh size of 0.40 mm x 0.40 mm, a drop of the strained suspension placed on a microscope slide, covered by a cover slip and the slides allowed to dry for one hour on a hot plate. Two slides were prepared for each sample. Measurement slides were not used because objects which could be recognized under light microscopy were large and difficult to fit within measurement grids. Ten non-overlapping fields were searched using 20x magnification (Leica light microscope DM LS2), similar to Reichman (1975), and objects seen were counted and identified using the reference slides. Seed fragments from each plant species had characteristic features, including shape, colour and size. Non-identified objects were discarded from the count. Photographs were taken of both reference and sample slides using a camera (Zeiss, CP-ACHROMAT) at 40x magnification (Zeiss light microscope Axioskop 40).

Data on native seed choices in the feeding arena were presented as the percentage of trials in which each seed species was eaten first. Data on native seed consumption in the laboratory were analyzed using a General Linear Model (GLM) for repeated measures. The net weight of consumed seeds of Rhanterium epapposum and Haloxylon salicornicum (weight of seeds offered – remaining seeds on the following day) was the dependent variable. Multivariate analysis of covariance (MANCOVA) was also used to explore the data. To analyze the data from the microscopic analysis of digestive tracts for native seeds, the number of seed fragments from each species was counted in each field, and the mean number for each plant species for each animal calculated using Microsoft Excel. Univariate analysis of variance (ANOVA), least significant difference (LSD), Sidak and Student-Newman-Keuls tests were used to compare the means. All statistical analysis was carried using SPSS (version 18).

Results and Discussion

Native seed choice in the feeding arena: the 12 lesser jerboas, in 48 trials, showed significantly higher feeding choices for H. salicornicum (0.46 ± 0.5). Other seeds showed no particular differences in their consumption rates; *S. parviflora* (0.21 ± 0.4), *B. tournefortii* (0.17 ± 0.38) and *R. epapposum* (0.16 ± 0.38). Data are reflected and summarized in Table 1. Post hoc test (LSD) was performed to examine significant differences among the tested seed groups.

Table 1. Post hoc test (LSD) Showing Levels of Feeding on the Seeds of *H. salicornicum*, *R. epapposum*, *B. tournefortii* and *S. parviflora* in the feeding areans reflected by 12 lesser jerboas.

Multiple Comparisons

Consumption
LSD

(I) Seed type	(J) Seed type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
H. salicornium	R. epapposum	.292*	.086	.001	.12	.46
	B. tornifortii	.292*	.086	.001	.12	.46
	S. parviflora	.250*	.086	.004	.08	.42
R. epapposum	H. salicornium	-.292*	.086	.001	-.46	-.12
	B. tornifortii	.000	.086	1.000	-.17	.17
	S. parviflora	-.042	.086	.628	-.21	.13
B. tornifortii	H. salicornium	-.292*	.086	.001	-.46	-.12
	R. epapposum	.000	.086	1.000	-.17	.17
	S. parviflora	-.042	.086	.628	-.21	.13
S. parviflora	H. salicornium	-.250*	.086	.004	-.42	-.08
	R. epapposum	.042	.086	.628	-.13	.21
	B. tornifortii	.042	.086	.628	-.13	.21

*. The mean difference is significant at the 0.05 level.

Native seed consumption in the laboratory: a total of 112 seed trials, 56 for R. epapposum and 56 for H. salicornicum, were conducted. Data were normally distributed (Kolmogorov–Smirnov test) and a GLM for repeated measures carried out to assess if the lesser jerboa has a preference towards the seeds of one of the two perennial plants. MANCOVA was used to investigate if there were differences between the 14 individual lesser jerboas used in this experiment. Within this test, there are several tests to calculate the differences in seed consumption. None of these tests were significant. Pillai's trace, Wilks's Lambda, Hotelling's Trace and Roy's Largest Root gave p = 0.22. Mauchly's test of sphericity gave p = 0.23. This indicated that sphericity exists in the data and results are accepted. The native seed consumption trials in the laboratory showed that the animals do not have a preference between *R. epapposum* and *H. salicornicum*.

Microscopic analysis of digestive tracts for native seeds: counts of identified seed fragments from a total of 840 microscopic fields were normally distributed (independent samples Kruskal-Wallis test; [stats] P<0.001). The univariate ANOVA for digestive tract contents of lesser jerboas revealed significant differences between the mean number of seed fragments per plant species, per digestive tract of seeds of *Rhanterium epapposum* (mean ± standard deviation: 2.1 ± 1.0), *Brassica tournefortii* (0.8 ± 1.0) and *Savignya parviflora* (3.0 ± 2.0). Post-hoc tests including the least significant difference (LSD) test and Sidak showed that the mean number of seed fragments found for each plant species was significantly different (Table 2). The 21 lesser jerboas showed individual variation in the number of seed fragments which could be identified as belonging to each of the three plant species.

Table 2. Post-hoc Tests Following a Significant ANOVA Show Significant Differences Between the Numbers of Seed Fragments Identified as Belonging to Three Plant Species in the Digestive Tract of Lesser Jerboas Caught from Arid Ecosystems of Kuwait.

Multiple Comparisons

Dependent Variable:Number of identified pieces

	(I) Name of tested plant seeds	(J) Name of tested plant seeds	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	R epapposum	B tournifortti	1.3036*	.25756	.000	.7963	1.8108
		S parvriflora	-.7857*	.25756	.003	-1.2930	-.2784
	B tournifortti	R epapposum	-1.3036*	.25756	.000	-1.8108	-.7963
		S parvriflora	-2.0893*	.25756	.000	-2.5966	-1.5820
	S parviflora	R epapposum	.7857*	.25756	.003	.2784	1.2930
		B tournifortti	2.0893*	.25756	.000	1.5820	2.5966
Sidak	R epapposum	B tournifortti	1.3036*	.25756	.000	.6844	1.9227
		S parvriflora	-.7857*	.25756	.008	-1.4049	-.1666
	B tournifortti	R epapposum	-1.3036*	.25756	.000	-1.9227	-.6844
		S parvriflora	-2.0893*	.25756	.000	-2.7084	-1.4701
	S parviflora	R epapposum	.7857*	.25756	.008	.1666	1.4049
		B tournifortti	2.0893*	.25756	.000	1.4701	2.7084

Based on observed means.
The error term is Mean Square(Error) = 2.786.

*. The mean difference is significant at the 0.05 level.

Conclusions

Seed selection, removal and subsequent management by granivores animals is the result of complex interactions between factors including qualities of the seeds and features of the local habitat. In recognition of the urgent need to restore degraded ecosystems in the State of Kuwait, and redress the balance by mitigating the ecological damage largely caused by the Iraqi invasion and subsequent aftermath, the United Nations Compensation Committee (UNCC) has approved compensation funds to allow Kuwait to launch major restoration projects. The main focus of these projects is to establish 70 protected areas with a total area of 420 km2 (Omar 2011). *R. epapposum* and *H. salicornicum* are widely used for restoration projects in Kuwait, and their seeds will be produced in large quantities, in order to establish the shrubs to restore the newly protected areas. *R. epapposum* and *H. salicornicum* plants, once established, are capable of building up a large seed bank in deep sandy soils; seeds remain viable for at least 4 years (Omar and Bhat 2008).

Acknowledgements

Appreciation is extended to Kuwait University, Faculty of science for allowing to use the facilities of the Animal House Unit; University of Bristol for providing the scientific and technical advice; and to Kuwait Institute for Scientific Research for funding this study and allowing the use of laboratory and Agricultural Research Station for data collection and analysis.

References

Abramsky Z, 1983. Experiments on seed predation by rodents and ants in the Israeli desert. Oecologia 57, 328-332.

Archer S & Pyke D, 1991. Plant-animal interactions affecting plant establishment and persistence on revegetated rangeland. Journal of Range Management 44, 558-565.

Baskin C & Baskin J, 1998. Seeds Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego, USA.

Best T, Skupski M, & Smartt R, 1993. Food habits of sympatric rodents in the Shinnery Oak-Mesquite grasslands of southeastern New Mexico. The Southwestern Naturalist 38, 224-235.

Boogert N, Paterson D, & Laland K, 2006. The Implications of niche construction and ecosystem engineering for conservation biology. BioScience 56, 570-578.

Boulos L, & Al-Dosar M, 1994. Checklist of the flora of Kuwait. Journal of the University of Kuwait (Science) 21, 203-218.

Byers J, Cuddington K, Jones C, Talley T, Hastings A, Lambrinos J, Crooks G, & Wilson W, 2006. Using ecosystem engineers to restore ecological systems, Trends in Ecology and Evolution 21, 493-500.

Crain C, 2008. Interactions between marsh plant species vary in direction and strength depending on environmental and consumer context. Journal of Ecology 9,166-173.

Delima E, Peacock J, & Khalil E, 2002. Bibliographic list of the wildlife fauna of Kuwait.Technical Report. Kuwait Institute for Scientific Research, Kuwait.

Graetz R, 1981. Animal-animal interactions. Arid-land Ecosystems: Structure, Functioning and Management, 2nd edn. (eds D. Goodall, R. Perry, & K. Howes) pp. 83-103. Cambridge University Press, Cambridge.

Gutierrez J, & Whitford W, 1987. Chihuahuan desert annuals: importance of water and nitrogen. Ecology 68, 2032-2045.

Heske E, Brown J, & Guo Q, 1993. Effects of kangaroo rat exclusion on vegetation s tructure and plant species diversity in the Chihuahuan Desert. Oecologia 95, 520-524.

Howe H, & Brown J, 1999. Effects of birds and rodents on synthetic tall grass communities. Ecology 80, 1776-1781.

Hulme P, 1998. Post-dispersal seed predation and seed bank persistence. Seed Science Research 8, 513-519.

Janzen D, 1971. Seed predation by animals. Annual Review of Ecology and Systematics 2, 465-492.

Kotler B, & Brown J, 1999. Mechanisms of coexistence of optimal foragers as determinants of local abundances and distribution of desert granivors. Journal of Mammalogy 80, 361-374.

Lobo N, Duong M, & Miller J, 2009. Conifer-seed preferences of small mammals. Canadian Journal of Zoology 87, 773-780.

Longland W, & Bateman S, 1998. Implications of desert rodent seed preferences for range remediation. Journal of Range Management 51, 679-684.

Martin G, 2003. The role of small ground-foraging mammals in topsoil health and biodiversity: Implications to management and restoration. Ecological Management & Restoration 4,114-119.

Nowak R, 1999. Walker's Mammals of the World, 6th edn. The John Hopkins University Press, Baltimore.

Omar S, "Restoration and remediation of Kuwait's damaged natural resources as a consequence of war" in The 15th Meeting of the Subsidiary Body on Scientific, Technical and Technological Advice of the Convention on Biological Diversity, 2011. Montreal, Canada. 7-11 November, 2011.

Omar S, & Bhat N, 2008. Alteration of the Rhanterium epapposum plant community in Kuwait and restoration measures. International Journal of Environmental Studies 65,139-155.

Omar S, Bhat N, & Asem A, 2009. Critical assessment of the environmental consequences of the invasion of Kuwait, the Gulf War, and the aftermath. Handbook of Environmental Chemistry 3U, 141-170.

Reichman O, 1975. Relationship of desert rodent diets to available resources. Journal of Mammalogy 56 (4), 731-751.

Reichman O, 1979. Desert granivore foraging and its impact on seed densities and distributions. Ecology 60, 1085-1092.

Schlesinger W, Reynolds J, Cunningham G, Huenneke L, Jarrell W, Virginia R, & Whitford W, 1990. Biological feedbacks in global desertification. Science 247, 1043-1048.

Scott D, Waite S, Maddox T, Freer R, & Dunstone N, 2005. The validity and precision of spotlighting for surveying desert mammal communities. Journal of Arid Environments 61, 589-601.

Shmida A, 1985. Biogeography of the desert flora. Ecosystems of the World 12A. Hot Deserts and Arid Shrublands (eds M. Evenari, I. Noy- Meir & D. Goodall), pp. 23-77. Elsevier, Amsterdam.

Shmida A, & Wilson M, 1985. Biological determinants of species diversity. Journal of Biogeography 12, 1-20.

Vander Wall S, & Longland W, 2004. Diplochory: are two seed dispersers better than one?. Trends in Ecology and Evolution 19, 297-314.

Weltzin J, Archer S, & Heitschmidt R, 1997. Small mammal regulation of vegetation structure in a temperate savanna. Ecology 78, 751-763.

Xuehua L, Xiaolan L, Deming J, Zhimin L, & Qinghe Y, 2008. Annual plants in arid and semi-arid desert regions. Frontiers of Biology in China 3, 259-264.

Yoshihara Y, Okuro T, Undarmaa J, Sasaki T, & Takeuchi T 2009. Are small rodents key promoters of ecosystem restoration in harsh environments? A case study of abandoned croplands on Mongolian grasslands. Journal of Arid Environments 73, 364–368.

Zaman S, Peacock J, Al-Mutairi M, Delima E, Al-Othman A, Loughland R, Tawfiq H, Siddiqui K, Al-Dossery S, & Dashti J, 2005. Wildlife and vegetation survey (Terrestrial Cluster 3) Addendum 2. Kuwait Institute for Scientific Research, KISR 7885, Kuwait.

Zohary M, 1973. Geobotanical Foundations of the Middle East. 2nd edn. Gustav Fischer Verlag, Berlin.

Role of Management Practices for Successful Restoration of Degraded Ecosystems: A Case Study of Pastoral Ecosystems in Eastern Morocco

Taleb Mohammed Sghir[1] and Acherkouk Mohamed[2]
[1]InstitutScientifique, Mohammed V University in Rabat
[2]Centre Régional de la RechercheAgronomique de Tanger
e-mail: talebmsg@yahoo.com

Abstract

Pastoral ecosystems including forests, uncultivated land cover about 92% of the total area of Morocco. Nearly 97% of these ecosystems are located in the arid and semiarid zones in the Oriental, pre-Sahara, and Sahara regions occupying about 51 million ha and covering between 30 and 60% of livestock needs by region and year. Eastern Morocco has about 4.7 million ha, about 9% of the national level, of which the arid steppes of the Highlands of Eastern Morocco represent more than 5.6 % of the arid steppes (> 3 million h) and satisfy in a good year up to 80% of the feed needs of more than 1.5 million heads of sheep and goats. The natural vegetation is mainly composed of steppes based on *Stipa tenacissima*, *Artemisia herba alba*, *Hammada scoparia*, *Noaea mucronata* and pre-Saharan steppes. These pastoral ecosystems, which play an important role in the economy of the region and in the establishment of the pastoral populations, are traditionally the main pastoral resources for the populations in these areas. Pastoral ecosystems are increasingly subjected to major degradation pressures caused by clearing and cultivation, abusive harvesting of firewood and permanent constructions, etc. To these human impacts are added severe and structural effects and impacts of climate change, marked in particular, by the spread of desertification and recurrent droughts, accentuating degradation of these areas and aggravating the fragility of the ecological and environmental balances and the living conditions of the local populations. In the presence of this situation of almost universal and continuous degradation, several programs for the development, restoration, and enhancement of these pastoral ecosystems have been undertaken for their rehabilitation. These programs are aimed at, among other things, pastoral improvement through the rehabilitation and enrichment of the vegetation cover in order to conserve the soil, increase pastoral productivity, and reduce degradation.

Keywords: Restoration, management practices, pastoral ecosystems, eastern Morocco.

Introduction

In Eastern Morocco, arid pastures cover more than three million hectares and support more than 1.5 million head of sheep and goats (High Commission for Planning, 2005). They are dominated by steppe ecosystems based on *Stipa tenacissima*, *Artemisia herba alba*, *Noaea mucronata*, *Hammada scoparia*, *Ziziphus lotus* etc. Overgrazed and cleared by population growth and irrational management, grazing area in Morocco currently contributes only 26% of the national food needs, compared with 60% in 1970 (Food and Agriculture Organization, 1996). Several studies have shown that these pastures are being degraded due to the synergy between anthropogenic impact (overgrazing, clearing, excessive firewood harvesting and extension of cultivated land) and climate (recurrent droughts). (Acherkouk et al., 1998 ; Mahyou et al., 2000 ; Rahmi et al., 2000 ; Acherkouk et al., 2006 ; Dutilly-Diane et al., 2007 ; Maatougui et al.,2011). To reverse trends, Morocco has developed a Strategy for the Development of Agriculture (Green Morocco Plan) adopted by the Moroccan Government in April 2008.This strategy is based on the analysis of the constraints of the agricultural sector to develop agriculture and fight against poverty. Several studies have been carried out to assess the impact of overgrazing on the evolution of pastoral ecosystems and their restoration (Acherkouk et al., 2011; Acherkouk et al., 2012; Acherkouk et al., 2013; Hachmi et al., 2015; etc.).

Materials and Methods

In order to assess the impact of overgrazing on the degradation of pastoral ecosystems and emphasize the role of Management Practices for Successful Restoration of Degraded Ecosystems in Eastern Morocco (Jfira, Karkour, Sidi Moussa, Mabarek El Ibil, Gaâda, Boumaâzouz, Irzaine sites), three restoration techniques were tested:

- Resting
- Alley-cropping (planting of forage (*Atriplex nummularia* plantation) and grass shrubs)
- Controlled grazing with or without long-lasting resting

The estimated vegetation parameters by management type are Phytomass, botanical composition and vegetation cover.

The results obtained from experiments and field measurements have been compared to free grazing chosen as a reference.

Results and Discussion

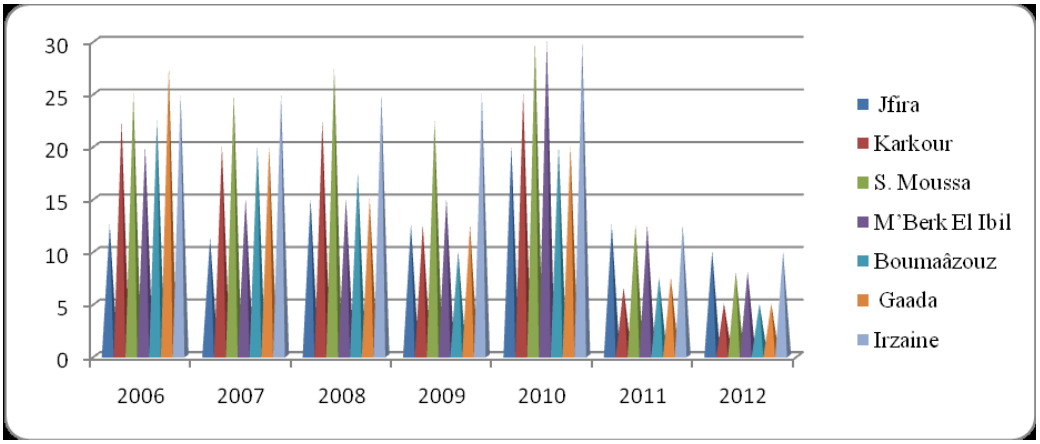
The analysis of the evolution of the various parameters studied indicates that the elimination of the grazing by the rest and Alley-cropping has allowed the installation of favorable conditions favoring a revitalization of the vegetation by the increase of the specific richness, shrubs mainly represented by *Artemisia herba alba*, *Stipa tenacissima*, *Noaea mucronata*, *Hammada scoparia*, *Ziziphus lotus* etc., indicating a progressive evolution towards the climax of origin or at least towards the para climax according to the duration of closure. The suppression of grazing has also allowed the regeneration of certain species such as *Anthyllis cytisoïdes*, *Artemisia herba alba*, *Atriplex nummularia* etc.

Evolution of Vegetation Cover. The vegetation cover provides a range of interpretations useful for pastoral management: forage availability, value of wildlife habitats, and trends in pasture status and represents a useful indicator to evaluate the degradation of vegetation. From a spatial point of view, Figure 1 shows that vegetation cover recorded in managed sites generally remain higher than those of unmanaged sites, regardless of year and site. Indeed, the vegetation cover of the improved vegetation is of the order of 17%.

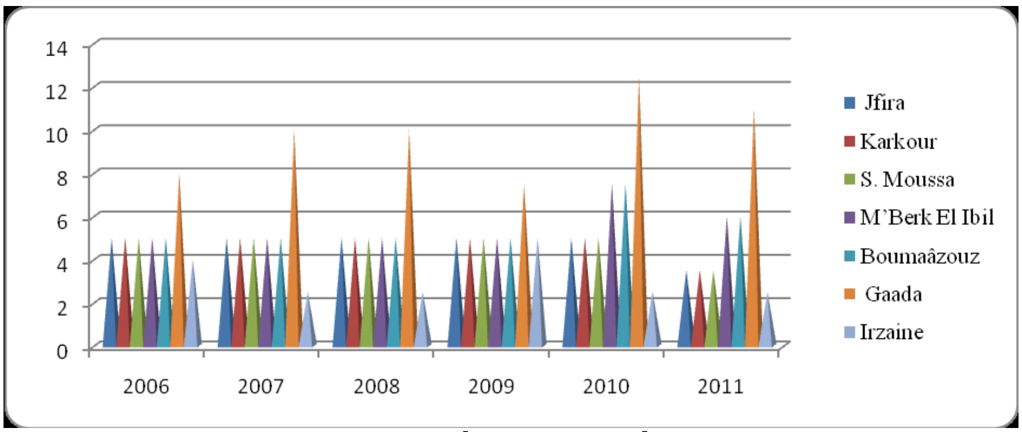
It is almost twice as high as the current 10% of undeveloped pasture in the Eastern of Moroccan (Maatougui et al., 2011), although it is slightly lower than the 20% recorded at the same sites between 2006 and 2008. However, it remains significantly lower than that of 34.5% obtained in a pastoral restoration of the Moroccan highlands (Acherkouk et al., 2012).

So in unmanaged sites, the vegetation cover is three times lower (3%). According to Berkat et al. (1990) and Berkat et al. (1992), the vegetation cover of unmanaged steppe vegetation in the highlands of the Eastern of Morocco does not generally exceed 15%.

The analysis of Figure 1 shows the positive impact of pastoral developments on the restoration and natural regeneration of vegetation. While it is well known that vegetation cover (especially chamaephytic species) in unmanaged arid grassland is low, slightly above 15% (Berkat et al., 1990; Le Houérou 2002; Aidoud et al., 2006 Nedjraoui et al., 2008, Maatougui et al., 2011).



a. Managed site



b. Unmanaged site

Figure 1 (a and b). Evolution of Average vegetation covers per site (2006-2012).

Figure 2. Very good vegetation cover in Jfira managed site (2010)

Evolution of Phytomass Production

The analysis of Figure 3 shows average annual phytomass production (kg DM / ha and UF / ha) according to the managed sites versus the unmanaged sites. The results show that, at the level of the whole zone, the production of the managed sites, both dry matter and energy, is clearly higher (> 5 times) than that of the unmanaged sites (368 against 69.50 Kg MS / ha and 149 versus 28 UF / ha). In addition to the type of management (resting, forage shrub planting, Alley-cropping), this superiority would revert to the (assumed to be) rational grazing (open and closed to grazing) mode, which allows plants to restore and produce a new biomass. This also depends on the type of vegetation and rainfall (Le Houérou, 1995).

In the unmanaged sites, production remains very low (69.50 Kg DM / ha) and much lower than similar situations (unmanaged sites and free and continuous grazing). This is the case of the Moroccan steppes with 150 kg DM / ha (Berkat et al., 1990, El Gharbaoui et al., 1996), or the majority of continually grazed North African steppes and where average production does not exceed not 200 kg MS / ha (Le Houérou, 1995). The main causes of this low production are overgrazing, cultivation and clearing of pastoral lands (Bourbouze et al., 2001).

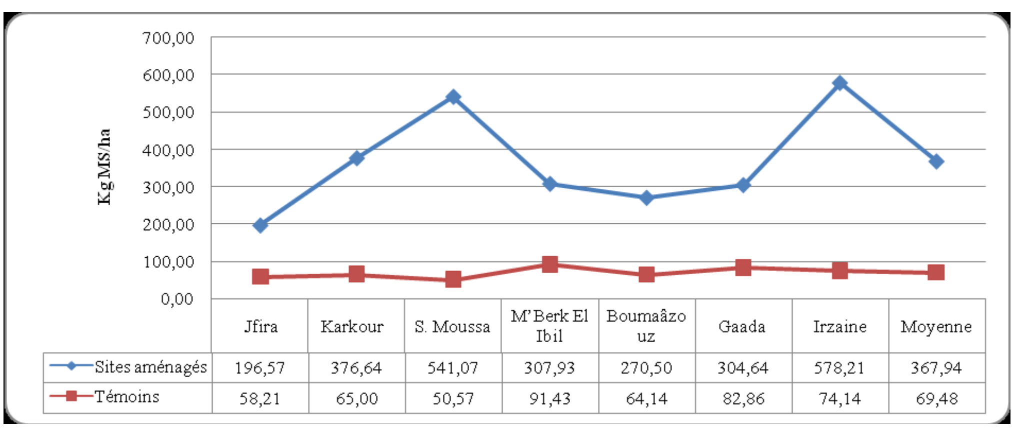


Figure 3. Average annual phytomass production of managed versus unmanaged sites between 2006 and 2012

Conclusions

The results obtained from experiments and field measurements make it clear that the regeneration and restoration of degraded pastures is possible through appropriate pastoral management actions. So, pastoral managements significantly re-establish original biological processes occurring before the disturbance by improving:

- enrichment of floristic diversity
- general vegetation cover
- production both in terms of phytomass and forage units.

These techniques have demonstrated their efficiency in the restoration and rehabilitation of degraded pastoral ecosystems and should be extrapolated to other pastoral areas of Morocco.

References

Acherkouk, M. ; Maatougui, A. et El Houmaizi, M. A. 2011. "Communautés végétales et faciès pastoraux dans la zone de Taourirt-Tafoughalt du Maroc oriental : Écologie et inventaire floristique". *Acta Botanica Malacitana*. *Vol.* 36. 125-136.

Acherkouk, M. ; Maâtougui, A. et El Houmaizi M. A. 2012. Etude de l’impact d’une mise en repos pastoral dans les pâaturages steppiques de l’Oriental du Maroc sur la Tire_s a_ part : M. Acherkouk restauration de la vegetation. *Secheresse* 23 : 102-12. doi : 10.1684/sec.2012.0340.

Acherkouk, M. et El Houmaizi, A. 2013. Évaluation de l’impact des aménagements pastoraux sur la dynamique de la production des pâturages dégradés au Maroc oriental. *ecologia mediterranea*. Vol. 39 (2).

Aidoud, A. ; Le Floc’h, E. et Le Houérou, H. N. 2006. "Les steppes arides du nord de l’Afrique". Sécheresse, 17(1) 19-30.

Berkat, O. et Hammoumi, M. 1990. "Etude de synthèse cartographique sur les parcours des communes rurales de Mérija, El Ateuf, Tendrara et Bouârfa". *Ministère de l’Agriculture et de la Réforme Agraire, Rabat (Maroc).*

Bourbouze, A. ; Lhoste, P. ; Marty, A. et Toutain, B. 2001. " Problématique des zones pastorales". *In* : Etude sur la lutte contre la désertification dans les projets de développement. CSFD/AFD. 12 p.

Dutilly-Diane, C.; Acherkouk, M. ; Bechchari, A. ; Bouayad, A. ; El Koudrim, M. et Maatougui, A. ; 2007. "Dominance communautaire dans l’exploitation des espaces pastoraux: impacts sur les modes de vie et implications pour la gestion des pâturages du Maroc oriental". Etude originale; Epreuves, *Cahiers Agricultures*, Vol. 16, n°4, juillet-août 2007. Cirad, Montpellier (France). 9 p.

El Gharbaoui, A. ; El Yamani, A. ; El Maghraoui, A. ; Boutouba, R. Alaoui, M. et Kabak, A. 1996. "Projet de Développement des Parcours et de l'Elevage dans l'Oriental: Stratégie de développement des terrains de parcours". Terre et Vie, Vol. 24.

Le Houérou, H. N. 1969. "La végétation de la Tunisie steppique : avec références aux végétations analogues d'Algérie, de Libye et du Maroc". *Ann. Inst. Nat. Rech. Agron.* Tunis (Tunisie), 624 p.+ Photos + Annexes.

Le Houérou, H. N. 1995. "Considérations biogéographiques sur les steppes arides du Nord de l'Afrique". *Sécheresse* 6 : 167-82.

Le Houérou, H. N. 2002. "Man-Made deserts: Desertization processes and threats". Arid Lands Res Manage; 16: 1-36.

Maatougui, A. ; Acherkouk, M. ; EL Fadili, M. ; El Houmaizi, M. A. 2011. "Les Pâturages Steppiques de l'Oriental Marocain : l'essentiel sur l'état de dégradation actuel et les voies d'amélioration". Rabat (Maroc) : INRA-Edition 2011.

Mahyou, H.; El Koudrim, M.; Bouayad, A.; Acherkouk, M.; Rahmi, M.; Bounejmate, M.; Thomas, N.; Kamal, M. et El Mourid, M. 2000. "The rangelands cultivation phenomenon in Eastern Morocco: Aïn Béni Mathar case". Reg. Workshop: Degradation and rehabilitation of marginal lands in the arab region. CEDARE, Cairo (Egypt), 2-4 July, 12 p.

Nedjraoui, D. et Bedrani, S. 2008. "La désertification dans les steppes algériennes : causes, impacts et actions de lutte". *Revue électronique* : *Vertigo*. Sc. Env.**,** Vol. 8 Numéro 1. [http://vertigo.revues.org/5375].

Rahmi, M.; Acherkouk, M.; Kamal, M.; Bouayad, A.; El Koudrim, M. and Mahyou; H. 2000. "The experience of the PDPEO and the contribution of INRA Morocco to the development and rehabilitation of marginal lands". *Reg. Workshop on "Degradation and rehabilitation of marginal lands in the arabe region". CEDARE*, Cairo (Egypt) 2-4 July, 2000. 12 p.