Propagation Studies on Selected Keystone Plant Species for Use in Restoration Projects in Kuwait

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Abstract: One of the main reasons for desertification and native flora extinction in Kuwait is the anthropogenic activities including over-grazing, camping and military activities coupled with poor law enforcement. The loss of natural habitats hinders the ability of plants to self-sustain due to the lack of seed source in the soil, which consequently affects plant recovery. This, in turn, will severely affect native fauna populations and, ultimately the health and functioning of ecosystems. Executing studies on seed germination and vegetative propagation of native plants plays a significant role in their use in conservation and restoration projects. Kuwait Institute for Scientific Research (KISR) is making concerted efforts to standardize mass propagation techniques and seedling establishment of a number of native plants. This study focused on the seed propagation and seedling establishment of selected keystone native plants. The deliverables of this study are being used for efficient propagation and establishment techniques that are crucial for both conservation of native plants and large-scale use in restoration programs.

Key-words: - Biodiversity, conservation, desertification control, native plants, seed germination, restoration

1 Introduction

Kuwait is an arid country with minimum natural resources, mainly water and vegetation cover; the majority of native plants have been damaged by overgrazing, cutting down the woody plants for fuel, abuse of off-road vehicles, urbanization, mining, pollution and activities of the Gulf War. The native plant communities are crucial constituents of the ecosystems. Desert plants are under tremendous pressure and are subjected to large fluctuations over time due to highly unpredictable environment with respect to water availability, a relatively short growth period and extreme aridity [1]. Arid regions are the most vulnerable systems to land degradation and desertification [2], [3].

In the arid ecosystems, native plants have evolved and are naturally selected to tolerate and adapt to the harsh environmental conditions of high temperatures, drought and high salinity. Kuwait's native vegetation is of huge importance to the desert ecosystem biodiversity, and it provides sustainable resources for the society. Native plants are natural forages for livestock, source of genetic diversity, and the massive strong roots stabilize the soil, add organic matters and enhance water percolation through the soil [1].

Restoration and revegetation programs are needed to conserve the wildlife biodiversity, and replanting the damaged area with the suitable native plants will enhance its survival and trigger the extension of the natural vegetation. Moreover, native plants species have the potential to be used in urban landscape projects. As 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. When native plants are properly blended with naturalized exotic ornamental plants in a landscape, they can improve social and cultural benefit, and it expands diversity in plant resources to be used in landscape and greenery projects in Kuwait [4]. Nevertheless, further studies are needed to assess the response of native plants to intensive growing environment in urban areas.

In this study some selected native plants, namely, *Lycium shawii, Nitraria retusa, Ochradenus baccatus, Peganum harmala and Rhanterium epapposum,* were evaluated for their field performance in urban landscape for greenery development. Seed viability and seed germination

studies were done to evaluate the quality of seeds for two species of those propagated native plants, after eight years of transplantation. The deliverables of this study are being used for efficient propagation and establishment techniques that are crucial for both conservation of native plants and large-scale use in landscape programs.

2 Materials and Methods

2.1. Field Transplanting

The objective of this task was to acclimatize hardened seedlings in the field for evaluation of plant performance and irrigation study. This task was initiated during May 2008. Seeds of the selected plants from which seedlings were raised for field transplanting were collected from the following locations:

- Seeds of *Ochradenus baccatus* were collected from Sabah Al- Ahmad Natural Reserve, Kuwait, on 4.4.07
- Hardwood cuttings of *Lycium shawii* was collected from Sulaibiya, Kuwait, on 11.11.08.
- Seeds of *Nitraria retusa* were collected from Nuwaiseeb, Kuwait, on 12.6.06.
- Seeds of *Peganum harmala* were collected from Shidadhiya, Kuwait, on 4.12.06.
- Seeds of *Rhanterium epapposum* were collected on 11.5.04 from Agricultural Research Station, Sulaibiya, Kuwait.

The seeds were pretreated as per the results of the previous studies [4-7]. Seeds of *Ochradenus baccatus*, *Rhanterium epapposum* and *Nitraria retusa* were pretreated with 500, 750 and 1000 ppm GA_3 respectively, cuttings of *Lycium shawii* were treated with Hormex (1000 ppm IBA) in quick dip method. Untreated seeds of *Peganum harmala* were used for raising seedlings. Hardened seedlings of all the selected species were transferred to the field at the KISR waterfront site, Kuwait. Seedlings were hardened by slowly exposing them to external environment, and then were transferred from green house to the shade house and then partially exposed to the external environment for 3-4 h for 10 d prior to transplanting them in the field. Soil was irrigated before planting to leach salts from the root zone. A complete fertilizer mix was incorporated into the soil to improve its fertility. Forty-five hardened seedlings of each species were transplanted. Plant spacing for each species was determined based on the growth pattern and nature of the plant (Table 1). Plants were irrigated immediately after planting, and further re-watered thoroughly to allow settling of the soil around the root ball [8]. Plant maintenance was scheduled to improve the aesthetic view and promote vigorous growth. Plants were irrigated based on their age and season according the recommendations of [9]. These plants were maintained for eight years after transplanting. Survival, flowering and fruiting in these species were observed. Relative growth in plant height, canopy and number of branches was calculated as:

$$Relative\ growth\ (\%) = \frac{\text{Final value} - \text{Initial value}}{\text{Final value}} \times 100 \quad (1)$$

Table 1. Plant Spacing of Selected Native Plants

Species	Spacing (m)
Nitraria retusa	2 x 2
Rhanterium epapposum	1 x 1
Lycium shawii	2 x 2
Ochradenus baccatus	1.5 x 1.5
Peganum harmala	1 x 1

2.2. Germination Study

Two species out of the 10 native plants in the study were selected to further analyze the viability, germination percentage and hence the quality of the seeds of the mother plants after eight years of field transplantation. The seeds were collected from the site (Urban Development Garden), in 2014. Out of the eight selected species, seeds of *Ochradenus baccatus* and *Peganum harmala* were evaluated for their seed quality in terms of viability and germination. Viability percentages of the selected seeds were determined by 2, 3, 5-Triphenyltetrazolium chloride (TTC) test.

2.3. Viability Test.

In this test, viable embryos were stained red due to the reduction of TTC by respirative activity in the cell [10]. Accordingly, seeds were soaked in distilled water overnight, and the embryos were exposed by splitting the seed with a longitudinal cut. Seeds were soaked in 0.1% TTC solution in petri dishes covered with aluminum foil and kept for 24 h at room temperature (25^{o}C). Seeds were then washed thoroughly with distilled water to remove excess stain and examined under the microscope.

After determining the viability of the seeds, the following germination experiments were carried out.

2.4. Treatment Details.

Seeds of *Ochradenus baccatus* pretreated with 500 GA_3 prior to sowing, and seeds of *Peganum harmala* were not subjected to any pretreatment based on the previous research findings (Suleiman et al., 2011). The seeds were stored at room temperature prior to germination. *Ochradenus baccatus* and *Peganum harmala* were sown on 18.11.2014 in a medium comprising of peat moss: potting soil: sand (1:1 V/V basis) in jiffy pots and maintained under laboratory conditions at 25^{o}C. Germination data were recorded.

3. . Results

3.1. Field Transplanting

3.1.1. Plant Survival Rate.

Survival rate (Table 2) and growth parameters were recorded during the establishment period (150 days). Due to the harsh summer, mortality in *Horwoodia dicksoniae* and *Farsetia aegyptia* was high.

Table 2. Survival Rate of Selected Indigenous Plants of Kuwait in the Field at 150 DAP

Species	Survival (%)	Flowering (%)	Fruiting (%)
Lycium shawii	100.0	28.9	0.0
Nitraria retusa	100.0	0.0	0.0
Ochradenus baccatus	100.0	44.4	0.0
Peganum harmala	98.0	82.2	86.7
Rhanterium epapposum	97.8	0.0	0.0

Lycium shawii: Survival rate in this species was 100%, and flowering was observed in 28.9% of plants during second week of September 2008.

Nitraria retusa : Survival rate in this species was 100%.

Ochradenus baccatus: Survival rate in this species recorded 100% and flowering was observed in 44.4% of plants at the end of September 2008.

Peganum harmala: Ninety-eight percent of the transplanted seedlings survived in the field. Additionally, flowering and fruiting were observed in 82.2% and 86.7% of plants respectively.

Rhanterium epapposum: The survival rate recorded 97.8 %.

3.1.2. Growth Parameters.

Plant growth parameters like plant height, canopy, and shoot number were recorded biweekly.

3.1.2.1. Plant Height.

Average plant height recorded at 0, 30, 60, 90,120 and 150 d after planting is detailed in Table 3 and Fig.1. Highest relative growth in height was recorded in *Lycium shawii*. Declining trend was observed in the average plant height of *Lycium shawii, Ochradenus baccatus* and *Rhanterium epapposum* until 90 d after planting. It should be noted that average height of the plants did not exhibit rapid growth during the first three months after transplanting (May- July). However, average height in *Lycium shawii and Ochradenus baccatus* exhibited rapid growth even during the peak summer (August-October) indicating their growth potential in harsh summer at irrigated conditions.

Table 3. Average Plant Height of Selected Indigenous Plants

	Average Plant Height (cm)						Relative
Plant	Initial	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	Growth (%)
Lycium shawii	55.5	54.6	50.8	50.3	79.2	105.9	90.9
Nitraria retusa	30.9	33.8	35.1	35.4	43.5	46.3	49.8
Ochradenus baccatus	79.5	71.3	71.5	76.8	91.6	97.3	22.5
Peganum harmala	22.5	30.1	31.6	30.6	33.6	36.4	62.0
Rhanterium epapposum	19.0	17.1	17.8	18.0	20.9	24.6	28.9

DAP – Days after planting.

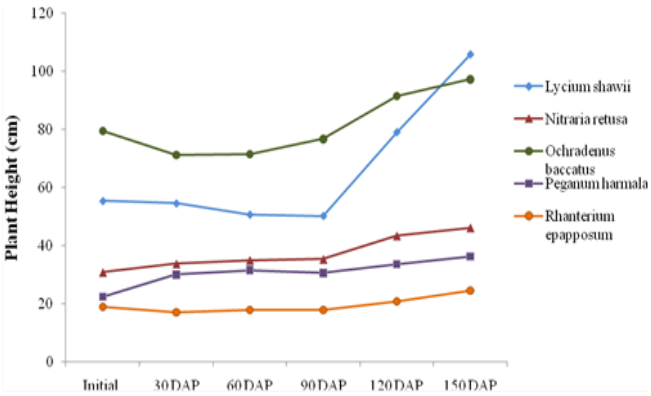

Fig.1. Comparison of height of selected indigenous plants.

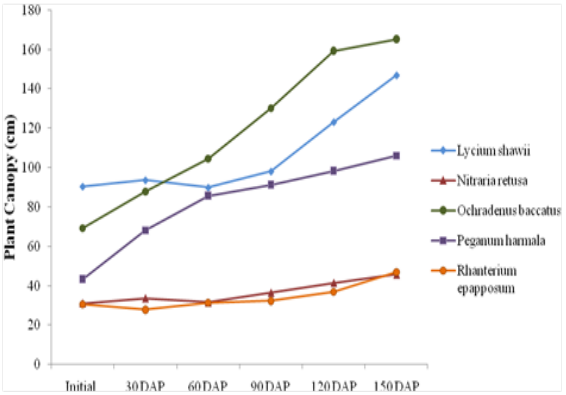

Fig.2. Comparison of plant canopy of selected indigenous plants

3.1.2.2. Plant Canopy.

Table 4 and Fig. 2 illustrate the average plant canopy of all the selected indigenous plants at 0, 30, 60, 90, 120 and 150 DAP. Highest relative growth of 144.49% was observed in *Peganum harmala*, and the lowest relative growth recorded was in *Nitraria retusa.*

Table 4. Average Plant Canopy of Selected Indigenous Plants

Plant	Canopy (cm)						Relative Growth (%)
	Initial	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	
Lycium shawii	90	94	90	98	123	147	62
Nitraria retusa	31	34	32	36	41	46	47
Ochradenus baccatus	69	88	105	130	159	165	139
Peganum harmala	43	68	86	91	98	106	144
Rhanterium epapposum	30	28	31	32	37	47	54

DAP – Days after planting.

3.1.2.3. Number of Shoots.

Number of shoots increased in all the selected species at the end of 90 d. Highest relative growth in number of shoots was in *Peganum harmala* (Table 5).

Table 5. Average Number of Shoots of Selected Indigenous Plants

Plant	No. of shoots						Relative Growth (%)
	Initial	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	
Lycium shawii	3	4	4	5	8	10	184
Nitraria retusa	2	3	2	3	4	4	66
Ochradenus baccatus	3	3	5	6	7	9	182
Peganum harmala	3	5	7	8	9	10	240
Rhanterium epapposum	3	3	3	3	4	5	73

DAP – Days after planting.

3.2.Germination Study

3.2.1. Viability Test. Hundred percent of seeds of Ochradenus baccatus and Peganum harmala were stained with TTC, which indicated that seed viability was not a limiting factor.

3.2.2. Germination Studies. The experiment was conducted from 14.4.2008 to 19.5.2008, The final

germination percentage was determined at the termination of the experiment. Ochradenus baccatus was pretreated with 500 ppm GA3, which resulted in 98% germination, and untreated Peganum harmala resulted in 95% germination.

4. Summary and Conclusion

4.1.Field Transplanting

One-hundred percent survival rate was observed in *Lycium shawii, Nitraria retusa* and *Ochradenus baccatus*. In *Peganum harmala* and *Rhanterium epapposum,* the survival rate was 97.8%. Flowering was observed in *Ochradenus baccatus, Lycium shawii* and *Peganum harmala,* and seeds are being collected periodically. Plant height was the highest in *Lycium shawii*. Canopy and number of shoots were the highest in *Peganum harmala*. All the selected species recorded a positive relative growth in average height, canopy and number of shoots at 150 DAP.

4.2.Germination Study

In 2014, the quality of the seeds obtained from the eight-years- old mother was evaluated. Seeds of *Ochradenus baccatus* and *Peganum harmala* exhibited 98% and 95% germination respectively. This indicated the good quality of the seeds and the capability of the transplanted plants with irrigation to produce viable seeds with high germination percentage. Seed production of native plants for re-vegetation programs should consider growing the near extinct species under irrigation rather than using seeds collected from the field. Naturally growing plants with limited water resources might affect the seed quality.

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