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REPRODUCTIVE AND GERMINATION ECOLOGY OF SINAI PRIMROSE, *PRIMULA BOVEANA* DECNE. EX DUBY

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Abstract

The Sinai primrose, *Primula boveana* has been reported as one of the rarest and most endangered plant species worldwide. It is endemic to the St Katherine Protectorate (SKP) in southern Sinai, Egypt. Our objectives were to: (1) Determine the effect of the surrounding ecology on the morphological and reproductive characteristics among *P. boveana* populations. (2) To detect the seed germination dynamics among different environmental factors and to investigate the dormancy rate between mother plants and new adult generation (3) The present work focuses on *ex-situ* requirements for seed germination of *Primula boveana*, specifically comparing the use of untreated and gibberellic acid (GA3) treated to promote germination, as an initial step to their conservation. Total of1010 individuals of the target species were recorded within this study, 162 of them are adult plants (16%), only three locations from five containing adult individuals. Morphological characteristics showed great variation within and among different subpopulation in the three sampled levels (Parent, new adult generation and seedling). Results of reproductive characteristics obtained from field observation showed great variation between the three sampled levels as well as among different subpopulations. Seeds of *P. boveana* did not germinate within 90 days in media without gibberellic acid; while those who are treated by gibberellic acid showed germination rate ranged from 10% (S.G.P.) to 77% (A.F.). Results showed that in general, the rate of germination of the seeds comes from young adult generations (F) are higher than the rate of germination of parents (P) seedsy.

Key words: *Primula boveana*; reproductive ecology; Saint Katherine Protectorate; germination; *Ex-situ* conservation; environmental variability.

INTRODUCTION

When the environmental factor changes beyond a certain level; plants try to adapt. Adaptation is any morphological, anatomical, physiological or behavioral feature, which favour results from some environmental pressure to increase the ability of an organism under changing environment and favour the success of an organism in a given environmental condition. A given population shows different levels of tolerance to a given limiting factor over its geographic distribution. Such locally adapted populations are called ecotypes, which may have developed due to genetic changes resulting in different responses to varying environment (Agrawal, 2005).

An important consequence of the sedentary lifestyle of plants is that they cannot escape from the environment in which they grow or from any changes in this environment. To cope with this, many plants are able to alter one or more morphological characters in response to both abiotic (e.g., climate and weather) and biotic (e.g., grazing and competition) factors of the environment with a potential effect on resource acquisition. For example, leaf size and leaf area of many alpine plants change with altitude (Meinzer *et al.*, 1985; Ko¨rner *et al.*, 1989; Galal, 2011 and Omar *et al.*, 2012), and some arctic plants may produce more or larger leaves during warmer summers than during colder ones (Havstro¨m *et al.*, 1995; Stenstro¨m and Jo´nsdo´ttir, 1997). Knowledge of how ecologically important morphological characters vary within the distributional range of plant species, as well as the underlying control mechanisms for such variation, is essential to understand how the plants may respond to environmental change (Stenstro¨m *et al.*, 2002).

Knowledge of reproduction is crucial to our understanding of the causes of rarity and for conservation of rare plant taxa (Kruckeberg and Rabinowitz 1985). Herbaceous perennials that do not reproduce vegetatively depend on seeds to recruit new individuals into populations. In order for new plants to establish in a population, flowers must be pollinated to form fruits, ovules must be fertilized, sustained with nutrients, and escape predation to form viable seeds, and seeds must be dispersed to suitable substrates for growth, where they must germinate. Any weak link or break in this chain of events curtails a plant's ability to reproduce and, if constant over space and time, may contribute to a species' rarity and impede its conservation.

Many workers have investigated single or combined components of the reproductive ecology of rare plants, such as flowering frequency and vegetative reproduction seed germination (Baskin and Quarterman, 1969; Baskin and Baskin, 1979; Halse, 1988; Jacobs, 1993; Clark *et al.,* 1997; Florance, 1997), breeding system and germination (Clampitt, 1987; Menges, 1995), and seed production and predation (Menges *et al.,* 1986).

Reinforcement of wild plant populations using individuals raised *ex-situ* is considered a valid means of reducing the risk of extinction of threatened species or populations (Bowes, 1999). If plants are multiplied from seed the genetic diversity of local ecotypes is maximized (Fay, 1992). However, each species has particular requirements for seed germination as a result of adaptive radiation into patchy and changing environments (Schu¨tz and Milberg 1997). Thus, although propagation from seed is inexpensive and usually effective, germination requirements for native species are often unknown, particularly for rare and/or endemic species of which material is more difficult to obtain.

A dormant seed is one that is unable to germinate in a specified period under a combination of environmental factors that are normally suitable for the germination of the non-dormant seed (Baskin and Baskin 2004). Seed dormancy is a temporary failure of a mature viable seed to germinate under environmental conditions that would normally favour germination (Li and Foley, 1997). Plants have evolved several dormancy mechanisms to optimize the time of germination (Jones, 1999), seed dormancy enhances survival (Foley, 2001). Since it is a physiological adaptation to environmental heterogeneity, seed dormancy is a primary factor that influences natural population dynamics (Bewley and Black 1994).

Seed survival and longterm viability enhances the fitness of the maternal parent. Seed dormancy allows germination to be timed to escape unfavorable conditions for seedling growth and development and to spread germination over many seasons, avoiding bad years. Dormancy is a block to germination under otherwise favorable moisture, temperature and light conditions (Baskin and Baskin 2001). These blocks to germination may occur at the level of the embryo, seed coat, or seed covering. According to the hormone balance theory (Le Page-Degivry, 1990), physiology of seed dormancy is mediated by the plant hormones abscisic acid and gibberelins (Finch-Savage and Leubner-Metzger et al., 2006). The production of abscisic acid (ABA) is known to induce dormancy in developing seeds and the continued presence of high levels of ABA serves to maintain the dormant state (Kucera *et al.*, 2005). Endogenous increases in gibberrellic acid (GA3) production is known to play a role in breaking dormancy and degrades in the presence of high levels of ABA. It is the ABA:GA3 ratio that determines the dormant or non-dormant state of a seed, as GA3 appears be antagonize to ABA production. External stimuli can act on this hormonal system, inducing genes for either GA3 or ABA biosynthesis (Kucera *et al.*, 2005). Abiotic signals such as temperature, gas concentrations or light can also induce changes in ABA:GA3 ratio, causing dormancy release. For example, cold stratification Arabidopsis seeds induced the transcription of GA3 biosynthesis genes GA3 oxidase, stimulating spikes in endogenous GA3 and a subsequent increase in germination (Yamauchi *et al.,* 2004). Increases in GA3 are associated with the weakening of both endosperm and testa tissues at the micropylar end of the seed, which restrict radicle protrution (Kucera *et al.,* 2005). Soaking in growth promoters e.g. as GA_3 or in water for prolonged times in the case of the presence of growth inhibitors and the immature embryos.

The intensity of dormancy in a given species exhibits high degrees of variation at several levels: among populations, within populations and even between seeds collected in different years from the same population (Andersson and Milberg 1998; Meyer and Pendleton 2000). The observed variability in seed dormancy has a genetic and an environmental basis, and the genetic basis of variability in dormancy levels has been documented in several species (Li and Foley 1997). Seed dormancy is a typical quantitative trait, since it is controlled by multiple loci and highly influenced by genotype versus environment interactions (Foley and Fennimore 1998; Koornneef *et al.*, 2002). Temperature, oxygen, light and moisture are some environmental factors present during seed development that influence the germination phenotype (Li and Foley 1997). Other factors that may lead to differences in germination percentages among or within populations are the age and the nutritional status of the mother plant during seed maturation (Fenner, 1991), seed position on the mother plant (Tieu *et al.*, 2001), seed size and shape (Jones and Nielson, 1999; Baloch *et al*., 2001), the time since seed harvest and the duration of seed storage (Santarém and Aquila 1995).

Our objectives were to: (1) Determine the effect of the surrounding ecology on the morphological and reproductive characteristics among *P. boveana* populations. (2) To detect the seed germination dynamics among different environmental factors and to investigate the dormancy rate between mother plants and new adult generation, and (3) The present work focuses on *ex-situ* requirements for seed germination of *Primula boveana*, specifically comparing the use of untreated and gibberellic acid (GA3) treated to promote germination, as an initial step to their conservation.

MATERIAL AND METHODS

Target species:

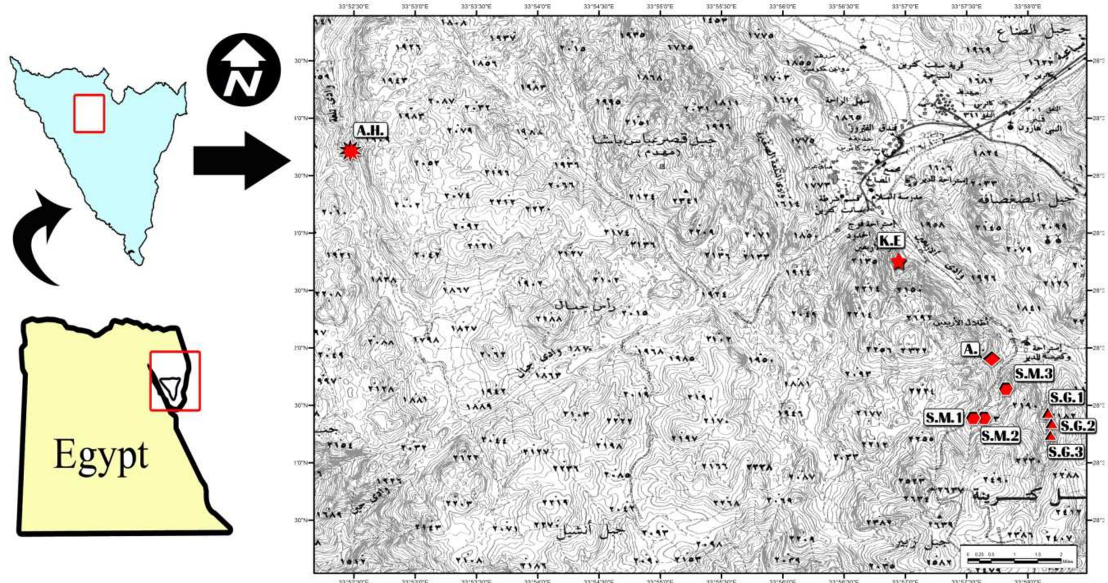
The genus *Primula* L. is the most important one in the Primulaceae family. Considering the latest evaluations, it includes 500 species, mainly located in the temperate and cold regions of the northern hemisphere and in the tropical mountains (Hao *et al.,* 2002). *Primula* is a complex and varied genus, with a range of habitats from alpine slopes to boggy meadows. Plants bloom mostly during the spring, with flowers often appearing in spherical umbels on stout stems arising from basal rosettes of leaves; their flowers can be purple, yellow, red, pink, blue, or white. Some species show a white mealy bloom (farina) on various parts of the plant. Many species are adapted to alpine climates.

The Sinai primrose, *Primula boveana* Decne. ex Duby is the only species from genus *Primula* in Egyptian flora, it has been reported as one of the rarest and most endangered plant species worldwide (Richards, 2003). It is endemic to the St Katherine Protectorate (SKP) in southern Sinai, Egypt, and has high medical importance because of substances extracted from its roots. This species is severely threatened by both natural (aridity of the area) and human factors (scientific research). All these factors are pushing *P. boveana* to the brink of extinction. The St Katherine Protectorate is one of the most floristically diverse spots in the Middle East with 30% of Egypt's endemic plant species. *Primula boveana* located in only five clearly delimited localities, This species is restricted to montane wadis fed by melted snow and distributed in moist ground in the vicinity of wells and sheltered mountain areas. Because of climate change, the wild population of this species could be in extreme danger in the near future.

Study Area:

The Saint Katherine Protectorate (SKP) is one of Egypt's largest protected areas and includes the country's highest mountains. This arid, mountainous ecosystem supports a surprising biodiversity and a high proportion of plant endemics and rare plants. The flora of the mountains differs from the other areas, due to its unique geology, morphology and climatic aspects. The soil is formed mainly from mountains weathering, thus it is mainly granitic in origin. The soil layer is generally shallow were the bed rock is close to the surface. Annual rainfall is less than 50 mm. However, rainfall is not of annual character, rather 2 to 3 consecutive years without rainfall is common. Rain takes the form of sporadic flash floods or limited local showers, thus highly spatial heterogeneity in received moisture is also common. The Saint Katherine region is situated in the southern part of Sinai and is a part of the upper Sinai massif. It is located between 33˚ 55' to 34˚ 30' East and 28° 30' to 28° 35' North (Hatab, 2009).

Primula boveana distributed inside SKP in five main localities; Sahk Musa (S.M.), Shak Elgragenia (S.G.), Alahmar (A.), Kahf Elghola (K.E.) and Sad Abu Hebeik (A.H.) (Map 1).



Map 1. Location of five main studied locations and nine subpopulation of *Primula boveana* sampled on Saint Katherine Protectorate, Egypt.

Population, morphological and reproductive characteristics:

This study was carried out in the period between September 2013 to May 2014. Number of individuals per site, adult plants, population size and range of spatial distribution and presence will be described for *P. boveana* in order to reflect its demography. Morphological aspects were recorded for all *P. boveana* individuals by using morphological indices attributed on each parameter as plant Height (cm), plant width (cm), number of branches per plant, number of leaves per plant, leaf area (cm^2) and size index to evaluate the variations that exist among different locations under study. Depending on the oval shape of the leaf, the leaf area was estimated according to the following equation: Oval shape area = π*a*b where a = half-length of major axis (horizontal) and b = half-length of minor axis (vertical) (Omar *et al.*, 2012). The size index was calculated using the following formula: Size index (cm) = (Plant height + Plant diameter) / 2 (Omar *et al.*, 2012).

Reproductive characteristics were recorded for adult *P. boveana* individuals, number of flowering individuals, number of inflorescence per plant, number of flowers per plant, seed weight per plant and total number of seeds per plant. Topographic features (altitude, aspect, slop), climatic variables (Min. Temp., Max. Temp., Precipitation) and threat degree will be detected for testing the effect of environmental variation.

Seed germination:

Seed collection

The seeds for *in vitro* germination were collected from all sites that containing adult individuals; three locations were recorded as containing adult species Shak Elgragenia, Shak Musa and Alahmar. Seeds from five subpopulation (one from Shak Elgragenia, 3 from Shak Musa and 1 from Alahmar) were collected at September 2013 (fruiting season), at each subpopulation we collect 2 samples one from parent individual and one from new adult generation to detect the seed vitality among generations. The samples were coded as fellow: Shak Elgragenia (S.G), Shak Musa (S.M.) and Alahmar (A.), Parent plant (P), new generation (F) and seedling (S) (Map 1). To avoid major damage to the populations themselves only a relatively small amount of seeds per plant were collected (collection of no more than 20% of the available seed is recommended to assure natural regeneration).

In vitro seed germination

Seeds underwent a drying treatment at room temperature to avoid damage or rotting during cold storage, when germination tests were performed.

Germination test without gibberellic acid:

Seeds samples were placed on three wetted Whatman No. 3 filter paper discs in Petri dishes. Each petri dish represent one subpopulation, each containing 100 seeds. Seeds were incubated for 50 days in a germination chamber in the following environmental regime: 16/8 h light/dark cycle at 15–20 _C (January 2014). To ensure no systematic effects due to position within the chamber, Petri dishes were re-randomised every two days (Yang *et al.,* 1999). Seeds showing radicle emergence were recorded daily as 'germinated' (Come, 1970). For each trial, germination percentages after 50 days were recorded, along with the germination delay (i.e. the number of days until the first germination record) and half-germination time calculated (i.e. the number of days until 50% of the final germination percentage was achieved). Non-germinated seeds were maintained in the same conditions for a further 90 days, to verify the possibility of any delayed germination.

Germination test with gibberellic acid:

Seeds samples were placed on three wetted Whatman No. 3 filter paper discs in Petri dishes. Each petri dish represent one subpopulation, each containing 100 seeds. Seeds were soaked in 1000-PPM gibberellic acid (GA3) for 24 hour to break the dormancy. Seeds were then incubated for 50 days in a germination chamber in the following environmental regime: 16/8 h light/dark cycle at 22–28 _C (April 2014). For each trial, germination percentages after 50 days were recorded, along with the germination delay (i.e. the number of days until the first germination record) and half-germination time calculated (i.e. the number of days until 50% of the final germination percentage was achieved). Non-germinated seeds were maintained in the same conditions for a further 90 days, to verify the possibility of any delayed germination.

RESULTS AND DISCUSSION

Population, morphological and reproductive characteristics:

Most of the *P. boveana* subpopulations were small and the individual plants occurred sporadically in space, as little groups were conjugated with wet soil. A total of 1010 individuals of the target species were recorded within this study, 733 were recorded at Shak Musa (72.6%), 246 were recorded at Shak Elgragenia (24.5%), 20 were recorded at Alahmar (2%), 6 were recorded at Abu Hebik (0.6%) and 4 were recorded at Kahf Elghola (0.3%). 162 adult plant (16%) were recorded within this study, only three locations from five containing adult individuals. Kahf Elghola and Abu Hebeik not recorded any adult individuals (Figure 1).

Eighty adult individuals were recorded at Shak Elgragenia (32.4% from total individuals in the same site), 74 were recorded at Shak Musa (10% from total individuals in the same site) and 8 were recorded at Alahmar (40% from total individuals in the same site). At the micro-site level, *P. boveana* plants occupied only few of the high altitude habitats in SKP such as cliffs (67%), slope (22%), and cave (11%) micro-habitats. There are no records in wadi beds, terraces, farshs microhabitat. This indicates that this species has a very restricted narrow range of spatial distribution and presence.

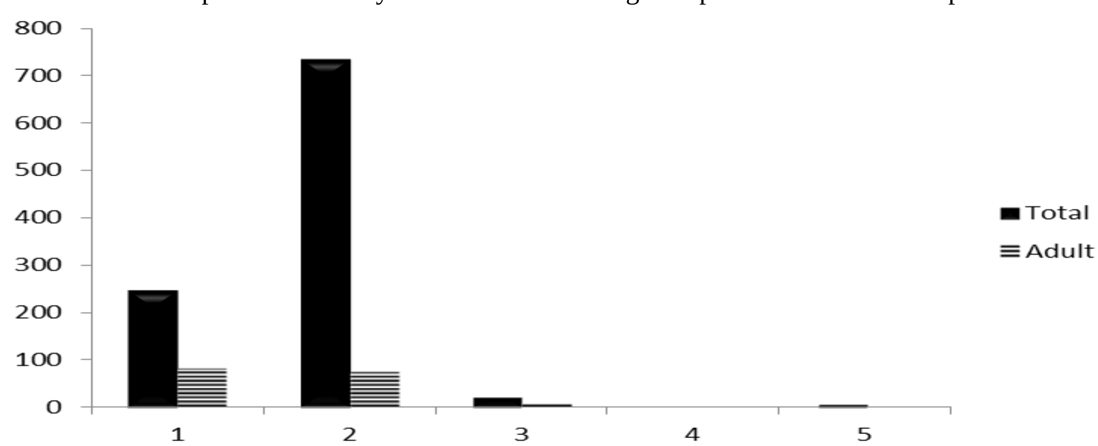


Figure 1 Adult individuals' ratio to total individuals per site; 1- Shak Elgragenia, 2- Shak Musa, 3- Alahmar, 4- Kahf Elghola, and 5- Sad Abu Hebik.

Morphological characteristics showed great variation within and among different subpopulation (See Table 1) in the three sampled levels (Parent, new adult generation and seedling). On parent level, number of leaves per individual plant ranged from six (K.E) to 42 (S.G.2) with average of 22 leaf per individual. Number of branches per plant ranged from zero (K.G & A.H.) to two (S.G.3) with average one branch per plant. Leaf length ranged from 5 cm (A.H) to 16 cm (S.M.2) with average of 10.4 cm. Leaf width ranged from 3 cm (A.H.) to 10 cm (S.M.2) with average of 6.1 cm. Leaf area showed great variation ranged from 11.8 cm^2 (A.H.) to 125.7 cm^2 (S.M.2) with average of 55.8 cm^2. *P. boveana* individuals showed also a great variation in size index ranged from 10 cm (A.H.) to 47.5 cm (A.) with mean of 30.4 cm. On new adult generation (F) and seedling levels, the variation is still alive with smaller degree than in parent (P) level (See Table 1 and Figure 2).

Results of reproductive characteristics obtained from field observation showed great variation between the three sampled levels as well as among different subpopulations. Seedling level will eliminate from this comparison because it is not containing any adult individuals. Only 3 locations from five containing adult individuals (K.E. & A.H. containing only seedlings); 16% of total recorded individuals are adult that able to produce seeds. Field observation showed that *P. boveana* starting the flowering season from the early of March and finish at the end of July when the fruiting season started in July and finish at the end of September (Figure 2). On parent level, number of flowers per individual plant ranged from 6 (S.G.2) to 28 (A.) with average of 13 flower per individual. Number of inflorescence per plant ranged from 1 (All except S.G.3) to two (S.G.3) with average one inflorescence per plant. Number of seed per site ranged from 715 (S.M.3) to 5200 (S.G.3) with average of 1807 seeds per site. Seed weight ranged from 0.04 gm. (S.M.3) to 0.29 gm. (S.G.3) with average of 0.1 seeds per site (See Table 2).

Table 1. Morphological variations among different *P. boveana* subpopulation

Location	Subpopulation	Sample level	No. Leaf	No. Branch	Leaf Length	Leaf Width	Shape Index	Leaf Area	Plant Width	Plant Height	Size Index
Shak Elgragenia	S.G.1	P	13	1	7	4	1.75	22.00	8	13	14.5
		F	10	1	4	2	2.00	6.29	6	7	9.5
		S	6	0	2	1	2.00	1.57	3	3	4.5
	S.G.2	P	42	1	12	6	2.00	56.57	22	26	35
		F	24	1	6	4	1.50	18.86	7	8	11
		S	16	0	3	2	1.50	4.71	4	5	6.5
	S.G.3	P	40	2	12	6	2.00	56.57	25	26	38
		F	12	1	6	4	1.50	18.86	9	9	13.5
		S	8	0	3	2	1.50	4.71	5	4	7
Shak Musa	S.M.1	P	30	1	14	8	1.75	88.00	18	30	33
		F	10	1	4	4	1.00	12.57	5	6	8
		S	6	0	2	1	2.00	1.57	3	4	5
	S.M.2	P	14	1	16	10	1.60	125.71	25	38	44
		F	10	1	6	5	1.20	23.57	8	6	11
		S	5	0	4	3	1.33	9.43	4	2	5
	S.M.3	P	22	1	12	8	1.50	75.43	24	24	36
		F	24	1	6	4	1.50	18.86	10	6	13
		S	14	0	4	2	2.00	6.29	6	4	8
Alahmar	A.	P	24	1	10	6	1.67	47.14	30	35	47.5
		F	18	1	8	4	2.00	25.14	19	12	25
		S	12	0	4	3	1.33	9.43	12	7	15.5
Kahf Elghola	K.E.	P	6	0	6	4	1.50	18.86	12	7	15.5
		F	4	0	4	2	2.00	6.29	4	3	5.5
		S	3	0	3	2	1.50	4.71	3	3	4.5
Abu Hebik	A.H.	P	8	0	5	3	1.67	11.79	7	6	10
		F	4	0	3	2	1.50	4.71	4	4	6
		S	3	0	3	1	3.00	2.36	4	3	5.5

On new adult generation level (F); we found variation with the other sample level (P) and variation between different subpopulations. However, parent level containing more flowers than F level we observe that F level contain seeds more than the P level in count and weight; this may be come from plant vigor degree. Sometimes, old plants tend to be less healthy than the young adult generation and this may come from age and amount of endogenous hormones, which reflect its effect on plant organs (See Table 2).

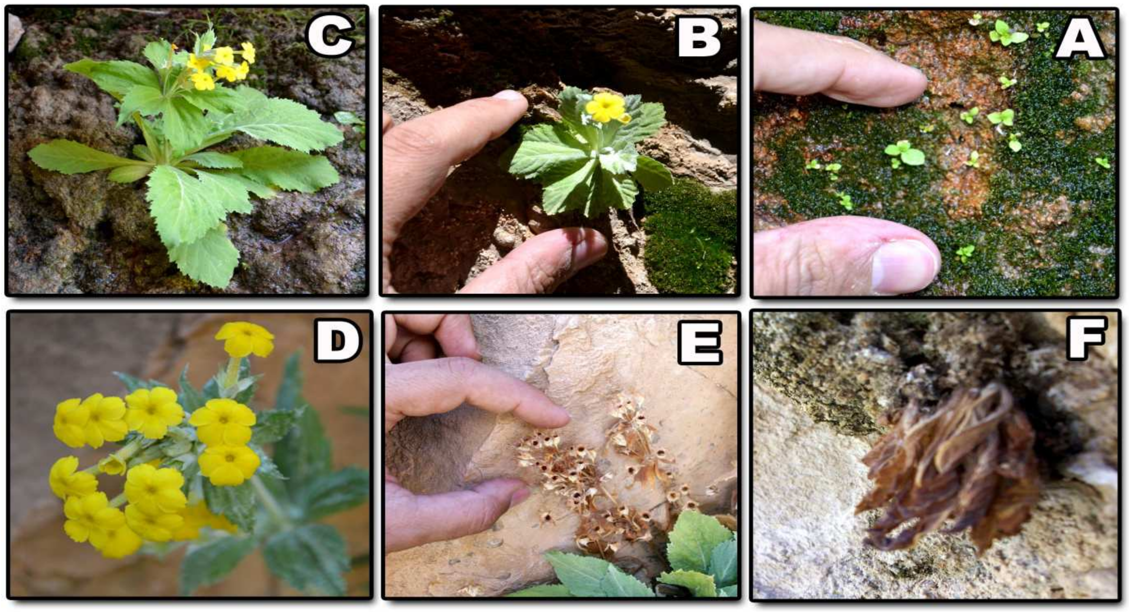


Figure 2 Morphological and reproductive characteristics of *P. boveana,* A- Seedling in wild, B- New adult generation (F), C-Adult plant, D- Flowering stage, E- Fruiting stage, and dead plant.

Table 2. Reproductive characteristics among different *P. boveana* subpopulation

Location	Subpopulation	Sample level	No. flowers	No. Inflorescence	No. Seed	Seed Weight (gm)
Shak Elgragenia	S.G.1	P	9	1	0	0
		F	6	1	620	0.002
		S	0	0	0	0
	S.G.2	P	6	1	3200	0.17
		F	6	1	5000	0.28
		S	0	0	0	0
	S.G.3	P	14	2	5200	0.29
		F	6	1	6250	0.35
		S	0	0	0	0
Shak Musa	S.M.1	P	16	1	2680	0.15
		F	6	1	1790	0.1
		S	0	0	0	0
	S.M.2	P	22	1	1965	0.11
		F	2	1	5350	0.3
		S	0	0	0	0
	S.M.3	P	18	1	715	0.04
		F	1	1	1250	0.07
		S	0	0	0	0
Alahmar	A.	P	28	1	2500	0.14
		F	6	1	4100	0.23
		S	0	0	0	0
Kahf Elghola	K.E.	P	0	0	0	0
		F	0	0	0	0
		S	0	0	0	0
Abu Hebik	A.H.	P	0	0	0	0
		F	0	0	0	0
		S	0	0	0	0

Environmental variability was observe during this study between locations and had been list in Table 3. It is well-known that following a change in environmental conditions, changes in morphological characteristics can occur from the scale of entire plants to the scale of individual leaves, buds, flowers, and fruits. Perhaps the most familiar morphological characteristics are those at the level of entire shoots (Horn 1971; Kramer and Kozlowski 1979). Morphological characteristics of stems as well as entire shoots include total leaf area, and the density and distribution of flowers, fruits, leaves and buds (Bonser and Aarssen 1994; Canham 1988; Dahlem and Boerner 1987; Huffman *et al.*, 1994a; Sipe and Bazzaz 1994; Stafstrom 1995; Tappeiner *et al.*, 1987; Wilson 1995). At smaller scales, differences can occur in the area, width, length, shape, orientation, and thickness of leaves, and the size, shape and seed characteristics of fruits (Abrams and Kubiske 1990; Collins *et al.*, 1985; Goulet and Bellefleur 1986; Harrington and Tappeiner 1991; Huffman *et al.*, 1994a; Jurik 1986; Niinemets 1996; Waller and Steingraeber 1995). Morphology of underground parts important in vegetative reproduction also change with environmental conditions and can be predicted to some extent from above-ground morphology (Huffman *et al.,* 1994a,b).

Table 3. Environmental variation among different *P. boveana* subpopulation

		Topographic feathers		Climatic variable			Threats		Edaphic Factors		
Location	Subpopulation	Altitude (m)	Aspect	TMIN	TMAX	PREC	Tourism %	Scientific Research%	pH	water content	Organic matter%
Shak Elgragenia	S.G.1	1886	NE	7.5	18.6	7.8	20	60	8.5	0.59	3.28
	S.G.2	2170	NE	7.5	18.6	7.8	0	70	8.7	0.55	3.05
	S.G.3	2202	NE	7.5	18.6	7.8	50	70	8.6	0.53	0.45
Shak Musa	S.M.1	2067	NE	8.83	20.13	7.92	20	30	7.7	0.31	2.76
	S.M.2	2065	NE	8.83	20.13	7.92	70	60	7.9	0.41	3.45
	S.M.3	1939	NE	8.83	20.13	7.92	70	80	7.2	1.13	4.14
Alahmar	A.	1914	E	8.09	19.46	9.25	0	20	8.7	0.98	8.03
Kahf Elghola	K.E.	1850	E	9.93	21.19	5.67	50	90	7.8	0.95	4.83
Abu Hebik	A.H.	1740	NE	10.5	21.78	4.75	20	10	8.1	1.01	2.07

Seed germination:

Seeds of *P. boveana* did not germinate within 90 days in media without gibberellic acid (data not shown); while those who are treated by gibberellic acid showed germination rate ranged from 10% (S.G.P.) to 77% (A.F.) See Figure 3. This may explain as that some endemic species specially those who are restricted to a very small area goes to be more safe in dormancy stage that protect the new generation to be more secure until the preferable environment condition for germination. Seed dormancy refers to seeds failure to germinate even when given favorable environmental conditions. i.e. adequate moisture, appropriate temperature regime, a normal atmosphere and in some cases light. This failure of a viable seed to germinate related to any causes such as Seed coverings, Chemical inhibitors include growth regulators etc., and Morphological aspects such as small, undeveloped embryos.

In natural habitats, dormancy can be broken by several factors; amongst these, the exposure to gastrointestinal secretions of animals and birds feeding on dormant seeds. This allows the softening of the hard seed coats testa while exposed to the action of gastric secretions and the micro-flora of animal gut. In addition, climatic fluctuations (high temperature during the day/summer versus very low temperatures at night/winter) and leaching off growth inhibitors by the action of heavy rain might participate in the breaking of the seed's dormancy.

Artificially, seed dormancy can broke also by one of the following methods: (a) Mechanical scratching of seeds by using abrasive materials such as sand or glass. (b) Chemical scratching by using strong inorganic acids such as H_2SO_4, Hcl, HNO_3, strong alkalis (NaOH: 4%) or salts ($CaCl_{2:}$ 11%); in the case of a hard testa; (c) cold/warm stratification; in the case of a hard testa and the immature embryo. (d) Soaking in growth promoters e.g. as GA_3 or in water for prolonged times in the case of the presence of growth inhibitors and the immature embryos (Schmidt, 2000).

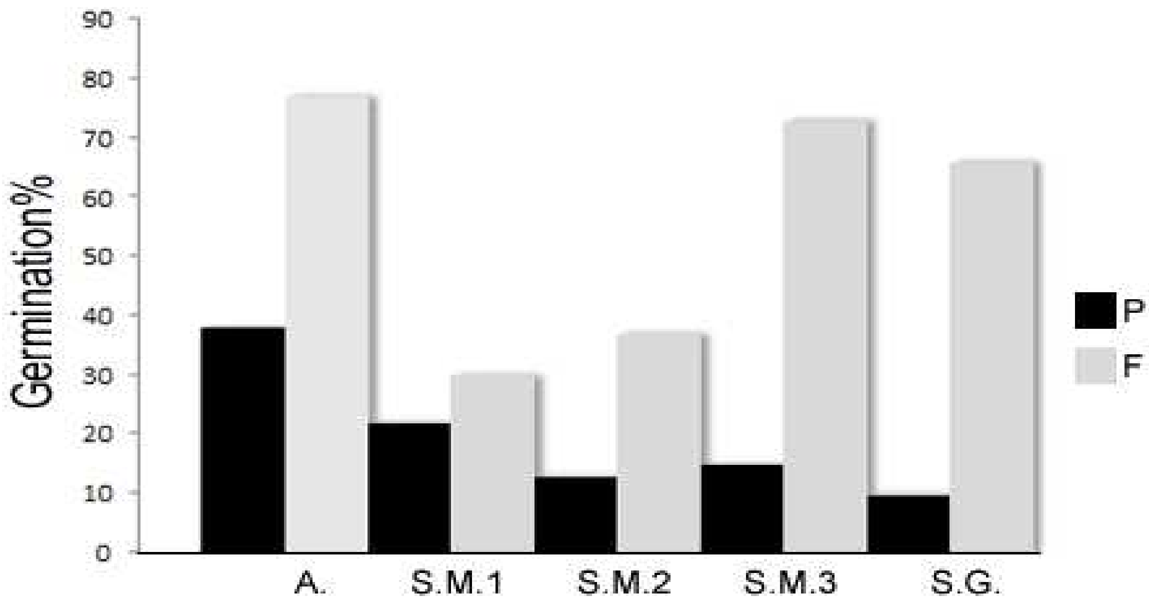


Figure 3 Germination rate between Parent (P) and new adult generations (F).

Results showed that in general, the rate of germination of the seeds comes from young adult generations (F) are higher than the rate of germination of parents (P) seeds. The rate of germination of the seeds comes from young adult generations (F) ranged from 33% (S.M.1) to to77% (A.) with average 57% (See Figure 4); while the rate of germination of parents (P) seeds ranged from 10% (S.G.) to 38% (A.) with average 20%. Seed germination varied in timing among the different subpopulations, it is observed that seeds of Ahmer (F) was the first germinated seeds, which began to germinate rapidly after 10 days of incubation and contained until the end of incubation period of 50 days, followed by seeds collected from Site Shak Elgragenia (F) while the seeds collected from Ahmer (P) were the last that sprouted (40 days) (Table 4). The high mountains of SKP support mainly Irano-Turanian steppe vegetation. Smooth faced rock outcrops supply sufficient runoff water to permit the survival of the unique flora.

Long period of *in-situ* conservation (Enclosure) in Alahmar for this species may explain by lentering in deep dormancy that need a long period of treatment to break this dormancy. This silence mode may case weakness (laziness) of old plants (P) and this may reflected to its ability to produce vigor seed that can give high germination rate when dormancy broke by growth promoters like GA. In the same conditions, we recorded the highest germination rate all over the tested subpopulations (77% in A.F.); that may come from the ability to germinate in such environment, which was adapted genetically, and become healthier and more viable. Moustafa *et al.,* (2001) and field observations also agreed that *P. boveana* had lower vitality, cover, and productivity in fenced areas than in unfenced ones

Other factors that may lead to differences in germination percentages among or within populations are the age and the nutritional status of the mother plant during seed maturation (Fenner, 1991), seed position on the mother plant (Tieu *et al.*, 2001), seed size and shape (Jones and Nielson, 1999; Baloch *et al.*, 2001), the time since seed harvest and the duration of seed storage (Santarém and Aquila, 1995).

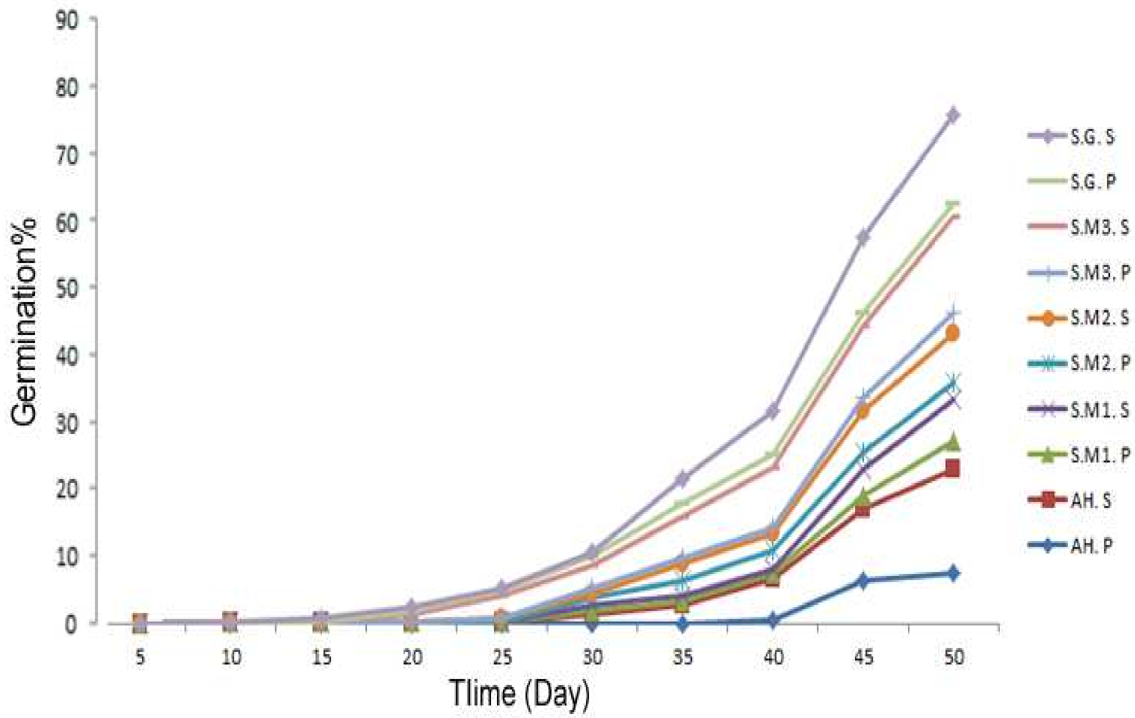


Figure 4 Germination dynamics of *P. boveana* seeds from different subpopulations in Saint Katherine Protectorate.

No. of days	AH. P	AH. S	S.M1. P	S.M1. S	S.M2. P	S.M2. S	S.M3. P	S.M3. S	S.G. P	S.G. S
5	0	0	0	0	0	0	0	0	0	0
10	0	1	0	0	0	0	0	0	0	0
15	0	1	0	0	0	0	0	0	0	2
20	0	1	0	0	0	0	0	5	4	2
25	0	1	0	0	0	3	0	17	4	2
30	0	6	4	4	5	3	4	17	8	2
35	0	13	4	4	11	13	4	31	10	18
40	2	31	4	4	13	13	4	46	10	32
45	32	53	10	20	13	31	10	54	10	56
50	38	77	22	30	13	37	15	73	10	66
Germination %	38	77	22	30	13	37	15	73	10	66

During the germination test we recorded 3 samples containing musty seeds (S.G., S.M.2, and S.M.3); all of them from parent level (P) and from the field observation this may come from insect pests during the flowering stage and this may cause damage to seed vigor. Some kind of ants is another important threat that also observed during the field visits. These ants may cause problems in reproductive process by eating plant seeds or transmitting it to other un-preferable conditions for germination. At the end of the experiment, germination rate percentages of *P. boveana* seeds showed great variation ranged from 10% to 77%. Considering that the germination percentages of this seeds reflect the degree of seed dormancy in the populations, these results indicated that there was a significant variation in levels of dormancy among populations of this species. Seed number, seed weight, and germination rate of young adult generation (F) is higher than Parent generation (P). This revealed that *P. boveana* individuals loss its reproductive viability and become less healthy with age and surrounding ecology.

The preservation of genetic diversity is important, because it provides long-term evolutionary potential for changing environmental conditions. There is no clear-cut answer to the question, when losses of diversity become critical. At some point losses will affect local communities, and, at a higher level, they will affect global stability. 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. When choosing species for *ex-situ* conservation, priority should be given to endangered species of global rarity, morphologically and genetically isolated species, monospecific genera, and relict populations (Anonymous, 1995). It's clearly known that *P. boveana* facing many threats from drought, overcollicting and climate change that may lead to its extinction in the near future. It was observed that *P. boveana* number of individuals specially adult ones is too small that may reach to be listed it as endangered species and this need a high level of proficient care in dealing with this situation in the near future.

It was revealed from this study that propagation from seed is a viable method for the *ex-situ* conservation of *P. boveana*, although this species has stringent requirements for germination (i.e. growth promoters that may only be provided by specialist research or propagation facilities). However, seed storage and germination are only the first steps in the reinforcement of populations of these species: studies to attain baseline data on *ex-situ* plant development and establishment in the field following transplantation are now required. Seeds of this species are scarce, and should extensive population reinforcement be necessary, a subsequent phase of multiplication using rosette division or meristem culture could be performed (Wochok, 1981).

The seed-dormancy variation observed in the present study within and among natural populations of *P. boveana* may be advantageous for a number of reasons. Presenting long-lived dormant seeds that may form seed banks with a high intrinsic genetic variability, since it contains seeds of different generations with different genotypes (Bazzaz, 1996). These highly diverse seed banks would give rise to highly diverse populations that may be able to maintain their variation even when subjected to selection, related to short-term environmental changes (Templeton and Levin, 1979) or genetic drift (Loveless and Hamrick, 1984).

The results also suggest that germination behavior could differ between sites. A warmer and drier climate may contribute to reducing species fitness and increasing the risk of local extinction in the long term. Thus, as genetic variability (expressed as phenotypic and physiological plasticity) will underpin adaptation to a changing climate, a study of the genetic diversity within and between populations of *P. boveana*, comparing cDNA micro-satellite regions, is needed. In addition, a long-term study of the phenology and demographics of the *P. boveana* population is in progress, in order to evaluate the effectiveness of sexual reproduction *In-situ.*

CONCLUSION

In conclusion, the present study demonstrates that environmental variation may lead to change in demographic, morphological, and reproductive characteristics of endemic species *P. boveana.* Environmental conditions like topography, soil, climate, and threats that surround restricted species may cause changes in the behavior of this species in dealing with any recent changes in these conditions. These changes may cause genetic variation by long time, which reflect its effect on the plant response. It was observe that not all species individuals are equal in their response to the change in the surrounding ecology. Generally, this study found that *P. boveana* young adult generation is faster than the parent generation in adapting to the new environment. This study revealed that *ex-situ* propagation of *P. boveana* is possible from seeds, thereby maximizing genetic variability, although growth promoters (GA) conditions is important for successful germination of this species.

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