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Full Length Research Paper

Fitness and its variation among populations of *Acacia tortilis* subsp. *raddiana* in Southern Sinai, Egypt

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***Acacia tortilis* subsp. *raddiana* (Fabaceae) plays an important role in the life of desert animals and is a major source of livestock feed and firewood for the native Bedouin people in Southern Sinai, Egypt. High mortality and low juvenile recruitment has been reported in recent years leading to decline in population size and number of this species. Small populations tend to be subject to an increased probability of stochastic extinction due to demographic, environmental and genetic factors. Therefore, this study aims to determine if *A. tortilis* subsp. *raddiana* populations suffer reduced fitness and its correlation or association with genetic diversity and mating parameters. Correlations and association between fitness, population size, genetic variation, and mating system parameters were tested using Spearman correlation and simple regression analyses. Stepwise regression analyses were used to identify useful predictors for fitness. The results revealed that *A. tortilis* subsp. *raddiana* populations' fitness is generally low to very low (0.1 ± 0.11). There was no relationship detected between *A. tortilis* subsp. *raddiana* fitness or population size and observed or expected heterozygosity. While there was no evidence for reduced fitness in *A. tortilis* subsp. *raddiana* populations, the study indicated that fitness is negatively associated with population size. Population size was associated also with the number of alleles (*A*) and number of alleles per polymorphic locus (A_P). The study came to the conclusion that although the low to very low values, there is no evidence for reduced fitness in *Acacia tortilis* subsp. *raddiana* small populations. Some of the studied populations (L6, L2, L3, and M4) are exceptionally fit which makes them the first candidates as seed source in restoration and both *in situ* and *ex situ* conservation efforts.**

Key words: *Acacia tortilis*, Sinai, germination, establishment, fitness.

INTRODUCTION

Habitat destruction and fragmentation has been an increasingly dominant process shaping landscapes over the last 100 - 150 years (Buza et al., 2000). For species that are now restricted to such fragmented habitats determining the viability of small, isolated, remnant populations is a research priority if informed management decisions are to be made. In Egypt, *Acacia tortilis* subspecies *raddiana* grows in desert wadis and sandy plains, usually in water catchments areas in the Sinai, Red Sea coast, Eastern Desert, and Gebel Elba (Boulos, 1999). In recent decades, drought and human interference including over-grazing, over -cutting, over-collecting, and habitat destruction have threatened this species by increasing the mortality of mature trees and reducing natural recruitment. As a result, it has undergone severe fragmentation and reduction in population number and sizes (Moustafa et al., 2000; Zaghloul et al., 2007). Such populations are prone to extinction due to stochastic demographic, environmental, and genetic factors as well as the deterministic processes which caused lowered numbers in the first place (Primack, 1998). Small population sizes influence inbreeding level and the amount and distribution of genetic diversity within tree species (Hamrick and Godt, 1989). Reduced fitness because of a loss of genetic variation and increasing inbreeding levels is often considered factor that may increase the probability of extinction of small and isolated populations (Menges, 1992; Widén, 1993; Van Treuren et al., 1993a; Heschel and Paige, 1995). Therefore, studying the influence of inbreeding and loss of genetic diversity on fitness has be-

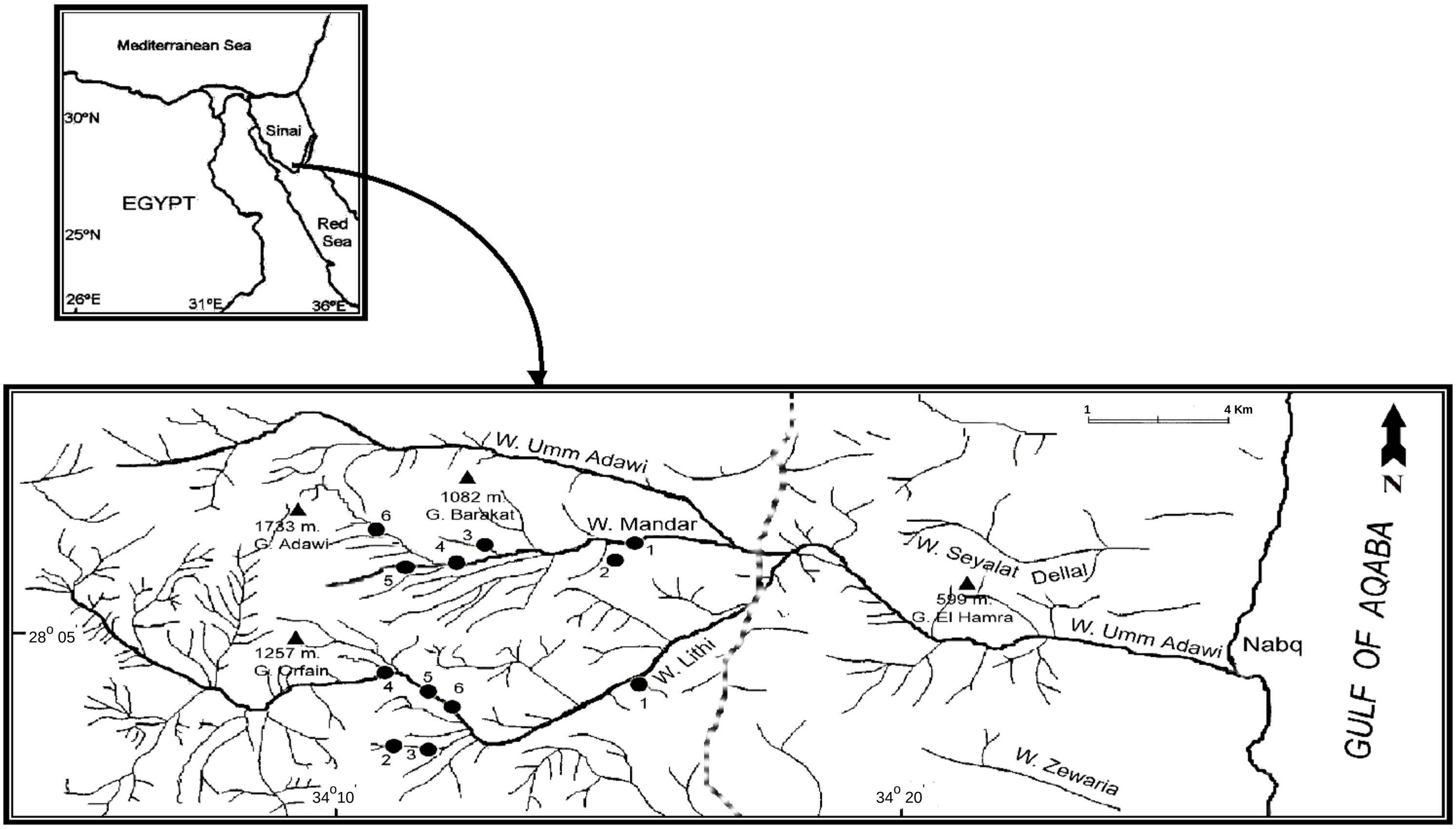


Figure 1. Location map showing the sampled populations of *Acacia tortilis* subsp. *raddiana*.

become a major component in conservation efforts for threatened species (e.g. Barrett and Kohn, 1991; Hedrick and Miller, 1992).

In Zaghloul et al. (2007), sixteen allozyme loci were used to examine regional genetic diversity within and among twelve natural populations of *A. tortilis* selected to repre-sent its geographic range in two large Wadis in Southern Sinai, Egypt. In Zaghloul and Hamrick (2007), the authors presented an analysis of outcrossing and inbreeding rates in *A. tortilis* subsp. *raddiana* for potential use in establishing an effective restoration and conservation strategy for natural populations from Southern Sinai, Egypt. In Zaghloul et al. (2008), using the age- radius rela-tionship, the ages of five hundreds and forty trees from thirty three *A. tortilis* subsp. *raddiana* populations was estimated. The estimated ages were used to determine the age distribution and construct a static life table. The age distribution of the studied populations was used as a predictive tool to determine if the *A. tortilis* subsp. *Rad-diana* populations in Southern Sinai are healthy or not.

In present study, the author addresses three questions; (1) do small, isolated *A. tortilis* subspecies *raddiana* populations suffer reduced fitness, (2) is there any correlation or association between fitness traits and any of genetic diversity and mating parameters in *A. tortilis* subspecies *raddiana* populations, and (3) are there significant differences in fitness between populations of *A. tortilis* subspecies *raddiana*.

MATERIALS AND METHODS Population samples and seed collection

To test for variation in fitness and its traits (germination, establishment, and un-infested seeds), twelve *A. tortilis* subsp. *raddiana* populations were sampled representing the geographic distribution of the species in Southern Sinai; six populations from Wadi Mandar and six populations from Wadi Lithi (Figure 1). Sample size from each population ranged from 3 to 30 trees with a total of one-hundred and fifty-eight trees (Table 1). The number of sampled individuals depended on the actual population size as well as seed availability.

Estimation of legume production and infestation rate

Legume production was estimated for forty-one fenced trees (19 in Wadi Mandar and 22 in Wadi Lithi) representing the sampled twelve populations (Table 1). The fallen pods under each tree (after been shaken strongly) were collected on a large sheet of cloth and weighed. The legume production was estimated as mean fresh weight (kg) for the population.

Infestation by bruched beetles was determined visually and infested seeds were separated manually in each seed lot for each of 158 sampled tree (Table 1). The rate of infestation was estimated as a mean on population base.

Seed viability and germination behavior

To quantify the viability of *A. tortilis* subsp. *raddiana* seeds, open-pollinated bulk un-infested seeds were chosen randomly representing studied populations. The seeds were soaked for 12 hours in water and then for another 12 h in diluted tetrazoluim salt solution (0.007 gm / L). The percentage of viable and non-viable seeds were counted. The viable embryos gain a pale pink color, while the non-viable embryos gain a yellow color (Smith and Throneberry, 1951).

A. tortilis subsp. *raddiana* seeds, like those of most acacias, have an innate dormancy due to presence of thick seed testa and a waxy layer which prevent the imbibitions of water. Physical and chemical techniques were evaluated as potential methods to break dormancy (Table 2). The following treatments were carried out: (1) Manual shaking for 5, 15, 20, and 25 min with gravel and coarse sand, (2) boiling in water for 0, 5, 10, and 15 min then leaving till cooling, (3) boiling in water for 0, 5, 10, and 15 min then leaving till cooling and soaking (in the same water) for 24, and 72 h, **(**4) soaking in hot water for 5 min and then in cool water for another 5 min for one, two, and three turns, (5) washing with detergent, (6) soaking in detergent for 24 h, and (7) soaking in concentrated H_2SO_4 for 5, 10, 15, 20, and 25 min then washing thoroughly. In all treatments ten (replicates) plastic pots (12 cm diameter) each containing 10 seeds were used.

Variation in seed germination and establishment

To test for variation among populations in germination and establishment rates, open-pollinated seeds were germinated in common environmental conditions (common garden experiment) to remove the environmental variance. Seeds were first treated with 95% sulfuric acid for 25 - 30 min and then rinsed thoroughly with water. They were sown on Fafard mix no. 3B (Canadian Sphagnum Peat, Vermiculite, Perlite, and Processed Pine Bark.) and placed in a greenhouse at the University of Georgia Plant Growth Facility. Seedlings were allowed to grow to 5 - 10 cm in height. Seedlings were considered to be established when they formed leaves and survived for at least a month after transplanting. The mean germination and establishment percentages were calculated and used to calculate the population mean and standard deviation.

Early life history fitness index

Un-infested (sound) seeds, germination, and establishment percenttages are the main and the most important factors in life history of *A. tortilis* subsp. *raddiana* affecting its early survivorship especially in such harsh desert environments. Since fitness is the probability of a plant to survive until the reproductive age and gives offspring, it is a net function of the total number of seeds that the mother plant produces, the viability of the produced seeds, the probability of germination and producing seedlings, and the probability of these seedlings to grow ahead of seedling (juvenile) stage and get established (adult). As the viability test revealed that all sound seeds collected from the studied populations are completely viable, the viability does not contribute to differentiate between populations in their fitness. Also the number of seeds been produced by mother trees are affected by different factors including the prevailing environmental conditions at each population. Therefore, sound seed, germination, and establishment percentages were used to construct the early life history fitness index as follows:

Fitness = sound seed x germination x establishment

The resulted fitness index was used to estimate the fitness distances between populations using Euclidean dissimilarity (Gower, 1985) algorithm in Genstat 10 (Payne et al., 2007) as $\{(x_i - x_j) / r\}^2$, where x_i and x_i are the values of the data variate *i* and *j* and *r* is the range of the variate. Correlation between the fitness and genetic distances matrices was assessed by Mantel test based on sums of squares and 1000 permutations (Manly, 1991).

Data analyses

Statistical analyses of fitness traits and fitness index were performed using Minitab release 14 computer program. One-way ANOVA or Kruskal-Wallis was used to test for significant differences between populations. Kruskal-Wallis test was used when an ANOVA could not be used due to a violation of its assumptions, normality and/or variance homogeneity. Correlations between fitness traits and genetic variation and mating system parameters were tested using a Spearman correlation analysis. Association between population size and genetic diversity, mating system, and fitness traits were analyzed with simple regression analysis. Genetic diversity parameters (Zaghloul et al., 2007) included: mean number of alleles per locus (*A*) and per polymorphic locus (*AP*), the effective number of alleles per locus (A_e) the percentage of polymorphic loci (*P*), and mean observed (H_o) and expected heterozygosity (H_e). Mating system parameters (Data from Zaghloul and Hamrick, in press) included; multilocus outcrossing rates (t_m), single locus outcrossing rates (t_s), biparental inbreeding (t_m – t_s), multilocus correlation of outcrossed paternity (r_{pm}) = single locus correlations of outcrossed paternity (r_{ps}), and effective number of pollen parents (N_{ep}), and fitness traits were analyzed with simple regression analysis. Stepwise regression analyses were used to identify a useful subset of the predictors (genetic and mating system parameters) for the investigated independent parameter (fitness traits, and fitness index).

RESULTS

Seed viability and breaking dormancy

Using Tetrazolium salt diluted solution as a test for viability revealed that open-pollinated bulked seeds randomly collected form the studied populations have 100% viability. The highest germination percentages of *A. tortilis* subsp. *raddiana* seeds (93 and 83%, respectively) were obtained by soaking in sulfuric acid for 20 and 25 min (Figure 4). Meanwhile, the manual shacking for 10/15 min or boiling in water for 5 min and soaking for 24 h gave the highest germination percentages among physiccal scarification treatments (28, 24, and 28%, respecttively). These treatments were almost equivalent to soaking in concentrated Sulfuric acid for 10 min (24%). The results showed also that treatment can affect the speed of germination (Figure 2). Boiling in water for at least 5 min accelerated the germination process to start, which in fact was faster than chemical scarification by Sulfuric acid even for long periods such as 20 and 25 min. On the other hand, the data showed that the highest mean of days at which highest germination occurred was 20 days found at physical treatments of manual shaking for 10 and 15 min using gravel and coarse sand with the seeds together. While as the lowest mean of days at which highest germination occurred was 1 - 2 days when seeds were boiled for 5 min until cooling.

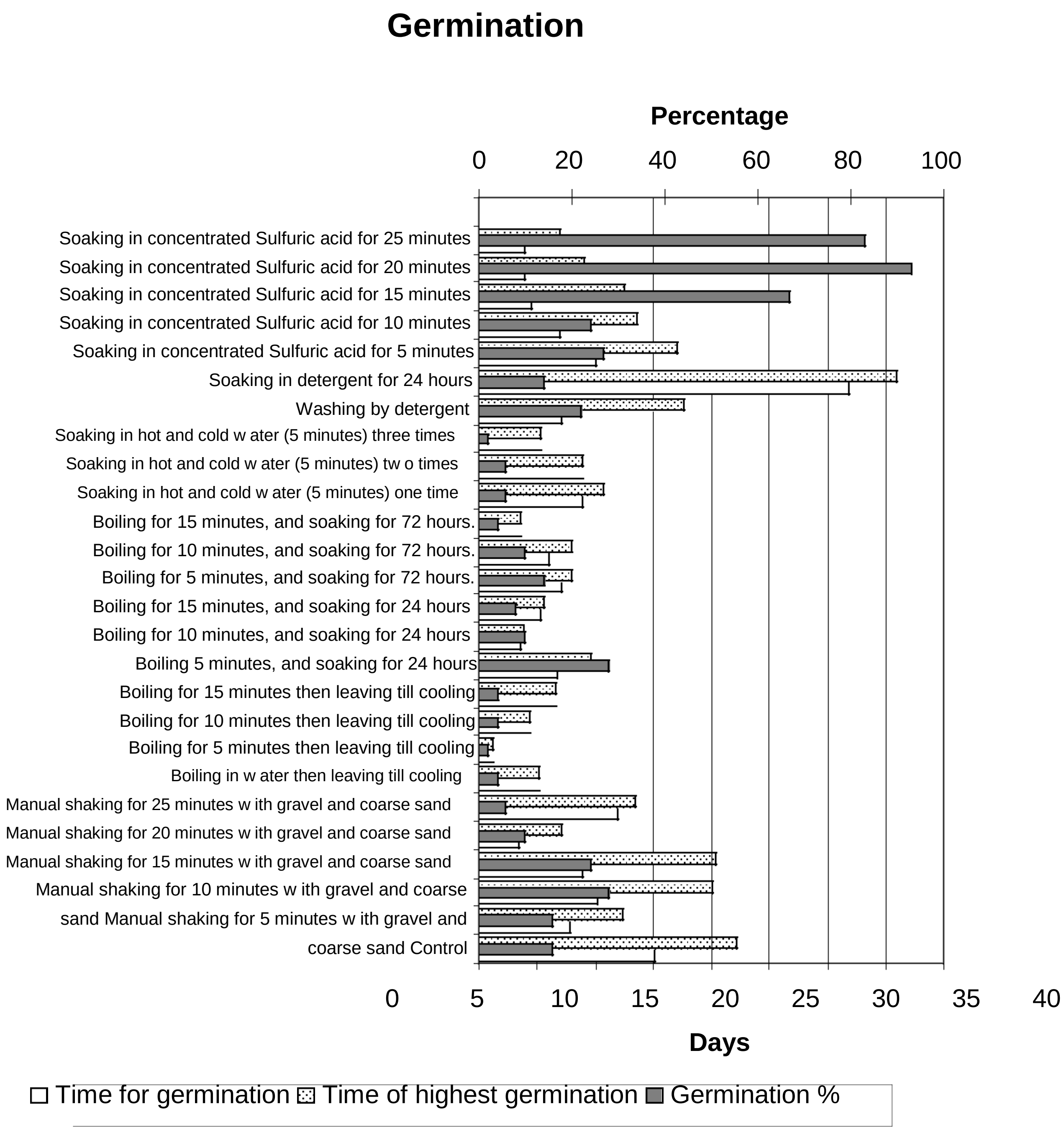
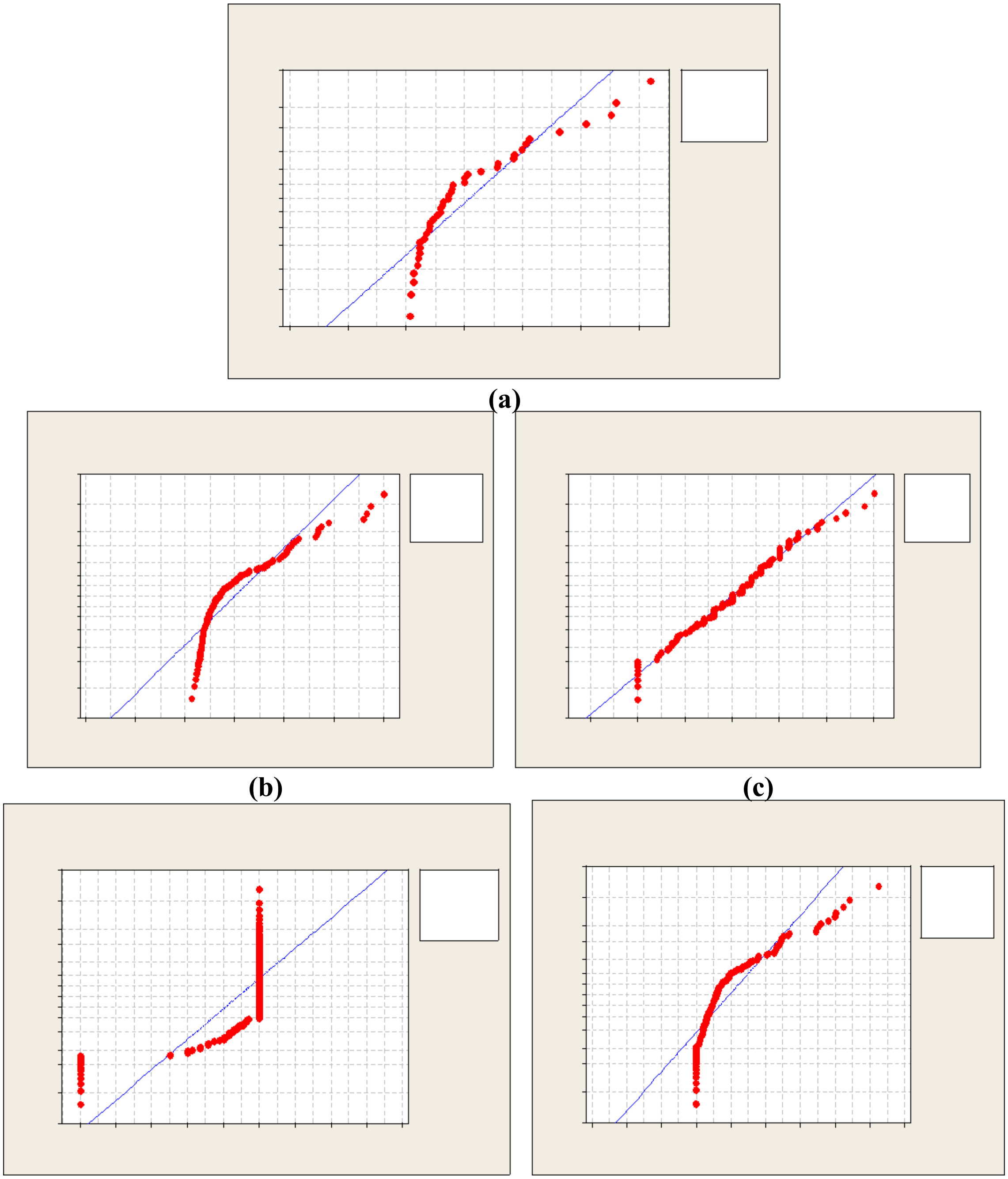


Figure 2. Treatments used to break dormancy in *Acacia tortilis* subsp. *raddiana* and response of germination percentage.

Legume production and seed infestation rate

As normality of distribution was violated (Figure 3), Kruskal-Wallis test was used to test significance of variation in legume production of *A. tortilis* subsp. *Raddiana* trees among populations (Figure 4). It revealed that legume production is significantly different between populations (*H* = 20.72 and *P* = 0.036). The population mean ranged from 1.43 ± 0.9 kg (M5 population) to 15.31 ± 3.16 kg (L6) with an overall mean of 5.45 ± 5.3 kg (Table 1). The mean legume fresh weight was higher for trees at W. Lithi (6.24 ± 6.08 kg) than for those at W. Mandar (4.54 ± 4.21 kg). Spearman rank correlation revealed that there is no significant correlation between canopy cover and legume production (Rho = 0.227). While legume production (Kg) was only significantly positively correlated with establishment percentage (Rho = 0. 7) (Table 2), the multiple stepwise regression equation associated



(a)

(b)

(c)

(d)

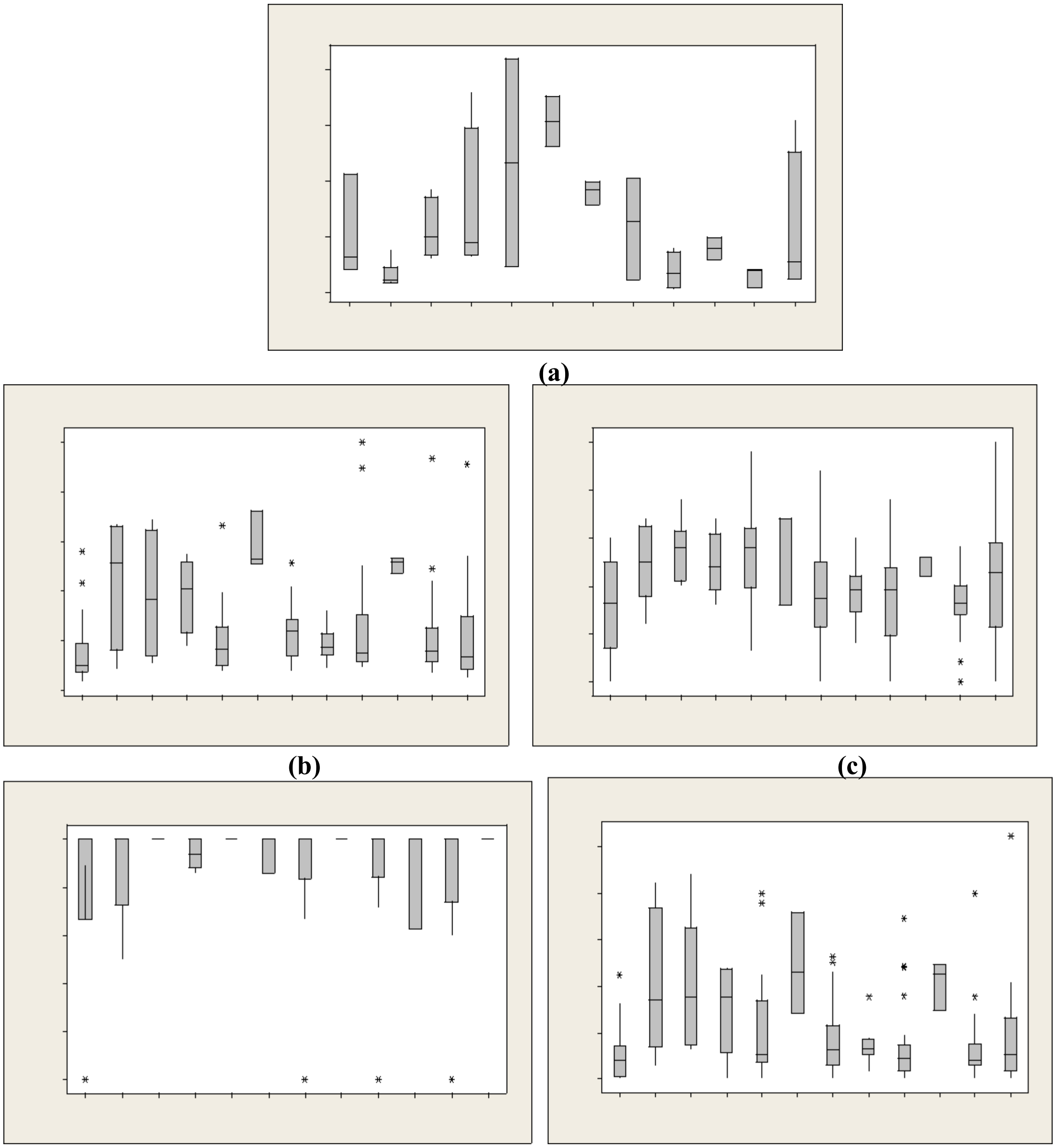
(e)

Figure 3. Normality test results for distribution of legume production (a), sound seeds (b), germination (c), establishment (d), and fitness index (e).

legume production with establishment percentage and single locus correlations of outcrossed paternity (r_{ps}) (r^2 = 51.61) as follows:

Legume production = -32.72 + 0.39 establishment + 32 r_{ps}

The results showed that seeds collected from W. Mandar were infested with insect (sound seeds = 23.40 ± 19.31%) much more than seeds collected from W. Lithi (sound seeds = 28.36 ± 21.93%) with an overall mean sound seeds of 25.05 ± 20.28%. The lowest infestation rate was recorded in L6 (sound seeds = 58.6 ± 21.93%) in W. Lithi and M4 (sound seeds = 50.69 ± 3.13%) in W. Mandar. In general, the lowest infestation rate was significantly higher than 46% (M4) (Table 1). Kruskal-Wallis test showed that variation in infestation rate among populations is significant (*H* = 31.79 and *P* = 0.001). The Spearman rank correlation showed a significance



(a)

(b) **(c)**

(c) **(e)**

Figure 4. Variation in legume production (a), sound seeds (b), germination (c), establishment (d), and Fitness index (e) among populations.

negative correlation between percentage of sound seed and population size (Rho = -0.72) and multilocus correlations of outcrossed paternity (r_{pm}) (Rho = -0.83) but positive with the fitness index (Rho = 0.88) (Table 2). In the meanwhile, the multiple stepwise regression equation associated sound seed percentage with fitness index, germination percentage, single locus correlations of outcrossed paternity (r_{ps}) and multilocus correlations of outcrossed paternity (r_{pm}) (r^2 = 98.02) as follows:

Sound seeds = 32.95 + 2.41 Fitness -

0.78 Germination + 0.127 r_{ps} -11.2 r_{pm}

Seed germination

The population mean of germination percentage of *A. tortilis* subsp. *raddiana* seeds ranged from 30.65 ± 18.9 % (L1) to 5.00 ± 12.42 % (L3) with a mean of 37.17 ± 19.28 % for W. Mandar, 44.72 ± 20.41 % for W. Lithi and 39.71 ± 19.92 % for pooled populations from the two wadis (Table 1). According to Kruskal-Wallis test, the less than 97%. The Spearman rank correlation showed that germination has significantly positive correlation with establishment (Rho = 0.67), fitness index (Rho = 0.74), and A_e (Rho = 0.59) and a negative correlation with Rpm (Rho = -0.65) (Table 2). The multiply stepwise regression

Table 1. Mean of fitness index and traits in studied populations.

Population		Germination %		Establishment %		Percentage of sound seeds		Fitness index		Pod Production (Kg)		
	No. of trees	Mean	St Dev	Mean	St Dev	Mean	St Dev	Mean	St Dev	No. of trees	Mean	St Dev
M1	18	36.61	21.83	88.49	23.95	23.18	11.89	0.09	0.08	3	8.98	1.04
M2	8	37.47	13.39	99.11	2.53	18.28	7.04	0.07	0.05	3	5.90	4.57
M3	22	35.12	18.01	86.07	29.14	26.83	25.95	0.07	0.09	4	1.88	1.72
M4	3	46.67	4.62	87.50	21.65	50.69	3.13	0.21	0.05	2	3.93	1.45
M5	24	33.60	13.47	84.43	28.90	22.03	18.88	0.07	0.08	3	1.43	0.90
M6	30	40.85	24.35	88.19	30.50	20.82	19.81	0.08	0.10	4	5.50	6.72
L1	18	30.65	18.99	77.16	42.52	15.74	14.62	0.05	0.07	3	5.22	4.66
L2	6	49.33	16.13	88.33	20.41	43.66	24.66	0.20	0.16	6	1.55	1.15
L3	8	55.00	12.42	98.53	4.16	38.18	24.83	0.20	0.14	5	5.71	2.72
L4	5	49.60	13.45	94.07	6.13	38.35	15.41	0.15	0.1	4	7.49	7.01
L5	13	54.04	21.95	95.96	11.34	21.17	16.93	0.11	0.13	2	11.60	13.15
L6	3	44.00	20.78	95.24	8.25	58.60	11.86	0.24	0.11	2	15.31	3.16
Mean W. Mandar	105	37.17	19.28	87.75	27.06	23.40	19.31	0.08	0.09	19	4.54	4.21
Mean W. Lithi	53	44.72	20.41	88.88	27.35	28.36	21.93	0.13	0.13	22	6.24	6.08
Overall mean	158	39.71	19.92	88.13	27.07	25.05	20.28	0.1	0.11	41	5.45	5.30

Table 2. Spearman rank correlations between fitness traits and population size and genetics and mating system parameters. Note: *P* = % polymorphic loci, *AP* = mean number of alleles per polymorphic locus, *A* = mean number of alleles per locus, A_e = effective number of alleles per locus, H_o = observed heterozygosity, and H_e = unbiased heterozygosity expected under Hardy-Weinberg assumptions, t_m = multilocus outcrossing rates, t_s = single locus outcrossing rate, $t_m - t_s$ = biparental inbreeding, r_{pm} = multilocus correlations of outcrossed paternity, r_{ps} = single locus correlations of outcrossed paternity, and N_{ep} = the effective number of pollen parents. * *P* 0.05.

	Pop size	Pod production (Kg)	Germination	Establishment	Sound seeds	Fitness index
Pop size						
Pod production (Kg)	-0.34					
Germination	-0.57	0.38				
Establishment	-0.44	0.70*	0.67*			
Sound seeds	-0.72*	0.05	0.48	0.13		
Fitness index	-0.79*	0.32	0.74*	0.41	0.88*	
P	-0.02	-0.32	-0.33	-0.46	-0.12	-0.07
A_p	0.70*	-0.01	0.00	-0.05	-0.49	-0.44
A	0.64*	-0.18	-0.17	-0.29	-0.54	-0.42
A_e	-0.19	0.10	0.59*	0.22	0.01	0.24
H_o	-0.32	-0.05	0.41	-0.04	0.01	0.25
H_e	-0.28	0.11	0.64*	0.31	-0.03	0.20
r_{pm}	0.71*	-0.26	-0.65*	-0.30	-0.83*	-0.78*
r_{ps}	0.17	0.14	-0.56	-0.09	-0.33	-0.35
t_m	-0.33	-0.15	0.11	-0.06	-0.03	0.15
t_m-t_s	0.00	-0.10	-0.24	-0.21	0.10	0.05
t_s	-0.09	0.00	0.12	-0.10	-0.34	-0.08
N_{ep}	-0.46	-0.10	057	0.06	0.48	0.51

associated germination percentage with fitness index, sound seeds percentage, and single locus correlations of outcrossed paternity (r_{ps}) (r^2 = 86.68) as follows:

Germination = 41.62 + 2.44 Fitness index -0.83 Sound seeds -38 r_{ps}

Seedling establishment

Establishment percentage is the only fitness trait that has non-significant (H = 5.55 and P = 0.901) difference among *A. tortilis* subsp. *raddiana* populations. It was significantly higher than 27.80 % in all populations but L1. It ranged from 77.16 ± 42.52 % (L1) to 99.11 ± 2.53 % (M2) with a mean of 87.75 ± 27.06 % at W. Mandar, 88.88 ± 27.35 % at W. Lithi, and 88.13 ± 27.07 % for pooled populations from the two wadis. The establishment percentage was not correlated with any of the population genetic diversity or mating system parameters. (Table 2). Although it was significantly positively correlated with both legume production (Rho = 0.70) and germination percentage (Rho = 0.67), the stepwise regression associated it with germination percentage only (r^2 = 43.68) as:

Seedling establishment = 68.01 + 0.52 Germination

Early life history fitness

The early stage fitness index was generally low to very low in almost all *A. tortilis* subsp. *raddiana* populations. Meanwhile it was very highly significantly (H = 31.41 and P = 0.001) different between populations (Table 1). Population L1 was the population that has the lowest fitness (fitness index = 0.05 ± 0.07), while population L6 has the highest fitness (fitness index = 0.24 ± 0.11). The mean fitness index for Lithi (0.13 ± 0.13) was higher than that for W. Mandar (0.08 ± 0.09), and even for pooled populations from the two wadis (0.1 ± 0.11). Fitness index was significantly positively correlated with popu-lation size and negatively correlated with (r_{pm}) (Rho = - 0.78) (Table 2). The multiple stepwise regression asso-ciated fitness index with sound seeds and germination percentages, single locus correlations of outcrossed paternity (r_{ps}), and multilocus correlations of outcrossed paternity (r_{pm}) (r^2 = 98.58) as follows:

Fitness = - 13.515 + 0.402 Sound seeds + 0.328 Germination – 0.051 r_{ps} + 4.4 r_{pm}

Mantel test detected very low association between the genetic and fitness distance matrices (association coefficient = 0.3621, percent permutations with equal or greater association = 2.10).

DISCUSSION

Small populations tend to be subject to an increased probability of stochastic extinction, due to demographic, environmental and genetic factors (Shaffer, 1981, 1987). In such populations, the limited number of mating partners and reduced levels of genetic variation as a result of genetic drift may further increase the likelihood of inbreeding (Lacy, 1987; Ellstrand and Elam, 1993). Theory predicts that high levels of inbreeding and genetic drift will occur in small populations, and that these processes will, respectively, lead to increased homozygosity and the random loss of alleles. In *A. tortilis* subsp. *raddiana*, although it occurs in small populations that have a risk of demographic extinction (Zaghloul et al., 2008), no decrease in genetic diversity was observed (Zaghloul et al., 2007). The observed heterozygosity (H_o = 0.264) is high compared not only to estimates for other tropical acacias but for plants in general. One factor with clear potential to delimit genetic changes in small isolated populations, and their effects on population persistence, is ploidy level. Theory suggests that, for a given populations size, auto-tetra-ploids are less subject to loss of allelic richness by genetic drift than diploids (Bever and Felber, 1992). Simi-larly, inbreeding is expected to erode heterozygosity more slowly in autotetraploids than in diploids (Glendinning, 1989). As with other African acacias, *A. tortilis* is a polyploid complex (Fagg, 1991), most are tetraploids (2n = 4x = 52) but subsp. *raddiana* is an octoploid (2n = 8x = 104). Also, mating system analysis revealed that *A. tortilis* subsp. *raddiana* is highly out-crossing plant (Zaghloul and Hamrick, in press). Further-more, it is probable that the small populations of *A. tortilis* subsp. *raddiana* seen today are a rather recent occur-rence.

Plants in populations that have experienced reductions in the number of individuals only recently may show reduced fitness after inbreeding, whereas individuals of populations that have been small during several generations may exhibit a much lower response to inbreeding because of purging of the genetic load (Van Treuren et al., 1993b). Although previous estimates indicated that *A. tortilis* spp. *raddiana* is predominantly outcrossing, suggesting the presence of a strong self-incompatibility system (Zaghloul and Hamrick, in press),this study shows that fitness values of *A. tortilis* subsp. *raddiana* is generally low to very low. Meanwhile, there was no relationship detected between fitness and heterozygosity suggesting that genetic drift has not yet had the chance to act to reduce diversity in these populations. This result was strengthened by the very low association that had been detected by Mantel test between the genetic and fitness distance matrices. On the other hand, fitness and sound seed have signify-cant negative correlation with population size and hence the detected negative correlation with correlations of outcrossed paternity (r_{pm}) becomes very logic. Also, we found no correlation between population size and observed or expected heterozygosity. Positive correlations have frequently been found between individual heterozygosity and fitness component (Mitton and Grant, 1984; Allendorf and Leary, 1986; Zouros, 1987; Bush et al., 1987; Wolff and Haeck, 1990). Koehn et al. (1988) and Booth et al. (1990) reported that such relationships could not be demonstrated. The relationship between heterozygosity and fitness traits seems to be restricted to growth rate and developmental homeostasis of some characters and is only occasionally reported for other fitness characters (Ouborg and Van Treuren, 1995).

The results revealed that germination percentage of *A. tortilis* subsp. *raddiana* seeds at pooled population mean is 39.71 ± 19.92 % which more or less similar to results of Moustafa et al. (1996). The results showed that germi-nation percentage and expected heterozygosity are significantly correlated (Table 2). Germination has been postulated to be under control of one or only a few loci (Husband and Schemske, 1996). The difference between populations in germination was highly significant (H = 23.82 and P = 0.014) . Germination differences among populations could well reflect inbreeding effects, and germination differences among individuals have important fitness consequences (Menges, 1995). Population diffe-rences in seed germination and populations' responses to environmental conditions have been documented for many species (Farmer et al., 1986; Wu et al., 1987; Meyer et al., 1989), but to consider the possible effects of possible frag-mentation-caused reduced genetic variation, these germina-tion differences must be correlated with population size and isolation which is not the case here (Table 2). These results strengthen the suggestion that the inbreeding and genetic drift have not yet had the chance to act to reduce diver-sity in the studied populations.

As might be expected, population size is correlated with both the number of alleles (A) and number of alleles per polymorphic locus (A_P). The relationship between population size and the amount of genetic variation within populations has been extensively studied and it has frequently been found that small plant populations exhibit lower allelic diversity (Moran and Hopper, 1983; Lesica et al., 1988; Billington, 1991; Van Treuren et al., 1991).

Seeds of *Acacia* species possess an innate dormancy caused by an impermeable seed coat. Field germination cannot occur unless this dormancy is broken, allowing the entry of water. Four basic dormancy breaking mechanisms have been recorded or inferred in the literature for hard-seed species. These are natural scarification through the action of fungi, bacteria and soil acids (Robbertse, 1974; Cavanagh, 1980), passage through the gut of birds or mammals which feed on the seeds (Lamprey, 1967; Janzen 1969), unsuccessful pre-dispersal seed predation, where the larval seed predator enters the host seed breaking the impermeable seed coat and perishes leaving the seed viable and non-dormant, (Halevy, 1974; Lamprey et al., 1974), and fire (Stone and Juhren, 1953; Floyd, 1966; Gill and Groves, 1981; Auld and Myerscough, 1986).

Some *Acacia* seed germinate under favorable conditions, usually after rain, but very few make it to the mature stage. Scarification by physical or chemical means was recommended in promoting germination of *Acacia* species seeds in laboratory. Grime et al. (1981) studied the germination of leguminous species and found that abrasion by sand and chemical scarification by concentrated sulfuric acid for short periods induced a rapid germination percent. Tran (1981) stated that dis-turbing the aril of *Acacia* seeds by prolonged heating at 60^{O}C or by a scalpel is the most satisfactory method in germination of *Acacia* . Immersion of *Acacia* seeds in sulfuric acid showed the highest percent of germination 89% (Hendawy, 1990; Abd El-Wahab, 1995).

In conclusion, there was no evidence for reduced fitness in *A. tortilis* subsp. *raddiana* small populations. Similar results were obtained for *Ballota* species (Zaghloul, 2003), *Scabiosa columbaria* (Van Treuren et al., 1993a), *Lychnis flos-cuculi* (Hauser and Loeschcke, 1994), and *Salvia pratensis* (Ouborg and Van Treuren, 1995). The L6, L2, L3, and M4 populations have high levels of legume production, and germination and esta-blishment percentages, and hence is exceptionally (relatively) fit over all other studied populations which makes them the first candidates as seed source in restoration and *ex situ* conservation efforts. Based on the presence of rare alleles and higher genetic diversity, previous study (Zaghloul et al., 2007) suggested that populations M4 and M6 should get the first priority for conservation at Wadi Mandar and L6 and L3 at Wadi Lithi. These results coupled with the information got from present study imply that population L6 should get the top priority of conservation efforts at the two wadis due to its unique genetic composition.

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