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Full Length Research Paper

Genetic diversity in coastal and inland desert populations of *Peganum harmala* L. (Peganaceae)

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This study compared the genetic diversity within and among six naturally growing coastal and inland populations of Peganum harmala by using random amplified polymorphic DNA (RAPD) technique. Seven primers generated a total of 63 RAPD bands (loci) of which 60 (95.24%) were polymorphic across all individuals. The genetic diversity of *P. harmala* at the population level and species level were percentage polymorphic loci (*PPL*) = 42.59%, Nei's gene diversity (*h*) = 0.1892, Shannon information index (*I*) = 0.2711 and *PPL* = 95.24%, *h* = 0.3116, *I* = 0.4723, respectively. The value of differentiation (the coefficient for gene divergence, *G*st = 0.3925) and analysis of molecular variance (AMOVA) indicated that there was a relatively high genetic differentiation within populations, and about one-six of the genetic variations occurred among populations. Analysis of fixation indices (*FST*) = 0.15500, *p* < 0.00196 showed low degree of differentiation among populations. The genetic variation in the coastal populations was higher than the variation in the inland populations. The present study suggests that the gene drift may play an important role in the differentiation of *P. harmala* populations. The *in situ* conservation of the species should focus on establishing more sites to protect the natural populations and their habitats, while the *ex situ* conservation needs to focus on enhancing the exchange of seeds and seedlings among populations to facilitate gene flow, exchange and recombination.

Key words: Random amplified polymorphic DNA (RAPD), gene diversity, soil factors, arid lands, Egypt.

INTRODUCTION

Peganum harmala L. (Peganaceae) is a perennial herbaceous, glabrous plant, which grows in different coastal and inland habitat types including sandy, rocky and gravel soils. The species is widely distributed in Middle East, India, Mongolia and China (Frison et al., 2008; Boulos, 2009). In Egypt, plant populations are distributed mainly along the Mediterranean coastal strip, Eastern desert and Sinai. P. harmala populations grow in slightly salt affected and non-salty habitat types. The plant occupies niches that receive runoff water in addition to the recorded rainfall. The species has long been used for medicinal purposes, as fungicide and herbicide due to the presence of harmine (Bertin et al., 1993). In addition, P. harmala play an important role in restoring the local ecosystem as drought resistant species.

The decreasing number of natural populations due to over collection and the rapid fragmentation of natural habitats have a severe impact on genetic diversity of the species. Genetic diversity plays an important role in conservation of the species. Researchers generally consider that loss of genetic variation is usually accompanied by reduction of the species' ability to cope with changes in the environment, which causes the species to become rare (Frankham et al., 2002; Hogbin et al., 2000). Estimation of genetic diversity of the populations has therefore been recognized as basic topic not only to describe *in situ* and *ex situ* conservation measures (Morden and Loeffler, 1999; Hegazy, 2000, 2001) but also to establish forms of reasonable sustainable management (Lacerda et al., 2001).

The conservation and management of biodiversity are complex challenges that require a basic understanding of

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Table 1. Location of the collection sites of the six study populations of *Peganum harmala* and their respective geographic coordinates in Egypt.

Population	Population site	Longitude (N)	Latitude (E)	Elevation (m)
1	Coastal desert, Borg Al-arab, Beer Bremly	30°54'33.50"	29°31'2.70"	13
2	Coastal desert, Borg Al-arab, Medan El-senya	30°54'7.50"	29°32'42.20"	13
3	Coastal desert, Omayed' train station	30°47'3.31"	29°12'9.30"	12
4	Inland desert, Marsad Al-kattamya	29°58'22.90"	31°48'36.20"	293
5	Inland desert, Wadi Al-ratam	29°48'38.40"	31°44'57.60"	395
6	Inland desert Wadi Al-reshrash	29°25'8.30"	31°31'10.40"	236

the distribution and abundance of species, their mutualistic interactions, their reproductive biology, and the genetic structure of their population (Williams et al., 1990; Barbosa et al., 2010). Therefore, the aim of the present study was to characterize the genetics of *P. harmala* utilizing random amplified polymorphic DNA (RAPD) molecular markers, focusing on their genetic variability, population structure and gene flow. The study provides some molecular information to understand the genetic background and further to support formulation of effective measures for the germplasm resources conservation, genetic improvement and sustainable utilization of the species.

MATERIALS AND METHODS

Plant populations

A total of 18 accessions of P. harmala were collected from six populations growing naturally in coastal and inland desert, Egypt (Table 1). Samples were collected from six populations. Three populations in the Mediterranean coastal land: Two from Borg Al-Arab (Medan El-Senya and Beer Bremly) and one population in Omayed. The other three populations were collected from the Eastern inland desert; from Wadi Al-Reshrash, Wadi Al-Ratam and Marsad Al-Kattamya. Soil physical and chemical characteristics were analysed following the procedures used by Hegazy et al. (2007). Soil characteristics supporting the six study populations are shown in Table 2.

DNA analysis

Fresh leaves of plants were collected and total genomic DNA was extracted using Wizard genomic DNA extraction kit promega (USA). 10- to 21-mer arbitrary primers were used for RAPD analysis. Seven primers were screened for their amplification (Table 3). PCR amplification was performed in total volume of 25 µl containing 10× reaction buffer, 2.5 mM dNTPs, 5 mM $MgCl_2$, 10 pmol/reaction primer, 100 ng of genomic DNA and (0.5 U μl^{-1}) of Taq polymerase (promega, Germany) in Thermocycler Gene Amp 9700 (Applied Biosystems (ABI), USA). After a denaturation step for 5 min at 95°C, the amplification reactions were carried out for 40 cycles. Each cycle comprised of 1 min at 95°C, 1 min of annealing temperature ranged from 28 to 30°C in the primers used and 1 min at 72°C. The final elongation step was extended to 10 min. Amplification products were separated on agarose gel electrophoresis using 1.5% (w/v) agarose in 0.5× TBE buffer and stained with ethidium bromide and photographed by using gel documentation system. Amplification products were compared with molecular weight marker I × (100 to 1000 bp).

Statistical analysis

RAPD bands were scored as binary presence (1) or absence (0) characters to assemble the matrix of the RAPD phenotypes. Then, the indices of genetic diversity, such as the percentage polymorphic loci (*PPL*), observed number of alleles (N_a), number of effective alleles (N_e), Nei's gene diversity (h), Shannon information index (I), the coefficient for gene divergence (Gst) and gene flow (N_m), were calculated using POPGENE 3.2 software (Yeh et al., 1999) on the basis of gene frequencies. Hierarchical analysis of molecular variance (AMOVA) within and among populations was estimated using allele frequencies with ARLEQUIN V. 1.1 (Excoffier et al., 1992). Genetic similarity was calculated on the basis of genetic distances coefficients using the NTSYS-pc program (Rohlf, 2002). The similarity matrix was subjected to cluster analysis by the unweighted pair group method with arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

The Pearson correlation between the genetic diversity index within population and ecological factors was analyzed using the SPSS 17.0 software. Meanwhile, the Mantel test was applied using XLSTATARTVIS software to test the significance of the association between the genetic distance and geographic distance matrices.

RESULTS

Genetic diversity in plant populations

Seven primers (Table 3) generated a total of 63 RAPD bands (loci), 11.1 bands per primer on average. The number of amplification products per primer varied from 6 to 14, and these primers produced fragments ranging from 10 to 300 bp in size (Figure 1). The genetic diversity of *P. harmala*, including *PPL*, gene diversity within population (h_S) and total gene diversity (h_T) was 95.24%, 0.1893 and 0.3116, respectively (Tables 4 and 5). The genetic diversity among populations *of P. harmala* from different habitats showed obvious differences, where the genetic parameters (*PPL%, I, h, N_a, N_e*) at mean population level were *PPL* = 42.59%, I = 0.2711, h = 0.1892, N_a = 1.4259, N_e = 1.3407, respectively (Table 4). It was found that the genetic variation in population 4 growing in Marsad Elkatamya reached the highest (I = 0.4344) and population 6 growing in Wadi El-Reshrash attained the lowest (I = 0. 1819) value.

The number of polymorphic loci (*PPL*), gene diversity

Table 2. The soil characteristics supporting the six study populations of Peganum harmala

Soil factor	Population					
	1	2	3	4	5	6
Coarse sand (%)	58.7	51.8	52.2	50.8	60.2	65.3
Fine sand (%)	28.5	33.7	23.9	20.5	30.8	18.4
Silt (%)	7.4	8.5	12.6	15.4	02.9	08.6
Clay (%)	4.6	5.7	5.2	05.5	03.5	04.9
pH	6.64	7.28	7.8	6.00	7.6	7.4
Conductivity (µs/cm)	137.2	520	74.9	35	38	41
Total nitrogen (mg/L)	1.87	2.75	2.46	0.526	0.295	0.644
Nitrate (mg/L)	8	12	19	7	4	8
Ammonia (mg/L)	0.08	0.05	0.01	0.00	0.00	0.00
Phosphate (mg/L)	2.30	1	4	0.291	0.214	0.255
Phosphorous (mg/L)	0.75	0.326	1.30	0.073	0.058	0.061
Chloride (mg/L)	6.88	7.56	6.88	3.6	2.5	3.1
Carbonate (mg/L)	57.63	41.38	38.41	41.2	36.5	34.6
Bicarbonate (mg/L)	0.00	0.00	0.10	0.15	0.12	0.00
Total alkalinity (mg/L)	57.63	41.38	38.41	31.05	30.27	30.61
Carbon (%)	7.73	6.64	7.51	1.14	0.83	0.96

Table 3. Sequences of the seven primers used in this study.

Primer	Sequences of primer (5→ 3`)
RAPD 6	AAA GCT GCG G
RAPD 10	GAG AGC CAA C
NS2	GGC TGC TGG CAC CAG ACT TGC
A9B7	GGTGACGCAGGGGTAACGCC
E1F	TCC GGG GTA TGT TAT GGA AGA
RAPD5	ATGCCC CTG T
B2	GGG AAA ACG ACA ATT GC

Table 4. Genetic diversity parameters in plant populations of *P. harmala* at the different study sites.

Population	Sample size	Polymorphic loci	Percentage population level (PPL %)	Observed number of alleles (N_a)	Number of effective alleles (N_e)	Shannon's index of diversity (I)	Nei's gene diversity (h)
Population 1	3	29	46.03	1.4603	1.3683	0.2930	0.2046
Population 2	3	33	52.38	1.5238	1.4190	0.3334	0.2328
Population 3	3	19	30.16	1.3016	1.2413	0.1920	0.1340
Population 4	3	43	68.25	1.6825	1.5460	0.4344	0.3030
Population 5	3	19	30.16	1.3016	1.2413	0.1920	0.1340
Population 6	3	18	28.57	1.2857	1.2286	0.1819	0.1270
Mean		26.83	42.59	1.4259	1.3407	0.2711	0.1892
Total overall loci	18	60	95.24	1.9524	1.5252	0.4723	0.3116

within group populations (h_S) and total gene diversity (h_T) of *P.* harmala groups was lower in group (2) populations (51, 0.1881 and 0.272) than group (1) (55, 0.1905 and 0.3073) (Table 6). The genetic parameters (*PPL%, I, h, N_a, N_e*) at population group level was lower in group (2) populations (80.95, 0.4140, 0.2720, 1.8095, 1.4482) than

Table 5. Genetic differentiation in populations of *P. Harmala* at the different study sites.

Parameter	POPGENE			
	h_S	h_T	N_m	G_{st}
Species level	0.1893	0.3116	0.7740	0.3925
SD	0.0111	0.0243	-	-

h_S, Gene diversity within population; h_T, total gene diversity; N_m, estimate of gene flow; and G_{st}, coefficient of gene differentiation.

Table 6. Genetic differentiation in the two population groups of *P. harmala* at different study sites.

Population group	POPGENE			
	h_S	h_T	N_m	G_{st}
Group (1): Coastal desert (pop. 1, 2 and 3)	0.1905	0.3073	0.8154	0.3801
SD	0.0159	0.0264	-	-
Group (2): Inland desert (pop. 4, 5 and 6)	0.1881	0.2720	1.1215	0.3084
SD	0.0200	0.0286	-	-

h_S = Gene diversity within population; h_T = total gene diversity; N_m = estimate of gene flow; and G_{st} = coefficient of gene differentiation.

Table 7. Genetic diversity parameters of the two population groups of *P. harmala* at the different study sites.

Population	Sample size	Polymorphic loci	Percentage population Level (PPL %)	Observed number of alleles (N_a)	Number of effective alleles (N_e)	Shannon's index of diversity (I)	Nei's gene diversity (h)
Coastal desert (pop. 1, 2 and 3)	9	55	87.30	1.8730	1.5191	0.4620	0.3073
Inland desert (pop. 4, 5 and 6)	9	50	80.95	1.8095	1.448	0.4140	0.2720

group (1) populations (87.30, 0.4620, 0.3073, 1.873, 1.5191) (Table 7).

Genetic structure within and among populations

The estimate of genetic structure of populations is significantly different from zero (p <0.001). The analysis by AMOVA implied that only 15.50% of genetic variation occurred among populations and most of the variation (84.50%) occurred within population (Table 8), which was in accordance with the G_{st} (39.25%). The estimate of gene flow N_m based on G_{st} was 0.7740, which indicated that gene flow among populations was low (Table 5). This result was equivalent to fixation indices (*FST*) 0.15500 p < 0.00196 calculated with Arlequin program, which implies a low degree of differentiation among population.

To identify the source of the highest genetic variation, AMOVA analysis was also performed among groups and separately for each group populations; Group (1) includes the inland desert populations and Group (2) includes the costal desert populations. Genetic variation among the two groups was found to be low, that reaching 1.8% of the total variation. The value of genetic variation was higher among Group (1) populations (21.86%) than Group (2) populations (10.00%) (Table 8). The gene flow N_m based on G_{st} among populations of Group (1) was lower (0.8154) than Group (2) populations (1.1213).

The genetic distance and genetic identity among populations are shown in Table 9. The variation of the genetic distance ranged from 0.1092 (between population 3 and population 6) to 0.3261 (between population 1 and population 6).

Correlation between genetic distance and geographic distance

On analyzing the obtained dendrogram (Figure 2), no clear relationship can be seen between genetic and geographic distances. Populations geographically close, such as populations 1 and 2 growing in Borg El-Arab far from each other only 2.79 Km are genetically distant from each other and clustered in different groups.

The Mantel test showed insignificant correlation between genetic distance and geographic distance equal

Table 8. Hierarchical analysis of molecular variance (AMOVA) within and among populations as estimated by using allele frequencies with ARLEQUIN version 1.1.

Source of variation	df	Sum of square	Variance component	Percentage variation	*P*
Among populations	5	69.333	1.64074^{Va}	15.50	< 0.001
Within populations	12	107.333	8.94444^{Vb}	84.50	< 0.001
Total	17	176.667	10.58519		
Fixation index, *FST*	0.15500				
Among groups (inland and coastal desert)	1	12.444	0.1975	1.88	
Among coastal desert populations (1, 2 and 3)	2	33.11	2.5185^{Va}	21.86	< 0.001
Within coastal desert populations (1, 2 and 3)	6	54.00	9.000^{Vb}	78.14	< 0.002
Among inland desert populations (4, 5 and 6)	2	23.778	1.000^{Va}	10	< 0.001
Within inland desert populations (4, 5 and 6)	6	53.333	8.888^{Vb}	89.89	< 0.001

The *P* values are the probabilities of having a greater variance component than the observed values by chance alone and are based on 1023 random permutations of the data matrix.

Table 9. Genetic identity (above diagonal) and genetic distance (below diagonal) among populations of *P. harmala.*

Population	1	2	3	4	5	6
1	****	0.806	0.7523	0.8198	0.7841	0.7217
2	0.2157	****	0.7963	0.854	0.8244	0.8124
3	0.2847	0.2278	****	0.1823	0.1467	0.8966
4	0.1987	0.1578	0.8333	****	0.8175	0.8458
5	0.2432	0.1931	0.8635	0.2016	****	0.8783
6	0.3261	0.2077	0.1092	0.1675	0.1298	****

to 0.312, which was not significant (*P* = 0.260 with 1000 permutations).

Correlation between genetic diversity and soil factors

The correlation analysis (Table 10) indicated that the genetic diversity indices of different populations showed insignificant (*P* > 0.05) correlations between the genetic diversity indexes and the soil factors. Genetic diversity indices attained negative correlation with coarse sand, pH, nitrate, phosphate and phosphorous, but demonstrated negative correlation with the other soil variables. There was also insignificant correlation (*P* > 0.05) between the genetic diversity indices and site elevation.

DISCUSSION

The RAPD amplification results and POPGENE analysis indicated that 60 bands were marked for all individuals of the six study populations, and these were all polymorphic. This means that low levels of diversity can cause deleterious effects on the population adaptation and its persistence (Reed and Frankham, 2003). The percentage of polymorphic bands ranged from 28.57 in population 6 to 68.25 in population 4. The mean percentage of polymorphic loci in *P. harmala* was lower (42%) than that reported for other long lived perennial plant species (48·1%) reported by Hamrick and Godt (1996). This shows that *P. harmala* has extremely low genetic diversity and variability. Shannon's information measures of each population indicated that the populations 3, 5 and 6 comprise a lower genetic diversity than populations 1, 2 and 4. The genetic loss in these populations might be attributed to the over collection of the plants in these locations.

At the species level, the genetic diversity indices *PPL*, *I* and *h* were 95.24%, 0.4723 and 0.3116, respectively. In this study, the mean value of Nei's gene diversity index (*h*) was 0.18, near to the minimum *h* value (0.174 to 0.328) of nine out-crossing plants summarized by Schoen and Brown (1991), which was consistent with the general characteristics of *P. harmala* as the species pollinated by animals.

About 1/6 of the genetic variations existed among populations, and most of genetic variation settled within population, which was similar to some endangered species, such as Cathaya argyrophylla (Wang et al., 1997); Liriodendron chinense (Li et al., 2002). Levels of genetic diversity in plant species are also associated with the plant species family. A review of genetic diversity in

Table 10. Pearson correlation analyses for the relationships between genetic diversity parameters within populations of *P. harmala* and soil factors.

Soil factor	Genetic diversity parameter				
	PPL %	N_a	N_e	Shannon's diversity (*h*)	Nei's gene diversity (*I*)
Elevation (m)	-0.050	-0.050	-0.050	-0.050	-0.051
Coarse sand (%)	-0.648	-0.648	-0.648	-0.648	-0.648
Fine sand (%)	0.033	0.033	0.033	0.033	0.034
Silt (%)	0.552	0.552	0.552	0.552	0.551
Clay (%)	0.568	0.568	0.568	0.568	0.568
pH	-0.191	-0.191	-0.191	-0.191	-0.190
Conductivity (µs/cm)	0.296	0.296	0.296	0.296	0.297
Total nitrogen (mg/L)	0.042	0.042	0.042	0.042	0.043
Nitrate (mg/L)	-0.176	-0.176	-0.176	-0.176	-0.176
Ammonia (mg/L)	0.237	0.237	0.237	0.237	0.238
Phosphate (mg/L)	-0.253	-0.253	-0.253	-0.253	-0.253
Phosphorous (mg/L)	-0.251	-0.251	-0.251	-0.251	-0.251
Chloride (mg/L)	0.167	0.167	0.167	0.167	0.168
Carbonate (mg/L)	0.370	0.370	0.370	0.370	0.370
Total alkalinity (mg/L)	0.139	0.139	0.139	0.139	0.140
Carbon (%)	0.013	0.013	0.013	0.013	0.014

507 plant species (Hamrick and Godt, 1996) indicated that families with predominantly herbaceous species have less genetic diversity and higher genetic differentiation than families with predominantly long-lived, woody perennials. This study populations showed high levels of genetic differentiation, with an estimated G_{st} 0.399, where Nei (1978) classified $G_{st} > 0.15$ as high value. According to allozyme and RAPD data (Hamrick and Godt, 1989; Nybom and Bartish, 2000), G_{st} is about 0.2 to 0.23 for outbreeding species and 0.5 to 0.59 for inbreeding species. The apparent gene flow found for *P. harmala* populations was 0.77, which is considered low, and according to Raymond and James (2001), the resultant genetics will lead to population differentiation if the flow value is less than one migrant per generation. Studies on the rates of gene flow in species with different pollination patterns revealed that predominantly for self-pollinating species, the mean gene flow was 0.83, while for species pollinated by animals, the mean gene flow was 1.21, and for wind pollinated species, the mean gene flow was 2.91 (Govindaraju, 1988). The average values reported for mixed-mating N_m = 0.727, which appears close to *P. harmala*. The G_{st} value in *P. harmala* (0.393) was higher than that for insect pollinated species (0.197) (David et al., 1996). Thus, these values offer an indication of the possible reproductive system of *P. harmala* in which insect visitation in the entomophilous species is partial outcrossing (self-incompatible). Changes in pollination patterns should ultimately be reflected in mating systems and plants can govern their mating opportunities by manipulating the behavior of pollinators (Barrett, 2003). Population size also interacts with its pollinators, through which the species may experience certain variations in its mating system (Routley et al., 1999). Small populations seem to be less attractive to pollinators than large populations (Agren, 1996); there should be fewer pollinators, and as a consequence, higher selfing, biparental inbreeding and correlation of outcrossed paternity (the probability that siblings shared the same father) eventually appear in smaller populations (Yates et al., 2007).

Gene flow is the movement of genes within and between populations. In this study, the level of gene flow for *P.* harmala as based on G_{st} was low (N_m = 0.772), which mean that the high level of genetic diversity maintained within each population was liable to genetic drift. However, more evidence is needed to prove whether the genetic drift and inbreeding have occurred. In general, the inbreeding in *P. harmala* seems to result in a declining reproductive fitness and depression of fecundity and vital capacity. This tends to increase the risk of extinction in small populations (Frankham et al., 2002); but because of the lack of studies covering the breeding system of *P. harmala*, we have no direct evidence to prove this behaviour. Moreover, many researchers suggested that the average number of individuals exchanged among populations per generation (N_m) has some effect on genetic differentiation among and within populations (Raymond and James, 2001). The N_m would be low, with increased spatial isolation of small populations caused by habitat fragmentation (Chen, 2000). Furthermore, if N_m were lower than unity, different-tiation among populations would mainly result from the limited gene flow in the condition of high selective pressure or discontinuous distribution. Low levels or absence of gene flow among populations is also

characteristic of many rare species (Xiao, 2006). Many studies have found that seed dispersal is a primary factor influencing variation in gene flow and population structure (Kalisz et al., 1999). Therefore, the short seed dispersal distance of *P. harmala* may result in limited gene flow among populations.

Comparison of the costal desert populations (Group 1) with the inland desert populations (Group 2) showed that Group 1 attained higher genetic variation among populations than in Group 2. Also, higher Nei's gene diversity and Shannon's index were observed in Group 1 populations compared to group 2. This may be explained by habitat fragmentation in coastal desert compared to the inland desert. There was a correspondence between the results, that is, populations which attained high values of variation have demonstrated low gene flow and genetic diversity.

The Mantel test reveals that there is no correlation between the genetic and geographic distances; that is, genetic variability demonstrates that these populations are not structured in space. This result may be supported with the finding that genetic variation among regions (Groups 1 and 2) was low and reaching 1.8% of the total variation.

Other studies reached similar results, where genetic distances between populations were not significantly correlated with the geographic distances (Reisch et al., 2003; Jordano and Godoy, 2000). They assumed that genetic drift or natural selection rather than migration or gene flow was the main factors affecting the population genetic differentiation (Fahima et al., 1999). The result was confirmed by the UPGMA dendrogram, as based on Nei's genetic distance, which indicated that genetic differentiation was incompatible with the geographic distance.

There were insignificant correlations between soil factors and the genetic diversity indices. This result may suggest that the change in the alleles was not related to the soil variables, but the possibility that genetic differentiation of *P. harmala* populations was induced by genetic drift (Li and Lin, 2004). In the mean time, this result eliminated the possibility of genetic differentiation as affected by local natural selection. Meanwhile, Volis et al. (2001) pointed out that if the genetic differentiation was caused by genetic drift among populations, and then there would be no correlation between genetic differentiation and ecological factors. Further studies are needed to reveal the driving forces and factors which cause the genetic variation within and among *P. harmala* populations.

Conservation considerations

The assessment of genetic variability is the first step in evaluating the long term conservation status of species in natural habitats. This is particularly important in plant species with low population sizes exposed to the effects of inbreeding and genetic drift (González-Astorga and Castillo-Campos, 2004; Hegazy et al., 2010a, b). Human impacts, especially over collection of P. harmala populations, were considered one of the main factors to cause the loss of genetic diversity in plant populations. The most important conservation measure is to control plant collection to reduce the impact of human activities, especially for population 3, which has higher level of genetic diversity, so that both habitats and genetic variation are conserved. For the populations having low level of genetic variation, such as population 6, plant introduction from other populations will increase the chance of gene exchange and recombination and improve the level of genetic diversity over time. Moreover, new germplasm banks collected from different populations with different heredity backgrounds are necessary to decrease propagation from the same hereditary clone and to prevent homogeny of individuals. Due to the low gene flow among *P. harmala* populations, management to conserve genetic variability in this species should aim to preserve not only the most variable populations but also as many of the less variable populations as possible.

We suggest that in situ conservation efforts should focus on establishing more sites to protect the natural populations and their habitats. *Ex situ* conservation efforts should focus on enhancing the exchange of seeds and gene flow among populations to facilitate gene exchange and recombination to support the genetic diversity of the species.

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