



Genetic Structure and Diversity Within and Among Six Populations of *Capparis decidua* (Forssk.) Edgew. from Saudi Arabia

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Abstract

Capparis decidua is a rangeland plant species growing in isolated populations in Saudi Arabia. Genetic diversity within and among six populations (Madina, Farasan island, Hawayer Assos, Khor Assos, Raudhat Khuraim, and Taif) of *C. decidua* were studied using RAPD technique. A total of 25 random primers were used. Eighteen primers generated discernible and reproducible bands. A total of 152 reproducible RAPD bands across the 36 individuals of the six populations were amplified. Out of those, 117 (76.2%) RAPD bands were polymorphic. The number of polymorphic bands per primer ranged between 3 and 11 with an

average of 6.5 bands per primer. Populations differed in the level of genetic diversity as shown from the percentage of polymorphic bands. Farasan population had the highest level of genetic diversity (24.3%) and two populations Khor Assos (5.9%) and Taif (4.6%) had the lowest genetic diversity. Analysis of molecular variance (AMOVA) showed highly significant differences among populations. The among population variance accounted for a higher percentage of the total variance (average 77.67%, $SD \pm 8.21$) than the within populations (average 22.33%, $SD \pm 8.21$). No significant correlation between geographical distance and genetic distance was found. However, there was a significant positive correlation between molecular genetic variation and actual population size. The implication of the results of this study in devising strategy for conservation of *Capparis decidua* is discussed.

Keywords: *Capparis decidua* –Tandhab –Assos- Population size – RAPD markers – Genetic diversity - AMOVA

Introduction

An increasing number of plant species have been restricted to small and isolated populations due to the habitat destruction and fragmentation. These populations face an increased risk of extinction because of environmental, demographic and genetic stochastic threats, even in intact habitats (Fisher and Matthies 1998). Random fluctuation of environmental conditions that affect plant survival and reproduction are considered to be the most important stochastic factors (Boyce 1992; Menges 1992), whereas demographic stochasticity in infinite populations is considered to be of minor importance (Menges 1991).

Knowing the degree of genetic variation is of fundamental importance for species' conservation (Barrett and Kohn 1991; Ellstrand and Elam 1993; Gilpin and Soule 1986; Hamrick and Godt 1996a; Karron 1997; Lande 1999). Positive correlations between fitness- related characters and heterozygosity have been found in a number of plant species (Linhart and Mitton, 1985; Oostermeijer et al. 1995). Small populations always have low genetic diversity; consequently, their capacity to adapt to environmental change may be diminished. In addition, their ability to survive over the long term may be compromised (Lande 1999). Inbreeding and genetic drift affect population fitness through the increased expression of recessive deleterious alleles as homozygosity increases in small populations (Ellstrand and Elam 1993; Karron 1997; Lande 1999). Genetic drift is expected to randomly reduce variation within small populations, causing loss of low frequency alleles, which can be associated with population fitness (Barrett and Kohn 1991). Moreover, the random loss of self-incompatibility alleles may reduce the reproductive capacity of individuals (DeMauro 1993; Karron 1997; Young et al. 1999) and may lead to population extinction (Barrett and Kohn 1991; Holsinger et al. 1999). Gilpin and Soule (1986) stated that in small populations these genetic factors combined with demographic stochasticity, may result in “extinction vortices,” feedback process that result in reducing the number of individuals until populations become extinct. Although the above discussion is true, not all small populations are genetically going through “extinction vortices” (Ellstrand and Elam 1993; Frankham 1997; Gitzendanner and Soltis 2000; Godt and Hamrick 1998). There are other factors such as

species' life-history, biogeography, and gene flow into the population that could also play critical roles in determining the current genetic composition of populations (Hamrick and Godt 1996a,b; Holsinger et al. 1999). Therefore, understanding genetic factors that contribute to extinction risks for particular species is critically important for their conservation (Godt and Hamrick 1998; Hamrick and Godt 1996a).

Capparis decidua (Forssk.) Edgew., family *Capparidaceae* (vernacular name; Tandhab, Assos) is a bushy shrub occurring in dense tufts. It reaches a height of 4-5 m or more. The species is common in dry tropical areas of Africa and Asia. It is an important plant because of its excellent adaptation to arid conditions. *Capparis decidua* was found to be one of the best species for shelter belts to check the movement of sand in the Thar desert of India (Pandey and Rokad 1992). As a drought resistant, it has relatively good nutritive value (Assaeed et al. 1995) and withstands neglect. In Saudi Arabia, *C. decidua* is present in isolated populations over much of the country and some are limited in size (Miller and Cope 1996; Collenette 1985). The plant is also heavily browsed by camels and goats. Being under biotic and abiotic stresses, it is feared that genetic diversity of *C. decidua* populations in Saudi Arabia may decrease. The objectives of this study were to: (1) analyze the genetic diversity within and among six populations occurring over a wide area in Saudi Arabia with RAPD markers, (2) relate the population size to genetic diversity and (3) determine if the geographic distance is related to genetic similarity among populations.

Materials and methods

Population sampling

Sampling included materials from six populations (Madina, Farasan island, Hawayer Assos, Khor Assos, Raudhat Khuraim, and Taif). Details of the locations of these populations are given in Table 1 and Fig. 1. Six randomly chosen individuals from each of the six populations were sampled in spring 2005. Fresh twigs of current year growth of *C. decidua* were collected, placed into plastic sealable bags and transported to the laboratory in an ice box. Samples were washed several times with distilled water before being subjected to lyophilization. Lyophilized material was kept at -20°C until isolation of DNA.

Genomic DNA extraction

Lyophilized samples were ground to a fine powder. DNA extraction was extracted from 20-50 mg of powdered material using a common CTAB procedure (Doyle and Doyle 1990) modified by adding 5% polyvinylpyrrolidone (w/v) and 2% 2-mercaptoethanol (v/v) to the extraction buffer. DNA concentration was determined either by electrophoresis on 1% agarose gel stained with ethidium bromide with comparisons made to standard DNA ladders or by spectrophotometer readings at 260/280 nm. DNA samples were diluted to 25 ng/μl and kept at -20°C until use.

RAPD assay

Twenty five different RAPD primers were initially screened in this study. PCR amplification was carried out in a 25 µl reaction mixture with the following components: 1x supplied PCR buffer (10 mM Tris-HCl pH 9, 50 mM KCl, 2 mM MgCl₂ and 0.01% Triton X-100), 1 U FastStart *Taq* DNA polymerase (Roche), 200 mM of each dNTP, 30 nM of primer and 25 ng of DNA template. Optimal amplification conditions for RAPDs were one cycle of 15 min at 94°C, followed by 45 cycles of 30 sec at 94°C, 1 min annealing at 36°C and 2 min at 72°C followed by a final step of 10 min at 72°C. Samples were cooled at 4°C until recovery. RAPD fragments were separated in 2.0 % agarose gel, stained in ethidium bromide (0.5 µg/ml) and visualised by UV transillumination. For band sizing DNA molecular weight standard Gene Ruler (100 bp ladder, Fermentas Life Sciences, USA) was used.

Data analysis

To examine the genetic relationship within the population, an UPGMA dendrogram based on Nei's coefficient of genetic similarity (Lamboy 1994) was constructed using a SIMQUAL, SAHN and TREEPLOT routines as implemented by NTSYS-pc, Version 2.02c (Rohlf 1997). The PCR polymorphisms data generated from the thirty six individual plants were scored into binary matrices indicating absence (0) or presence (1) of particular RAPD fragment. Analysis of molecular variance (AMOVA) was carried out using Arlequin software 3.0 (Schneider et al. 2000) to statistically test the existence of differences among six *C. decidua* populations. All possible 15 pairwise population group comparisons were defined for AMOVA test. The significance level for AMOVA calculations was 0.01. Correlation co-efficients for the linear association between percentage of polymorphic bands and population size and between Nei's genetic similarity and geographical distance were calculated according to Steel and Torrie (1980).

Results

RAPD and statistical analysis

A total of 25 random 10-mer primers (Operon Co., USA) were screened against six individuals per population. Of these, 18 primers that could generate discernible and reproducible bands were selected for further amplification (Table 2). A total of 152 reproducible RAPD bands were generated with the 18 primers across the 36 individuals of the six populations of *C. decidua*. Out of these RAPD bands, 117 (76.2%) were polymorphic (Table 2). The bands ranged in size from 220 to 2000 bp. The number of polymorphic bands per primer ranged between 3 and 11 with an average of 6.5 bands per primers (Table 2).

Primers varied in their ability to detect variation both within and between populations. Some primers showed polymorphism in some population, and were monomorphic in other populations. These differences suggested that a sufficient number of primers are essential for a reliable evaluation of the genetic diversity.

Populations differed in the level of genetic diversity as shown from the percentage of polymorphic bands in each population. For example, Farasan had the highest level of genetic diversity (24.3%) followed by Madinah (19.7%). Hawayer Assos (15.1%) and Raudhat Khuraim (12.9%) had a moderate genetic diversity. However, both Khor Assos (5.9%) and Taif population (4.6%) had the lowest genetic diversity.

The genetic relationship of the 36 individuals in the six populations was illustrated using the UPGMA dendrograms based on Nei's genetic similarity coefficient (Fig. 2). The six populations are clustered into two major groups, each of them carrying three subgroups. Madina, Farasan, and Hawayer Assos populations formed a very closely related group. The second group includes Khor Assos, Raudhat Khuraim, and Taif. The last two populations are very closely related and the most related of the six populations. According to the dendrogram, Madinah and Taif populations are the most different of the six populations.

To analyze genetic structure within and among populations, AMOVA analysis (Table 3) was performed for 6 different populations (15 pair-wise calculations). In all cases, AMOVA test resulted in highly significant ($p < 0.01$) differences between populations. The variance among populations accounted for a higher percentage of the total variance (average 77.67%, $SD \pm 8.21$) than the variance within populations (average 22.33%, $SD \pm 8.21$).

Discussion

This study provides the first detailed analysis of genetic variability of *C. decidua* in Saudi Arabia. Previously, genetic variability was conducted in just one population using RAPD markers (Abdel-Mawgood et al. 2005, 2006). RAPD markers, along with appropriate statistical procedures, are suitable for genetic variation analysis at both intra- and inter-population levels and for devising strategies for conservation (Sun and Wong 2001; Pvingila et al. 2005; Li et al. 2008; Huang et al. 2008).

Genetic diversity

The used eighteen RAPD primers were highly informative and produced 152 bands with an average of 8.4 bands per primer. Of these bands, 117 were polymorphic across all populations (67.2% polymorphic bands) reflecting divergence between populations. The remaining bands were common in all populations. The level of genetic diversity for *C. decidua* is higher than expected based on geographical distribution and when compared with other plant species with similar life histories. The percentage of polymorphic bands in endangered plant species is reported to be 69% in *Changium smyrnioides* (Fu et al. 2003), 24.5% in *Lactoris fernandeziana* (Lactoridaceae), (Brauner et al. 1992), 22.5% in *Paeonia suffruticosa*, 27.6% in *P. rockii* (Pei et al. 1995), and 33% in *Dacydium pierrei* (Su et al. 1999). One possible explanation for high variability is the outcrossing or hybridization between individual plants within a population. It may be an important

component of recent evolution and may also be a factor contributing to the high level of genetic diversity. Moreover, the high number of polymorphic products generated by some primers- as shown in our data- might be attributed to the fact that in RAPD there are various reasons that can result in polymorphism, which are well documented (Scott et al. 2002, Juchum et al. 2007, Ge et al. 1999).

Genetic structure

Genetic structure of plant population reflects the interactions of various factors such as long term evolutionary history of species, genetic drift, mating system, gene flow and selection (Schaal et al. 1998). Farasan population was the most genetically diverse population with 24.3% polymorphic bands. The least genetically diverse populations were Khor Assos and Taif with percentages of polymorphic bands of 5.9 and 4.6, respectively (Table 1).

Geographical distances between each pair of the studied populations vary from 143 to 1185 km (Fig. 1). No significant correlation ($r=0.15$) was detected between the genetic and geographical distances among the six populations. However, some populations show certain correlation between genetic distance and geographical distance. For example, according to the dendrogram (Fig. 2), Khor Assos and Raudhat Khuraim were clustered in one group and they are geographically very close, with 246 km apart. It is possible that these two populations have had the same origin and still could maintain some level of gene flow between them. In contrast, Raudhat Khuraim and Taif were grouped together despite the long geographical distance of 822 km between them. The same is true for Khor Assos and Taif populations. The lack of such correlation could be partially explained by possible adaptive eco-geographical differentiation associated with habitat fragmentation or by severe bottlenecks in some populations in the past (Owuor et al. 1997). These could possibly result in forming a new allelic composition of the populations irrespective of their geographical location (Gaudeul et al. 2000).

Correlation between population size and genetic diversity

There was a significant positive correlation ($r=0.64$) between molecular genetic variation and actual population size in the six populations. The low level of genetic variability in smaller size populations and the observed correlation is most likely due to the loss of variation in small population through genetic drift. Similar results were obtained for small-size isolated populations in several other studies. For example, population size was significantly correlated with the proportion of polymorphic loci in the outbreeding perennials *Salvia pratensis* and *Scabiosa columbaria* (Van Treuren et al. 1991). Another study covering 25 populations of a rare perennial plant; *Gentiana pneumonanthe* showed similar results (Raijmann et al. 1994). In contrast, Dixon and May (1990) found no consistent relationship between heterozygosity and population size in *Aconitum noveboracense*.

Implication for conservation

As stated by Banares et al. (1993), "the main objectives of a recovery plan are the preservation of genetic diversity, and to insure the continuous survival of populations

especially by the perpetuation of species in their natural habitat without any specific human aid to maintain evolutionary potential". Information on current level of genetic diversity of a population is essential for designing appropriate strategies for conservation (Falk and Holsinger, 1991). The results of the current study showed that there is a positive correlation between population size and percentage of polymorphic loci. Moreover, most of these populations are small (with the exception of Farasan population) ranging in size from 35 to 183 trees (Table 1). In addition, these populations are isolated and fragmented. Ellstrand and Elam (1993) indicated that in isolated populations, genetic drift may reduce genetic variation, increase levels of inbreeding and consequently, reduce the potential to adapt to environmental changes. Reduced level of genetic diversity can affect plant fitness and population persistence in several ways. In the long term, populations with reduced level of genetic diversity have low potential for adaptation to changes in environmental conditions. While in the short term, low level of genetic diversity may affect fitness through increased level of inbreeding and inbreeding depression. This may suggest that these populations may be of particular conservation concern as it is unlikely for them to recover from any stochastic extinction events. The results of this study could be meaningful for devising strategy for conservation of *C. decidua*. For ex-situ and en-situ program, sampling should be representative of all populations giving the high level of within population diversity. Moreover, preservation of genetic variation should be through conservation of a large number of individuals especially for small size populations such as Madina, Hawayer Assos, and Khor Assos. In conclusion, it seems thus advisable to give to *C. decidua* a conservation priority on ecological grounds.

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Fig. 1 Map showing the locations of six analyzed populations of *C. decidua* in Saudi Arabia: Khor Assos, Raudhat Khuraim, Hawayer Assos, Medina, Taif and Farasan

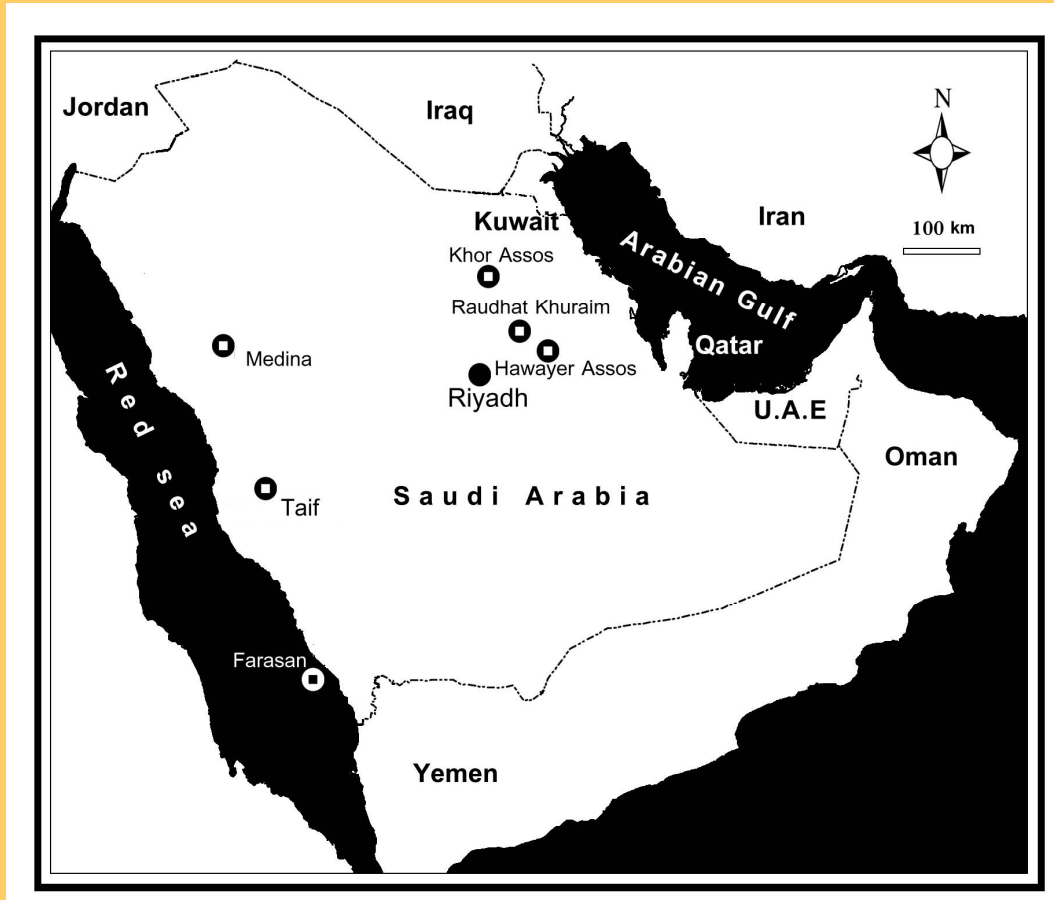


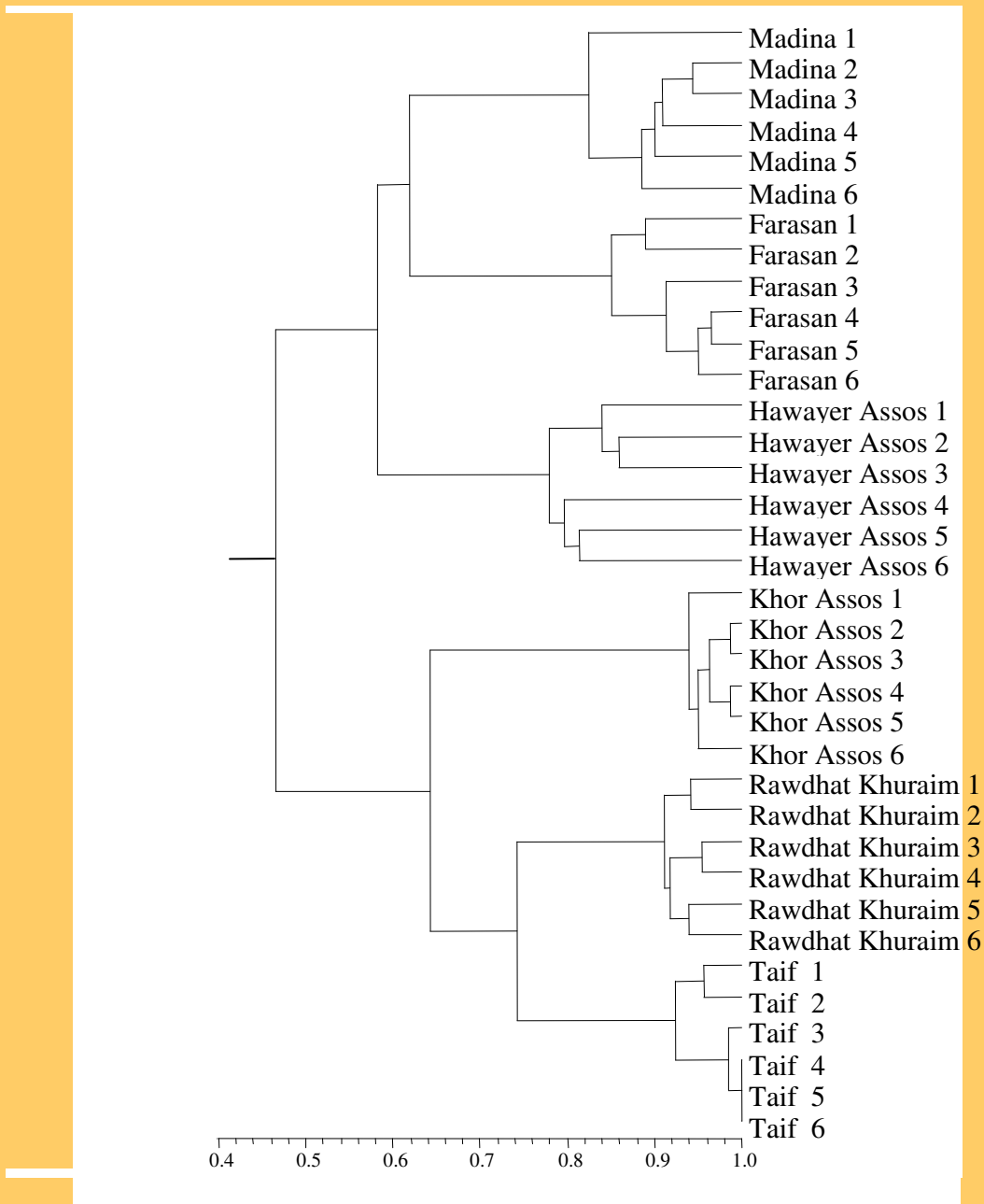
Fig. 2 UPGMA dendrogram of 36 *C. decidua* plants based on Nei's genetic similarity.

Table 1 Brief description of *C. decidua* populations

| Population | Population Size | Percentage of polymorphic bands | Longitude Latitude, & Altitude | Vegetation of the area |
|-----------------|-----------------|---------------------------------|----------------------------------|--|
| Madina | 45 | 19.7 | 24° 08' N 39° 34' E 761 m | <i>Capparis decidua</i> , <i>Acacia asak</i> , <i>A. tortilis</i> , <i>Ochradenus baccatus</i> , <i>Lycium shawii</i> , <i>Aerva tomentosa</i> , <i>Leptadenia pyrotechnica</i> and <i>Belpharis ciliaris</i> |
| Farasan | 950 | 24.3 | 16° 39' N 40° 29' E 7 m | <i>C. decidua</i> , <i>A. ehrenbergiana</i> , <i>Commiphora opobalsamum</i> , and <i>Salvadora persica</i> |
| Hawayer Assos | 35 | 15.1 | 25° 11' N 48° 37' E 357 m | <i>C. decidua</i> , <i>A. gerrardii</i> , <i>Calotropis procera</i> , <i>L. shawii</i> and <i>O. baccatus</i> |
| Khor Assos | 53 | 5.9 | 26° 59' N 45° 33' E 524 m | <i>C. decidua</i> , <i>A. gerrardii</i> and <i>L. shawii</i> |
| Raudhat Khuraim | 183 | 7.9 | 25° 26' N 47° 14' E 556 m | <i>C. decidua</i> , <i>Ziziphus nummularia</i> , <i>A. tortilis</i> , <i>A. ehrenbergiana</i> , <i>A. gerrardii</i> , <i>L. shawii</i> , <i>C. procera</i> , <i>Pulicaria crispa</i> , <i>Achillea fragrantissima</i> , <i>Zilla spinosa</i> , <i>Lasiurus scindicus</i> and <i>Pennisetum divisum</i> |
| Taif | 85 | 4.6 | 21° 18' N 40° 29' E 1577 m | <i>A. asak</i> , <i>A. tortilis</i> , <i>A. flava</i> , <i>L. persicum</i> , <i>Tamarix articulata</i> , <i>O. baccatus</i> , <i>P. crispa</i> , <i>Aerva tomentosa</i> , <i>Coccinia grandis</i> , <i>Cenchrus ciliaris</i> and <i>Panicum turgidum</i> |

Table 2 Primer sequences, total number of amplified fragments for each primer, number of polymorphic bands and percentage of polymorphism averaged over the six populations

| Primer name | Primer sequence | Number of bands | Polymorphic bands | Percentage of polymorphism |
|--------------------|------------------------|------------------------|--------------------------|-----------------------------------|
| OPA-01 | CAGGCCCTTC | 10 | 7 | 70.0 |
| OPA-02 | TGCCGAGCTG | 11 | 8 | 72.7 |
| OPA-03 | AGTCAGCCAC | 10 | 9 | 90.0 |
| OPA-04 | AATCGGGCTG | 13 | 11 | 84.6 |
| OPA-05 | AGGGGTCTTG | 8 | 7 | 87.5 |
| OPA-06 | GGTCCCTGAC | 5 | 3 | 60.0 |
| OPA-08 | GTGACGTAGG | 8 | 6 | 75.0 |
| OPA-09 | GGGTAACGCC | 10 | 7 | 70.0 |
| OPA-12 | TCGGCGATAG | 11 | 8 | 73.0 |
| OPA-13 | CAGCACCCAC | 8 | 7 | 87.5 |
| OPA-14 | TCTGTGCTGG | 7 | 5 | 71.4 |
| OPA-16 | AGCCAGCGAA | 6 | 5 | 83.0 |
| OPA-17 | GACCGCTTGT | 6 | 3 | 50.0 |
| OPA-18 | AGGTGACCGT | 5 | 4 | 80.0 |
| OPB-01 | GTTTCGCTCC | 7 | 5 | 71.4 |
| OPB-02 | TGATCCCTGG | 7 | 6 | 85.7 |
| OPB-03 | CATCCCCCTG | 11 | 9 | 81.8 |
| OPB-04 | GGACTGGAGT | 9 | 7 | 77.8 |
| Total | | 152 | 117 | 76.2 |

Table 3 Matrix of mean Nei's unbiased genetic similarity and -in parentheses-pairwise geographical distance (below diagonal) and percentage of total variation (between and within populations) values from AMOVA analysis for pairwise comparisons among population (above diagonal).

| | Madina | Farasan | Hawayer Assos | Khor Assos | Raudhat Khuraim | Taif |
|----------------------------|------------------|-------------------|--------------------------|-----------------------|----------------------------|----------------|
| Madina | | 58.81 41.19 | 68.85 31.15 | 82.22 17.78 | 82.61 17.39 | 85.32 14.68 |
| Farasan | 0.651 (867.7) | | 63.79 36.21 | 76.77 23.23 | 75.55 24.45 | 77.29 22.71 |
| Hawayer Assos | 0.621 (918.0) | 0.587 (1150.5) | | 83.44 16.56 | 81.22 18.78 | 84.24 15.76 |
| Khor Assos | 0.518 (671.8) | 0.462 (1185.4) | 0.487 (365.8) | | 81.90 18.10 | 87.49 12.51 |
| Raudhat Khuraim | 0.423 (780.4) | 0.401 (1099.9) | 0.458 (143.1) | 0.663 (242.6) | | 75.56 24.44 |
| Taif | 0.427 (325.5) | 0.449 (543.8) | 0.449 (931.2) | 0.631 (808.9) | 0.736 (822.7) | |