# GENETIC DIVERSITY AND POPULATION ANALYSIS OF *Melia azaderach* L. BY RAPD MARKERS IN BATHINDA AND ADJOINING REGIONS

A Dissertation submitted to the Central University of Punjab

For the award of

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In

Biosciences

BY
**Navgeet**

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September, 2013

## CERTIFICATE

I declare that the dissertation entitled “GENETIC DIVERSITY AND POPULATION ANALYSIS OF *Melia azedarach* L. BY RAPD MARKERS IN THE BATHINDA AND ADJOINING REGIONS” has been prepared by me under the guidance of Dr. Pankaj Bhardwaj, Assistant Professor, Centre for Biosciences, School of Basic and Applied Sciences, Central University of Punjab. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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## ABSTRACT

**Genetic Diversity and Population Analysis of Melia azedarach L. by RAPD markers in Bathinda and adjoining regions.**

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*Melia azedarach* L. is an ecologically imperative species growing in Thar Desert. Biological effects such as antiviral, anthelminthic, antibacterial etc. makes it important, yet a little is known about its genetic diversity and structure. In this study, 30 RAPD primers were employed for DNA profiling of 47 individuals representing 6 populations from different geographic locales. A total of 87 bands were scored with an average of 2.9 bands per primer. The PIC ranged from 0.1195 to 0.4998 with an average of 0.4160. Nei's genetic diversity (h) and Shannon's information index (I) ranged from 0 to 0.5 and 0 to 0.6931 with an average Nei's genetic diversity of 0.2422. The gene flow (Nm) 1.4381 and the genetic diversity of 0.2381 at species level demonstrated overall high level of genetic diversity. Cladistics analysis using DARwin and Bayesian cluster analysis using STRUCTURE placed 47 individuals into two main clusters or original genetic stocks (K=2) which showed little or no association with the geographic origin. The lack of clear assignment of individuals to geographic regions of sampling and consideration of different populations in the same genetic cluster suggests a recent common evolutionary history.


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## LIST OF ABBREVIATIONS

| Sr. No. | Full form | Abbreviation |
|---|---|---|
| 1. | Amplified Fragment Length Polymorphism | AFLP |
| 2. | Arbitrarily Primed PCR | AP-PCR |
| 3. | Deoxyribonucleic acid | DNA |
| 4. | Ethidium Bromide | EtBr |
| 5. | Inter Simple Sequence Repeat | ISSR |
| 6. | Polymerase Chain Reaction | PCR |
| 7. | Random Amplified Polymorphic DNA | RAPD |
| 8. | Restriction Fragment Length Polymorphism | RFLP |
| 9. | Tris-Acetate-EDTA | TAE |
| 10. | Unrooted Pair Group Method Analysis | UPGMA |

# Chapter 1

# Introduction

*Melia azedarach L.,* a forest tree, known as bead tree, Persian lilac, Paradise tree, Bakain, is native to north western India and China. It is a member of family *Meliaceae* which grows at a temperature range of -5 to 40°C, drought and frost conditions (Sheikh, 1993). It is grown widely in the world due to its high adaptability to a wide range of climatic conditions which naturalized it in other countries (Olmos *et al.*, 2002; Yulianti *et al.*, 2011). It is an important fast growing forest tree (Scocchi *et al.*, 2004) which grows well in deep and sandy clay soils with good drainage having an alkaline pH of around 5.5-6.5. It grows at hills with elevation of 700-1400m above sea level and with a rainfall of 600-2000 mm/year and in warmer conditions (Yulianti *et al.*, 2011). It attains a height of 5-15m and stem thickness of 110cm. Leaves are lobed, incised, serrate, oval, pinnate, binately compound and alternately arranged. Upper surface of leaves is dark and lower surface is pale yellow. Its purple flowers with pleasant fragrance later on form yellow, almost round, smooth and stone like hard fruits or berries having 5-6 black coloured seeds (El-Juhany, 2011). It is considered important for reforestation programs (Scocchi *et al.*, 2004) and commercial purposes because of its good wood characteristics (Scocchi *et al.*, 2004) and several biological effects including antifeedant (De Nardo *et al.*, 1997), antihelminthic (Hördegen *et al.*, 2003), antibacterial (Khan *et al.*, 2001), and antiparasitic (Szewczuk *et al.*, 2003) etc.

### 1.1 Distribution of *M. azedarach*

This tree is mostly grown because of its ornamental property. It provides shade as it is having high lateral branching. This plant is commercially used at a high level because of very good wood quality (El-Juhany, 2011). It has been cultivated in different regions of world because of its high climatic tolerance and used for treatment of dandruff, pimples, ringworms etc. as it has medicinal, insecticidal, and vermifugal, antifeedant, growth and development regulating, mosquito repellent properties etc. which have been detected in extracts of its fruits, leaves, seeds etc (Szewczuk *et al.*, 2003). The efficacy of methanol extracts of fruits, seeds and leaves showed larvicidal, pupicidal, adulticidal, antiovipositional effects etc. against malarial vector *Anopheles stephensi* under in vitro conditions (Nathan *et al.*, 2006). In humans, extracts of this plant has shown antibacterial, antiprotozoal, antiviral, antiulcer, antinephrolihiasis and antihelminthal properties against different organisms or diseases such as *Shigella flexneri, Herpes simplex*, foot and mouth

disease, *Trichoma vaginalis, Bacillus subtilis, Staphylococcus aureus* etc (Coria *et al.*, 2008; Nathan *et al.*, 2006; Szewczuk *et al.*, 2003). Thus this plant possess great importance for human health and for the researchers in the field of pharmacy and biotechnology because pharmaceutical companies basically depend upon plants for the production of drugs or pharmaceutical compounds (Samantaray *et al.*, 2010). Due to its distribution in wide range of conditions and importance in human health there is a need to study this plant on genetic level.

### 1.2 Genetic diversity

Due to restricted or limited mobility of plants, genetic structure refers to spatial structure or actual geographical distribution of plants which results from different environmental or ecological traits along with migration, dispersion, mutation, natural selection and genetic drift etc. Small, localized populations are more susceptible to genetic drift and limited gene flow as compared to widely distributed species resulting in an increased genetic diversity between populations and decreased genetic diversity within populations (Loveless *et al.*, 1984). Limited gene flow between populations of plant species cause genetic differentiation due to increased geographic distance among populations or distant spatial pattern of populations. Alteration and fragmentation of many habitats lead to increased geographic distance which may be the cause of low level of gene flow and high genetic diversity among populations (Fischer *et al.*, 2000).

Genetic variation in a species will allow itself to respond to environmental stress, adapt, evolve and survive for a long term. Severe environmental stress play an important role in revealing evolutionary history of forest trees (Sheng *et al.*, 2005). Information about genetic structure of forest species of desert region not only provides information regarding significance of evolutionary forces such as gene flow, genetic drift, mutation and migration under extreme environmental conditions but also provides basic information for designing plans related to restoration and rational exploitation (Doligez *et al.*, 1997; Fischer *et al.*, 2000).

As *M. azedarach*, a forest tree is of economical importance and its breeding is slow, so it is desirable to detect functionally important regions in the genome. A number of molecular markers can be used to analyse the genetic diversity and variations in plants in and among the species and are the most reliable source to

study genetic diversity because they reveal neutral sites of variation at the whole genome sequence level as they are not affected by environmental factors. They are much more numerous than morphological markers and do not disturb the physiology of the organism. The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms the basis for designing strategies to exploit for applied purposes. A marker must be polymorphic i.e. it must exist in different forms so that chromosome carrying the mutant genes can be distinguished from the chromosomes with the normal gene by a marker it also carries (Kumar *et al.*, 2009). Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two discontinuous variants or genotypes. This naturally occurring genetic polymorphism is the basis for studying various aspects of population genetics with the help of molecular markers. These markers include RFLP, AFLP, and RAPD etc.

### 1.3 Statement of purpose

*M. azedarach* L. is very important due to its fast growth in wide range of climatic conditions or wide geographical distribution, good wood quality and several biological effects (Olmos *et al*., 2002; Scocchi *et al*., 2004; Yulianti *et al*., 2011). These properties of *M. azedarach* L. make it ecologically and commercially important, but very less information is available about the genetic diversity and structure of this forest tree till now. So it will be very useful to explore its genetic diversity and structure considering its ecological and commercial values. Many molecular markers can be used to study the genetic diversity and structure of the trees. RAPD analysis is conceptually simple, easy, requires very less amount of genomic DNA and no prior knowledge of the genome. RAPDs are the reliable markers to study genetic diversity between closely related individuals or species as they are not influenced by environment (Campbell *et al*., 1999; Nkongolo *et al*., 2003; Radhika *et al*., 2012). In the current study RAPD markers will be used to explore its population genetic diversity and structure in Bathinda and its adjoining regions. Considering the importance of present study, following objectives have been designed:

- Sample collection of *M. azedarach L.* from different locations of Bathinda and adjoining regions.

- Analysis of genetic diversity and population structure with RAPD markers.

# Chapter II

# Review of Literature

*Melia azedarach* L. is fast growing plant along with high adaptability to different soil and climatic conditions (Scocchi *et al.*, 2004; Scocchi *et al.*, 2004). In different regions of world this plant is having different chemical and biological activities which may be due to genetic variations (Szewczuk *et al.*, 2003).

### 2.1 Genetic diversity of trees

Forests are the natural renewable resources which play important role in maintenance of ecosystem. They continuously improve the quality of environment by preventing pollution by sequestering carbon dioxide from environment at a lower cost. They are the main source of biodiversity i.e. genetic diversity being a habitat of more than half of the terrestrial plant and animal species which is prime requirement for the maintenance and improvement of forestry and agricultural products. Genetic diversity of tree species provides the raw material for industrial and scientific research (Brockerhoff *et al.*, 2008; Kettle, 2010). As forest species have long life span, so their genome levels evolve very slowly. Thus natural selection, genetic drift, habitat fragmentation etc. have strong effects on their genome.

Many commercially important and ecologically dominant forest trees undergo less domestication and artificial selection as they are mostly found in their natural populations (Savolainen *et al.*, 2007). Many trees are commercially exploited on a large scale resulting in decrease in their number. Because of its ecological and commercial importance there is need of conserving the germplasm of *M. azedarach* , but little is known about its genome so genetic diversity of this tree should be studied (Coria *et al.*, 2008).

Genetic diversity play important role in ecosystem functioning, predicting the vulnerability of a species towards extinction and determination of survival of populations. Genetic diversity influence survival of a population which is important for its adaptation in an ecosystem and the degree of genetic diversity in a population depends on various factors such as mating systems, evolutionary history of a species etc. (Booy *et al.*, 2000). A high level of genetic diversity is the cause of plant survival, stability and acts as a source of ecosystem functions. This is because genetic diversity contributes to different resistance abilities of plant populations to stresses such as disease, predation and physical disturbance

(Ehlers *et al.*, 2008; Hughes *et al.*, 2004; Johnson *et al.*, 2006; Leung *et al.*, 2003; Reusch *et al.*, 2005). Loss of genetic diversity leads to reduced fitness to environmental changes. Due to positive effects of high levels of genetic diversity, it plays important role in ecosystem restoration (Frankham, 2010; O'Grady *et al.*, 2006).

With low selection pressures seed dispersal is more effective in reducing population divergence as compared to pollination. Without selection pressure the gene flow is twice in case of seed as compared to pollen. But in the presence of selection pressure population divergence due to seed and pollen depends on fitness and dominance (Loveless *et al.*, 1984).

A large number of PCR based markers are used to study genetic diversity. The first and so far most commonly used method in this group is RAPD (random amplified polymorphic DNA) which was introduced in 1990 (Williams *et al.*, 1990). Later on almost similar ISSR (inter simple sequence repeats) (Prevost *et al.*, 1999; Zietkiewicz *et al.*, 1994) and the somewhat more technically demanding AFLP (amplified fragment length polymorphism) (Vos *et al.*, 1995) were introduced. With these markers within and among population diversity has been studied. These methods generate markers that are rapid, unaffected by the environment and reliably used for the analysis of genetic diversity in plant species. These DNA fingerprinting methods have been successfully used for the analysis of genetic diversity in many tree species like *Dracaena cambodiana* (Zheng *et al.*, 2012), *Jatropha curcas* (Basha *et al.*, 2009; Basha *et al.*, 2007), *Mangifera indica* (Schnell *et al.*, 1995), *Prosopis cineraria* (Sharma *et al.*, 2011), *Punica granatum* (Narzary *et al.*, 2009; Narzary *et al.*, 2010), *Sapindus mukorossi* (Mahar *et al.*, 2011) and *Sapindus emarginatus* (Mahar *et al.*, 2011) etc.

### 2.2 Genetic diversity and population structure

One of the most commonly used parameters used to study within population diversity is Nei's unbiased genetic diversity ($H_S$) (Nei, 1978). Dominant markers can be used to study population diversity in both selfing plant species in which heterozygotes are infrequent and out crossing species in which Hardy-Weinberg equilibrium must be assumed for each locus (Lynch *et al.*, 1994).

Among population diversity is estimated with $G_{ST}$ (genetic differentiation between subpopulations relative to the genetic diversity in the total population). $G_{ST}$ can be calculated in two ways: (i) $H_S$ and $H_T$ are first averaged across all loci and then $G_{ST}$ is calculated from these average values (Nei, 1973) (ii) $H_s$ and $H_T$ are first calculated for each locus and then averaged (Hamrick *et al.*, 1996).

RAPD, AFLP, SSR etc. produce a number of reliable and unambiguous bands, easily detectable with gel electrophoresis which can be scored as presence or absence matrices (Gorji *et al.*, 2011; Poczai *et al.*, 2011). Commonly used statistical approaches for measuring genetic diversity include following: Band based approaches which include band polymorphism, Shannon's information index (I), similarity coefficients such as Jaccard similarity coefficient, Nei and Li index etc (Hill *et al.*, 2004; Holsinger *et al.*, 2002; Lynch *et al.*, 1994). Allele frequency based approaches include observed and effective number of alleles, Nei's genetic diversity, $G_{ST}$ values and gene flow (Frankham *et al.*, 2002).

### 2.3 RAPD (Randomly amplified Polymorphic DNA)

Many markers are used to study genetic diversity within a population or between populations (Warghat *et al.*, 2012). But RAPD differ in some aspects from other markers such as level of polymorphism, cost requirement, locus specificity, reproducibility, time requirement, technical facilities etc (Du *et al.*, 2011; Snezana *et al.*, 2012). RAPD has been used in *Andrographis paniculata* due to easy detection and rapidity (Wijarat *et al.*, 2012). RAPD is random amplified polymorphic DNA in which unknown DNA sequences of genome of plants are amplified randomly with random oligonucleotide primers (Harris, 1995) i.e. to determine whether there is variation in nucleotide sequence in the genome or not; and no prior information of the genome is required for this (Badr *et al.*, 2012; Khurana *et al.*, 2012; Varsha *et al.*, 2012)). In RAPD single species of primer binds at different priming sites on complementary strands of DNA template. RAPDs are DNA sequences or fragments amplified with short synthetic oligonucleotide primers by PCR. These primers serve as both forward and reverse primers and can amplify DNA sequences or fragments from approximately 1-10 priming sites simultaneously. The polymorphism observed in RAPD is basically due to variation in the primer binding sites. RAPDs are randomly distributed and highly abundant in

the genome. They are the reliable markers to study genetic diversity on species or cultivar level as there is no influence of environmental conditions on RAPDs. They act as dominant markers (Radhika *et al.*, 2012) as they cannot distinguish between homozygous or heterozygous conditions and determine only the presence or absence of the trait. Each primer leads to amplification of approximately 10 scoreable markers (Khurana *et al.*, 2012; Lynch *et al.*, 1994; Olmos *et al.*, 2002; Pomper *et al.*, 1998).

RAPD is used for taxonomic identification. Amplification products of RAPD can be grouped into two categories: variable i.e. polymorphic or constant i.e. non-polymorphic. Both fragments constant for a genus as well as fragments which show polymorphism between species within genus can be determined which help in establishing systematic relationships. Species specific polymorphic fragments identify members of a species if the fragment is constant among all the members of a species. RAPD is used to detect hybrid populations or species which depends upon identification of RAPD markers for parental genotypes which can be done with modified form of RAPD i.e. AP-PCR. For parentage analysis RAPD markers are treated as Mendelian alleles and this approach is developed on the basis of allelic frequencies. For RAPD finger printing synthetic offsprings are prepared by mixing equal amounts of DNA from mother and potential father. The amplified product from this should consist of full complement of bands in any offspring i.e. it is complete representation of both of the parental genomes. The degree to which synthetic offspring clutch varies from actual offspring clutches indicates mixed paternity which is determined by quantitatively analysing mixed genome samples. RAPD markers are used to generate novel specific probes which may be used to avoid co-migration of DNA fragments of similar size but different sequences (Hadrys *et al.*, 1992). Seed dormancy decreases genetic variation. Genetic variation is more in species with short generation time than those with long generation time (Loveless *et al.*, 1984).

### 2.4 Molecular systematics

RAPD markers can be used to determine the genetic identity of individuals from different geographical locations, to correctly classify the misclassified populations of a species and considered a reliable source for estimation of genetic diversity or

identity between closely related individuals or species (Campbell *et al.*, 1999; Nkongolo *et al.*, 2003). Rapidity of RAPD data generation has made it a potential source of systematic features. Three important features to be considered for RAPD data to be valuable for molecular phylogenetics are character identification, homology and independence.

**Table1**. List of studies conducted on molecular systematics in plants using RAPD.

| S.No. | Title | Result | Reference |
|---|---|---|---|
| 1. | Genetic validation and characterization of RAPD markers differentiating black and red spruces: molecular certification of spruce trees and hybrids. | RAPD markers were applied to determine genetic identity of red, black spruce and their hybrids from different sympatric and allopatric regions. An extremely high level of genetic identity was determined by the random primers and primer annealing sites on the genome. Sequence analysis of species-diagnostic RAPD markers indicated that most of these markers are not species specific and they are also present in other species of *Picea* genus. | Nkongolo *et al.*, 2003 |
| 2. | Systematics and randomly amplified polymorphic DNA in the genus *Leucaena* (*Leguminosae, Mimosideae*) | Phenetic analysis of RAPD data of *Leucaena* produced phenograms inconsistent with clustering of the taxa based on affirmation obtained from morphological, crossing and cpDNA-RFLP data. Parsimony based analysis of *Leucaena* RAPD data revealed three clusters as (i) *L. pulverulenta* and *L. leucocephala* (ii) *L. gregi* WATSON and *L. retusa* BENTH (iii) *L. esculenta* subsp. *esculenta* and *L. esculanta* subsp. *matudae.* | Harris, 1995 |
| 3. | Comparisons of within - population genetic variation in sexual and agamospermous *Amelanchier* (*Rosaceae*) using RAPD markers. | *A. laevis* (asexually seed producing) and *A. bartramiana* (sexually reproducing) did not show significant difference in their mean genetic diversity. The genetic diversity within *A. laevis* may be because its genetic identity values exceeded those of *A. bartramiana* which reveal its agamospermous mode of reproduction. | Campbell *et al.*, 1999 |
| 4. | Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. | RAPD determined high level of genetic variation within population and at species level in case of *G. sepium* and *G. vaculata.* Most of the genetic variation was observed between *G. sepium* populations but RAPD primers differed in their ability to determine genetic variability between and within populations. | Chalmers *et al.*, 1992 |
| 5. | RAPD analysis in *Crocus* | Results of RAPD analysis were not | Caiola *et al.*, |

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|---|---|---|---|
| | *sativus* L. accessions and related *Crocus* species. | corroborating with geographic locations and indicated that *C. sativus* originated from *C. cartwrightianus*. | 2004 |
| 6. | Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congeners *W. bolanderi* (*Asteraceae*) using RAPD and allozyme technique. | *W. reticulata* had less genetic diversity as compared to its congeners. Frequent gene flow among *W. bolanderi* populations lead to low $F_{ST}$ value whereas high $F_{ST}$ value in *W. reticulata* resulted from limited gene flow, local movement of pollen and seed and reproductive dominance of large, long- lived individuals. | Ayres *et al.*, 1999 |
| 7. | Molecular markers reveal little genetic differentiation among *Aconitum noveboracense* and *A. columbianum* (*Rannunculaceae*) populations. | Both *A. novboracense* and *A. columbianum* represented single species because all plant populations of *A. novboracense* from different geographical locations showed high level of genetic similarity (> 80%) with those *A. columbianum,* but *A. unicatum* population was the most differentiated from other populations. | Cole *et al.*, 2001 |
| 8. | Genetic variation within and among three invasive *Prosopis juliflora* (Leguminosae) populations in the River Nile State, Sudan | RAPD analysis of *Prosopis juliflora* revealed high diversity within and similarities between groups of populations which may be due to limited seed source, extensive endozoic seed dispersal system, limited pollen dispersal and recent introduction of species into Sudan. | Hamza, 2010 |
| 9. | Assessment of genetic relatedness of the two *Amaranthus retroflexus* populations by protein and random amplified polymorphic DNA (RAPD) markers. | Results regarding individual plants of both populations suggested low level of polymorphism which may be due to amplification of conserved part of the genome of *A. retroflexus.* | Snezana *et al.*, 2012 |
| 10. | Analysis of genetic diversity and population structure of 135 dill (*Anethum graveolens* L.) accessions using RAPD markers. | Analysis of genetic diversity and population structure with RAPD markers indicated high genetic diversity within and among the populations and clustered 135 accessions of *Anethum graveolens* into two main clusters which showed no correlation with the geographic origin of the individuals. | Suresh *et al.*, 2013 |
| 11. | Genetic variation in natural populations of mate Â (*Ilex paraguariensis* A. St.-Hil., *Aquifoliaceae*) using RAPD markers. | Gene flow between populations of *I. paraguariensis* occurred as the populations were geographically closer. High level of within population variation was due to its life history characteristics. | Gauer *et al.*, 2000 |

## 2.5 Germplasm conservation

As more than 8000 tree species are on the verge of extinction due to deteriorative effects of anthropogenic activities on trees' gene pool and due to mismanagement adverse environmental changes and overexploitation for commercial purposes for a long time decreases genetic diversity of the species, there is dire need of their conservation for appropriate ecosystem structure and function. So conservation of genetic diversity is the most considerable objective for maximum conservation programmes and it can be studied easily on population and species level with RAPD markers (Allnutt *et al.*, 2003; Geburek, 1997; Runo *et al.*, 2005).

**Table2**. List of studies conducted on RAPD for germplasm conservation of important and threatened species.

| S.No. | Title | Result | Reference |
|---|---|---|---|
| 1. | Genetic differentiation and diversity analysis of medicinal tree *Syzygium cumini* (Myrtaceae) from ecologically different regions of India. | As genetic diversity within population is higher than among populations so regional approach for conservation of *S. cumini* should be preferred. Therefore maximum number of populations must be incorporated for conservation. | Khan *et al.*, 2010 |
| 2. | Analysis of genetic structure in *Melia volkensii* (Gurke.) populations using random amplified polymorphic DNA. | A high level of genetic variation has been found between the eastern and coastal regions. So to maintain the whole genetic variation among populations, both of the regions should be properly conserved. | Runo *et al.*, 2005 |
| 3. | Genetic variation of *Melia azedarach* in community forests of West Java assessed by RAPD. | As moderate levels of genetic diversity has been observed in West Java forests, populations exhibiting highest genetic diversity should be used as potential source to improve and increase productivity of *M. azedarach* in that region. | Syamsuwida, 2011 |
| 4. | Patterns of Genetic Variation in *in* and *ex situ* populations of the Threatened Chilean Vine *Berberidopsis corallina*, detected Using RAPD Markers. | Because *B. corallina* has been listed as endangered due to high rate of habitat loss and its stems are used commercially so it should be conserved (Walter and Gildt 1998; Smith- Ramirez 1996). To design *ex situ* and *in situ* conservation strategies genetic variation should be studied with RAPD markers. For ex situ conservation of *B. corallina* reintroduction must be undertaken at already protected sites rather than at degraded sites. | Etisham-Ul-Haq *et al.*, 2001 |
| 5. | Genetic diversity and population structure of *Dactylorhiza hatagirea* (Orchidaceae) in cold desert Ladakh region of India. | As the population number and size of *D. hatageria*, a critically endangered species is decreasing critically due to anthropogenic activities and habitat destruction, so this species should be preserved and protected. Low level of | Warghat *et al.*, 2012 |

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|---|---|---|---|
| | | genetic variation within the population and moderate level among the populations was reported. So each population should be conserved specifically. | |
| 6. | Genetic variability among *Coleus* sp. studied by RAPD banding pattern analysis. | *Coleus* species is widespread all over India and highly valuable due to its medicinal and nutritional values. So genetic variation is essential for its survival which is important for conservation. RAPD results revealed the need of conservation of more number of populations to preserve their genetic diversity for further conservation and management projects as only three populations were used in this study. | Govarthanan *et al.*, 2011 |
| 7. | RAPD-based assessment of genetic relationships among and within American ginseng (*Panax quinquefolius* L.) populations and their implications for a future conservation strategy. | Economically important wild species are frequently endangered due to overharvest. Cultivated populations of *Ginseng* were identified as locally derived and serve as *in situ* gene banks. | Schlag *et al.*, 2012 |
| 8. | Genetic characterization and gene flow in different geographical distance neighbouring natural populations of wild soybean (Glycine soja Sieb. & Zucc.) and implications for protection from GM soybeans. | As wild soybean populations were considered threatened due to habitat loss by pollution and reduced habitat area and gene flow from cultivated (genetically modified) to wild population s may lead to genetic escape. So to reduce this risk, GM soybeans should be grown away from wild. | Wang *et al.*, 2012 |
| 9. | Genetic Diversity of *Picea asperata* populations Based on RAPDs. | Molecular analysis of 10 natural populations of *Picea asperata* revealed high levels of genetic differentiation among populations which was due to restricted gene flow, founder effects etc. Thus to conserve this *P. asperata* populations in Western China, populations should show low to moderate levels of within population diversity, high genetic diversity among populations and high level of gene flow. | Xue *et al.*, 2007 |

## 2.6 Factors influencing the genetic diversity

The level of genetic variation among and within population occurs due to gene flow, genetic drift, selection, habitat fragmentation, mutation etc. So knowledge of present genetic structure provides insights into evolutionary history of the population.

**Table3.** List of studies conducted to determine factors shaping genetic diversity.

| S.No. | Title | Result | Reference |
|---|---|---|---|
| 1. | RAPD variation among and within small and large populations of the rare clonal plant *Rannunculus reptans* (Ranunculaceae). | Gene flow among populations of *R. reptans* was highly limited and genetic drift lead to reduced genetic diversity of smaller populations. Both large and small populations were required for the conservation of genetic diversity which can be increased by increasing the size of small populations. | Fischer *et al.*, 2000 |
| 2. | Genetic structure and diversity in *Ramonda myconi* (Gesneriaceae): effects of historical climate change on a preglacial relict species. | Paleoclimatic history, acogeographical heterogeneity and anthropogenic activities were the main cuse of high species diversity in Mediterranean Basin and *R. myconi* is one of these species. Three glacial regions fitted well with already recognized endemism and species richness centres. | Dubreuil *et al.*, 2008 |
| 3. | Genetic analysis of invasive plant populations at different spatial scales. | At local level, genetic structure in plant populations was determined mainly by unequal gene flow, while at continental and global scale, founder effect and propagule transport via human interference shapes the genetic structure of invasive plant populations. To find the complete information about genetic forces involved in invasion, a multidirectional approach including sampling at different spatial and temporal scales was applied. | Ward, 2006 |
| 4. | Geographical patterns of nucleotide diversity and population differentiation in three closely related European pine species in the *Pinus mugo* complex | Bayesian cluster analysis revealed no clear assignment of individuals to species or geographic locales. This suggested limited gene flow, common evolutionary history and recent divergence. | Wachowiak *et al.*, 2013 |
| 5. | Random amplified polymorphic DNA analysis of southern brown bandicoot (*Isoodon obesulus*) populations in Western Australia reveals genetic differentiation related to environmental variables. | Local climatic conditions and physical barriers interfere with gene flow between populations through selection against migrants. | Cooper, 2000 |
| 6. | Genetic diversity of the shea tree (*Vitellaria paradoxa* C.F. Gaertn), detected by RAPD and chloroplast microsatellite markers. | The 'Dahomey Gap' was the major factor leading to differentiation between Western and Eastern populations as it may be the region that exceptionally remained dry during glacial periods. Genetic structure of Western populations may have been due to human interference. | Fontaine *et al.*, 2004 |

Out crossing species have higher level of genetic diversity within populations but lower level of genetic diversity among populations than selfing species (Fischer *et al.*, 2000)

# Chapter III

# Material and Methods

### 3.1 Plant material:

A total of 6 populations consisting of 47 individuals of *M. azedarach* have been collected from 6 different geographically isolated regions of Bathinda and adjoining regions. Of the 47 samples, 10 were from Bathinda population, 10 from Hanumangarh, 5 from Dabbali, 9 from Ganganagar, 5 from Kesrisinghpur and 8 from Padampur. Within each region populations were isolated from each other at a distance of at least 50-60 Km and their size varied from 5-10 plants. Complete information regarding sample collection and location is given in Figure 1 and Table 4. Fresh leaves of each selected plant were collected and kept inside multiple layers of blotting sheets for transportation to the laboratory.

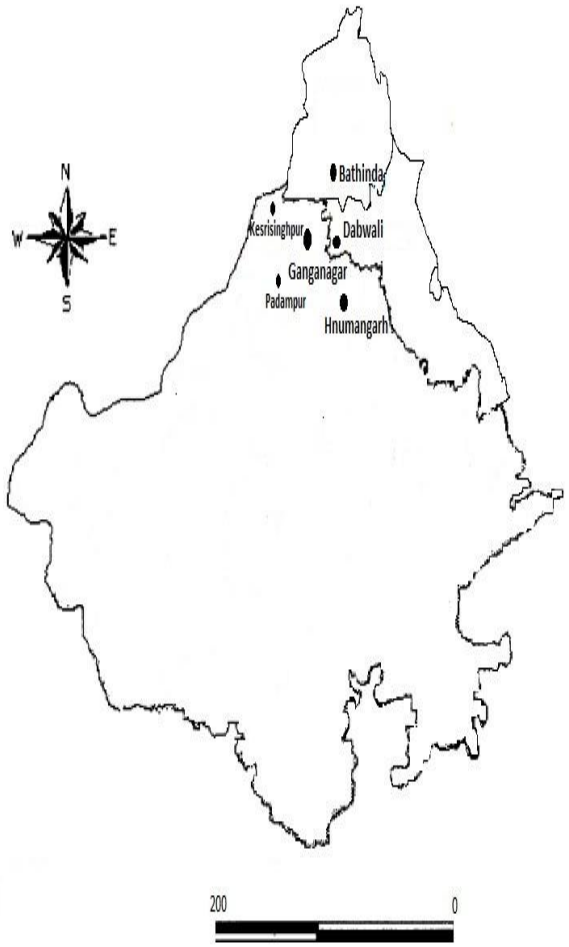


**Figure 1.** Sites of sample collection from Bathinda and its adjoining regions in India.

### 3.2 DNA isolation and purification

Total DNA was extracted from leaf tissue by the CTAB method (Doyle, 1990) with some modifications. The details of protocol for DNA isolation and purification are given in the Appendix A. The quality and concentration of the extracted DNA were estimated on 0.8% agarose gel and Nano Drop 2000 Spectrophotometer.

**Table 4.** Location of sample collection with longitude and latitude.

| Population | Sample Size | Longitude (E) | Latitude (N) |
|---|---|---|---|
| Bathinda (Bti) | 10 | 74°47'29.52"E | 30°08'26.96"N |
| Hanumangarh (Han) | 10 | 74°17'32.04"E | 29°36'48.33"N |
| Dabbali (Dab) | 5 | 74°38'46.09"E | 29°55'28.72"N |
| Ganganagar (Gng) | 9 | 73°51'32.55"E | 29°55'12.69"N |
| Kesrisinghpur (Kes) | 5 | 73°37'16.18"E | 29°56'51.68"N |
| Padampur (Pdm) | 8 | 73°37'34.83"E | 29°42'25.86"N |

### 3.3 RAPD analysis

Genomic DNA was analysed using standard 10 mer oligonucleotide RAPD molecular markers by Williams et al method with some modifications (Williams *et al.*, 1990). We standardized the DNA amplification conditions by assessing DNA concentration, primer concentration, different bands and concentrations of Taq polymerase as well as temperatures for PCR amplifications. Reactions were carried out in a final volume of 20uL containing 25mM $MgCl_2$ solution, 2.5mM dNTPs,10 picomoles primer, 25ng/uL of genomic DNA, 1.5 U of Taq polymerase (Bangalore Genei). Amplifications were performed in a thermal cycler (Applied Biosystems Veriti 96 well) programmed for initial denaturation cycle of 5 min at 94°C followed by 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 37°C and extension for 2 min at 72°C with a final extension of 7 min at 72°C.

PCR amplified products mixed with 6X loading dye were run on 2% agarose gel prepared in 1X TAE, stained with EtBr at 55V for 90 minutes; then visualized and photographed with the BIORAD Gel Doc X - imager.

### 3.4 Data analysis

Each fragment amplified using RAPD was treated as a binary unit character and scored 0 and 1 for absent and present bands respectively. Only intense and clearly visible bands were scored. Polymorphism information content (PIC) and percentage polymorphism were calculated for each primer which gives estimate about discrimination ability of a marker by considering number of alleles at a locus along with their relative frequencies (Table 5).

Genetic diversity was estimated using POPGENE software version 1.31 (Yeh *et al.*, 1999) to determine different parameters which include number of polymorphic loci and their percentage (Table 6), observed number of loci, effective number of alleles( $n_e = 1/\sum p_i^2$, where $p_i$ = frequency of the ith allele for the studied locus), Nei's genetic diversity ( $h= \sum h_k/r$, where $h_k$ = the value of h for the $_k$th locus and r = number of alleles studied) (Nei, 1973), Shannon's information index (Table 7) and dendrograms.

The phylogenetic relationship among populations was generated using software DARwin (Figure 3) and STRUCTURE version 2.3 (Pritchard *et al.*, 2000). It was used to perform a Bayesian analysis to identify hidden population structure by delineating individuals into genetically distinguishable clusters on the basis of their genotypes at multiple loci. The optimal value of K was identified using both the adhoc procedure introduced by Pritchard et al (2000) (Figure 5) and method developed by Evanno et al (2005) (Figure 4). Population assignment test based on a Bayesian approach was conducted for all the samples using the software STRUCTURE for assigned number of populations of K=1 to 6 and with 3 replicates with a random start for each K value and 1000 runs. The number of distinct population clusters were determined using Δ K method (Evanno *et al.*, 2005).

# Chapter IV

# Results

The study was designed to analyze the genetic structure and diversity of 6 populations of *Melia azedarach* L. from different geographic locales of Bathinda and adjoining regions.

### 4.1 Marker amplification and characterization

Out of the 80 primers that were evaluated in 6 populations, 39 primers were polymorphic and finally 30 primers were chosen on the basis of generation of stable and reproducible bands in all the samples. A total of 87 polymorphic bands were generated with an average of 2.9 bands per primer and 0.4160 average polymorphism information content (PIC). The highest number of polymorphic bands were achieved with primers OPA02, OPA04, OPA05, OPA10, OPA15, OPB08 OPB12, OPB18, OPD03 and OPD04, while the most informative primer were OPA01 and OPB11 with PIC value of 0.4998 (Table 5).

**Table5.** Data of RAPD primers used in the present study and the extent of polymorphism.

| S.No. | Primer Name | Primer Sequence (5'-3') | Amplified Bands | Polymorphic Bands | % Polymorphism | PIC* |
|---|---|---|---|---|---|---|
| 1. | OPA 01 | CAGGCCCTTC | 1 | 1 | 100 | 0.4998 |
| 2. | OPA 02 | TGCCGAGCTG | 4 | 4 | 100 | 0.4885 |
| 3. | OPA 04 | AATCGGGCTG | 4 | 4 | 100 | 0.4490 |
| 4. | OPA 05 | AGGGGTCTTG | 4 | 4 | 100 | 0.4964 |
| 5. | OPA 07 | GAAACGGGTG | 2 | 2 | 100 | 0.4919 |
| 6. | OPA 09 | GGGTAACGCC | 2 | 2 | 100 | 0.4002 |
| 7. | OPA 10 | GTGATCGCAG | 4 | 4 | 100 | 0.4836 |
| 8. | OPA 11 | CAATCGCCGT | 2 | 2 | 100 | 0.4183 |
| 9. | OPA 12 | TCGGCGATAG | 3 | 3 | 100 | 0.3183 |
| 10. | OPA 15 | TTCCGAACCC | 4 | 4 | 100 | 0.2825 |
| 11. | OPA 17 | GACCGCTTGT | 3 | 3 | 100 | 0.2917 |
| 12. | OPA 18 | AGGTGACCGT | 3 | 3 | 100 | 0.1674 |
| 13. | OPB 07 | GGTGACGCAG | 2 | 2 | 100 | 0.4964 |
| 14. | OPB 08 | GTCCACACGG | 4 | 4 | 100 | 0.3906 |
| 15. | OPB 10 | CTGCTGGGAC | 2 | 2 | 100 | 0.4980 |
| 16. | OPB 11 | GTAGACCCGT | 2 | 2 | 100 | 0.4998 |
| 17. | OPB 12 | CCTTGACGCA | 4 | 4 | 100 | 0.4855 |
| 18. | OPB 14 | TCCGCTCTGG | 3 | 3 | 100 | 0.4988 |
| 19. | OPB 15 | GGAGGGTGTT | 2 | 2 | 100 | 0.2789 |
| 20. | OPB 17 | AGGGAACGAG | 2 | 2 | 100 | 0.3696 |
| 21. | OPB 18 | CCACAGCAGT | 4 | 4 | 100 | 0.4964 |
| 22. | OPC 02 | GTGAGGCGTC | 3 | 3 | 100 | 0.4396 |
| 23. | OPC 08 | TGGACCGGTG | 2 | 2 | 100 | 0.1195 |
| 24. | OPC 11 | AAAGCTGCGG | 2 | 2 | 100 | 0.4817 |
| 25. | OPC 12 | TGTCATCCCC | 2 | 2 | 100 | 0.4889 |
| 26. | OPC 15 | GACGGATCAG | 3 | 3 | 100 | 0.4726 |
| 27. | OPC 18 | CACACTCCAG | 3 | 3 | 100 | 0.2012 |
| 28. | OPD 02 | GGACCCAACC | 3 | 3 | 100 | 0.4843 |
| 29. | OPD 03 | GTCGCCGTCA | 4 | 4 | 100 | 0.4993 |
| 30. | OPD 04 | TCTGGTGAGG | 4 | 4 | 100 | 0.4919 |

PIC* = Polymorphism Information Content

The number of polymorphic loci ranged from 33 (Kesrisinghpur) to 66 (Ganganagar) within 6 populations with an average of 50. The percentages of polymorphic loci ranged from 31.73% (Kesrisinghpur) to 63.46% (Ganganagar) with an average of 46.22 (Table 6).

**Table 6.** Different populations with Number of polymorphic loci and percentage polymorphic loci.

| Population | No. Of polymorphic loci | Percentage of polymorphic loci |
|---|---|---|
| Bathinda | 47 | 45.19% |
| Hanumangarh | 62 | 59.62% |
| Dabbali | 41 | 39.42% |
| Ganganagar | 66 | 63.46% |
| Kesrisinghpur | 33 | 31.73% |
| Padampur | 53 | 50.96% |

## 4.2 Within population diversity

Eight parameters were used to assess the genetic variation within populations and they included number of loci or bands, number and percentage of polymorphic loci, actual or observed number of alleles (na), effective number of alleles (ne), Nei's genetic diversity (h), Shannon information index (I), genetic diversity within population (intrapopulation diversity for subdivided populations). The highest number of observed alleles (na=1.6346) were present in Ganganagar. The highest effective number of alleles (ne=1.4047), Nei's genetic diversity (h=0.2286), Shannon's information index (I=0.3384) was found in Ganganagar population (Table 7).

**Table 7.** *Melia azedarach L.* populations from Bathinda and adjoining regions and their genetic variability detected by RAPD analysis.

| Population | Sample Size | na | ne | H | I |
|---|---|---|---|---|---|
| Bathinda | 10 | 1.4519 | 1.2788 | 0.1584 | 0.2356 |
| Hanumangarh | 10 | 1.5962 | 1.3621 | 0.2094 | 0.3119 |
| Dabbali | 5 | 1.3942 | 1.2984 | 0.1631 | 0.2358 |
| Ganganagar | 9 | 1.6346 | 1.4047 | 0.2286 | 0.3384 |
| Kesrisinghpur | 5 | 1.3173 | 1.1934 | 0.1125 | 0.1684 |
| Padampur | 8 | 1.5096 | 1.3318 | 0.1881 | 0.2779 |

na = Observed number of alleles

ne = Effective number of alleles

h = Nei's gene diversity

I = Shannon's Information index

### 4.3 Between population diversity

Five parameters were calculated to investigate the genetic variation among populations and these included total genetic diversity for all populations ($H_T$), genetic differentiation between subpopulations relative to total genetic diversity ($G_{ST} = 1 – H_s/H_T$ where $H_S$= population diversity and $H_T$ = total diversity), estimate of gene flow ($Nm = 0.5(1 – G_S)/G_{ST}$ ), Nei's genetic distance and Nei's genetic identity (Appendix C). The average genetic diversity was 0.1767 within population (Hs) and 0.2381 among populations or at species level ($H_T$). Average estimate of $F_{ST}$ or $G_{ST}$ across all loci were significantly different from zero (average $G_{ST}$ = 0.2580) which indicated limited gene flow and genetic structuring amongst sampling locales (Appendix B).

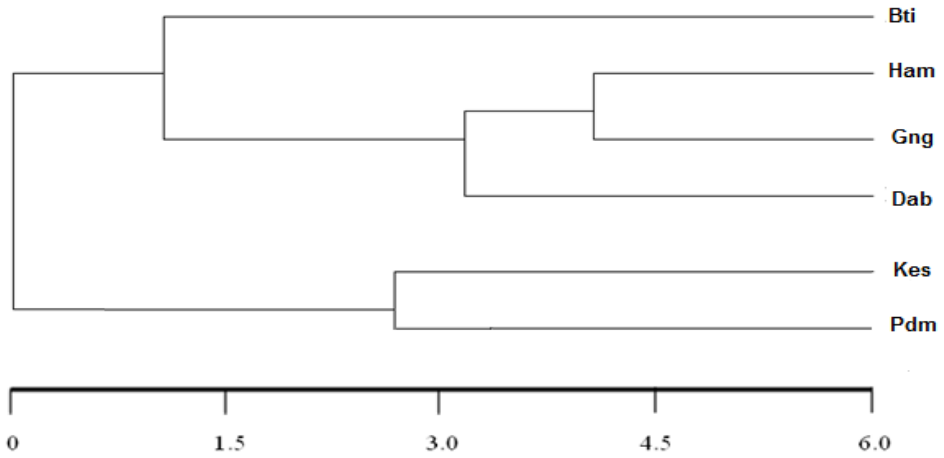


**Figure 2.** Dendrogram based on Nei's (1978) genetic distance for 6 popuations of *Melia azedarach L.* based on 30 RAPD primers.

### 4.4 Cladistic analysis

Dendrogrm was drawn by Dendrogram based on Nei's genetic distance method (UPGMA modified from Neighbor procedure of PHYLIP version 3.5) to visualize the relationships among 6 populations of *M. azedarach* L. (Figure 2). The results represented two clusters, one consisting of Kesrisighpur and Padampur and the

other consisting of the other 4 populations. Second cluster further consisted of two sub clusters, one of which included Bathinda and the other sub cluster consisted of Hanumangarh, Ganganagar and Dabbali.

**Table 8.** Populations with maximum gene flow.

| POPULATION | $H_T$ | $H_S$ | $G_{ST}$ | Nm |
|---|---|---|---|---|
| Han - Gng | 0.2374 | 0.2190 | 0.0775 | 5.9542 |
| Han - Dab | 0.2070 | 0.1862 | 0.1004 | 4.4825 |
| Dab - Gng | 0.2188 | 0.1958 | 0.1051 | 4.2580 |

$H_T$ = Total genetic diversity

$H_S$ = Subpopulation genetic diversity

$G_{ST}$ = Genetic differentiation between subpopulations relative to the total genetic diversity

Nm = Estimate of gene flow from $G_{ST}$

According to Nei's unbiased measures of genetic distance (1978) data, the highest genetic distance pairs were between Kesrisinghpur and Hanumangarh (0.1426), Kesrisinghpur and Bathinda (0.1065) and Kesrisinghpur and Dabbali (0.1052). Therefore, it indicates Kesrisinghpur population to be the most differentiated of all the populations. Data of Nei's (1978) unbiased measures of genetic identity validates the data of genetic distance and the highest genetic identity pairs were between Ganganagar and Hanumangarh (0.9682), Hanumangarh and Dabbali (0.9665) and Ganganagar and Dabbali (0.9624). Therefore it indicates that Hanumangarh, Ganganagar and Dabbali are highly similar on genetic level. Hence, these populations were showing strong relationships between them (Appendix C). The data of maximum gene flow agrees with the data of maximum genetic identity because populations having highest identity have high maximum Nm values (Table 8).

Unrooted tree constructed using DARwin determines phylogenetic relationship between the individuals on the basis of genetic distance and indicated two clusters of original genetic stocks and one cluster consisting of individuals migrated from the two original genetic stocks (Figure 3).

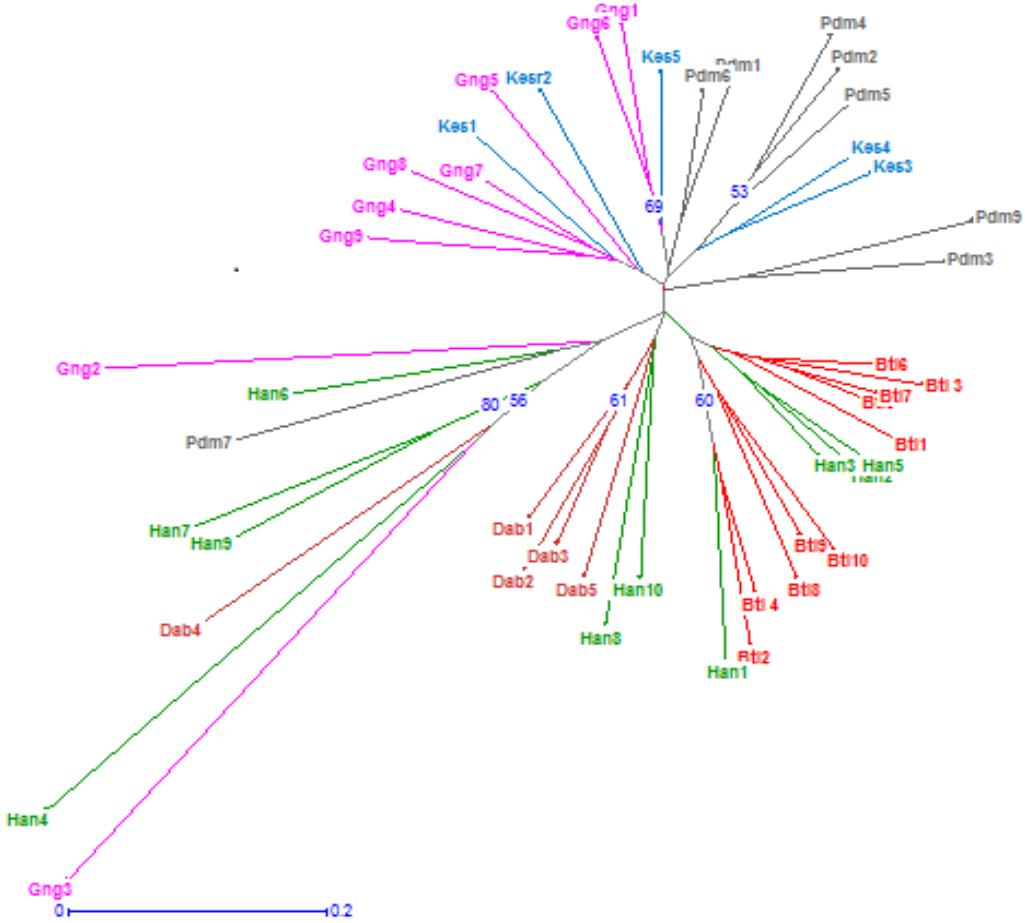


**Figure 3.**Unrooted tree construted by DARwin software. Each coloured branch represents one individual collected from corresponding inferred population.

STRUCTURE version 2.3 based on Bayesian approach was applied to search for hidden population structure among genotypes of different populations of *M. azedarach.* The relative distinctiveness of sampled populations was determined by assigning individuals on the basis of genetically homogeneous groups rather than on geographical locations of sampling. The highest value of ΔK identified 2 clusters or genetic stocks that were present among 6 sampled populations. This grouping is consistent with UPGMA dendrogram.

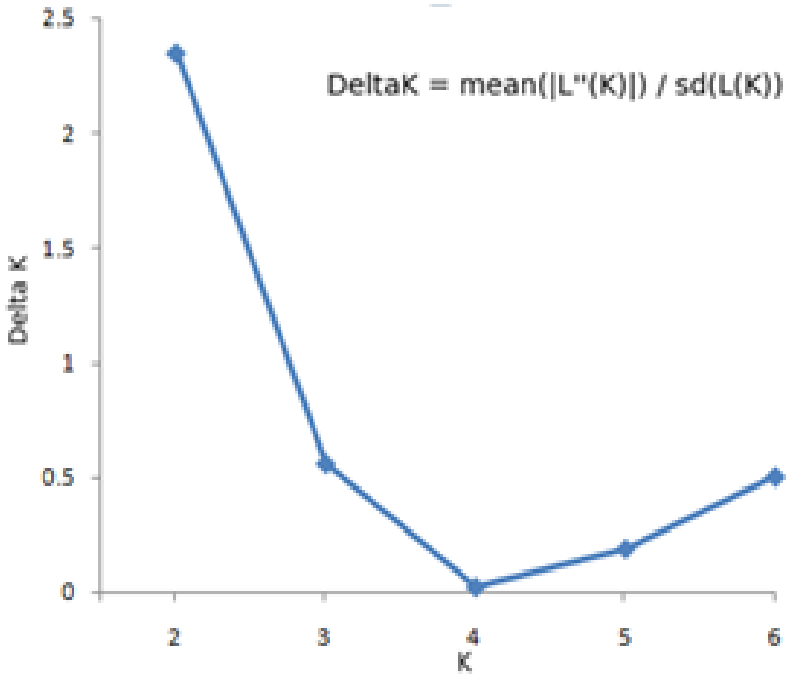


**Figure 4.** Most appropriate value of K from a range of 1-6 populations using the second order statistics (Δ K) given by Evanno et al. (2005)

Graphical method used to detect true number of populations i.e. K indicated mostly the number of genetic stocks at K=2 considering ΔK distribution (Figure 4). Out of 47 individuals, 34 individuals were observed in the admixed stock and 12 individuals in one original genetic stock and one single individual of Hanumangarh in the second original genetic stock (Figure 5).

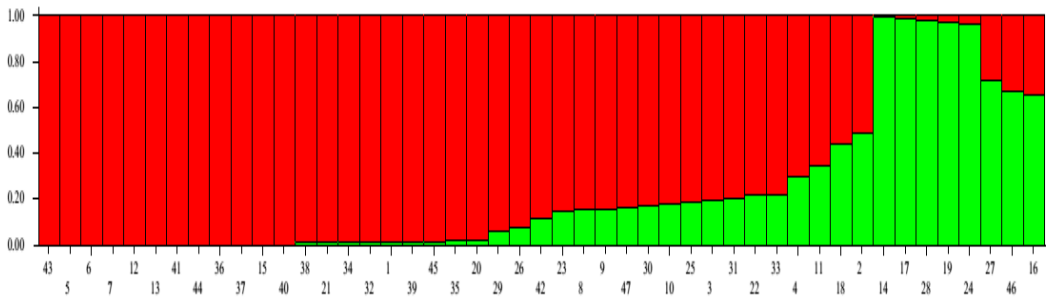


**Figure 5.** Structure plot with membership coefficient of individuals on y-axis. Two clusters inferred by Structure in combined clustering of all individuals. Each colour represents different genetic stock.

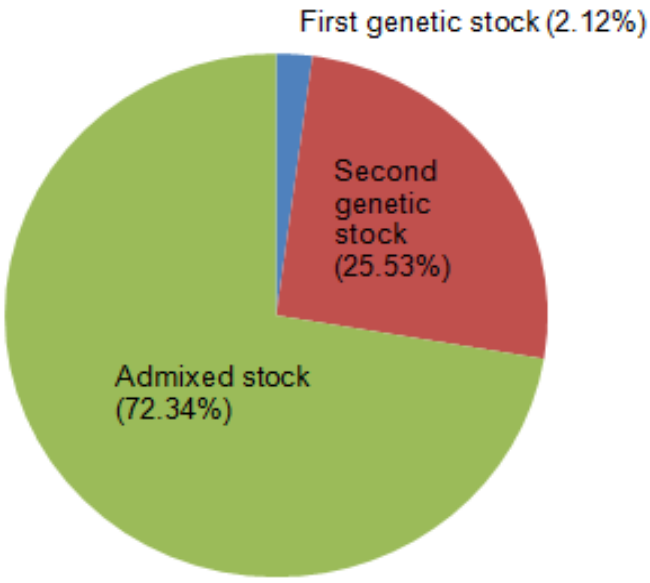


**Figure 6.** Pie chart representing two original and one admixed genetic stock revealed by software STRUCTURE version 2.3.

Of the 47 individuals sampled, first smaller genetic cluster occupied 2.12% (only 1 individual from Hanumangarh population), second larger genetic cluster occupied 25.53% (12 individuals from 4 populations) and the admixed cluster occupied 72.34% (34 individuals from all the 6 populations) (Figure 6).

# Chapter V

# Discussion

Genetic diversity, a basic source of biodiversity is the total number of traits leading to variation within or among populations of a species (Poczai *et al.*, 2012). Maintenance of genetic diversity and population distinctiveness plays an important role in biodiversity and conservation of species as it helps to understand the processes or factors involved in genetic variation in populations and species (Nongrum *et al.*, 2012).

RAPD has been used to detect genetic diversity within and between populations of *Populus euphratica* (Saito *et al.*, 2002)*, Vitellaria paradoxa* (Fontaine *et al.*, 2004), *Gliricidia sepium* (Dawson *et al.*, 1995), *Mangifera indica* (Díaz-Matallana *et al.*, 2009) and so many others. High values of percentage polymorphism for all the primers indicated that the main cause for high genetic diversity may be geographic isolation which regulates the process of genetic diversity and variation. High level of genetic diversity due to geographic isolation in *Anethum graveolens* populations and RAPD was used to reveal its genetic structure (Suresh *et al.*, 2013). Population wise highest value of percentage of polymorphic loci is of Ganganagar population (63.46%) which is geographically distant from other populations and dendrogram agrees with this data. This suggested that Ganganagar population was the most distant and differentiated population. Present investigation using RAPD marker revealed that in *M. azedarach* highest genetic variation was observed among populations ($H_T$ = 0.2381) as compared to within population ($H_S$ = 0.1767). Till now investigations regarding genetic diversity revealed low levels of genetic diversity within populations in case of populations of geographically restricted plant species but high levels of genetic diversity within population was observed in case of out-crossing species (Torres *et al.*, 2003; Wagner *et al.*, 2011).When genetic diversity is classified into within and among population genetic diversities, selfing species exhibit low levels of genetic diversity within population, but a considerable high genetic diversity among populations (He *et al.*, 2007).

Genetic diversity among the populations and geographic range are highly related (Warghat *et al.*, 2012). Several factors like geographical isolation, population fragmentation, breeding system and genetic drifts may be responsible for high population differentiation (Hogbin *et al.*, 1999; Zong *et al.*, 2008). $G_{ST}$ values ranging from 0 to 0.05 represent little or very little genetic diversity, from 0.05 to

0.15 represent considerable genetic diversity and that >0.25 represent very high genetic diversity. The results of average $G_{ST}$ (0.2580) indicated very high genetic diversity among populations. If populations are small and isolated from one another, the genetic drift could be capable of influencing the genetic structure and increasing differentiation among populations (Ellstrand *et al.*, 1993). High genetic diversity indicated limited gene flow among *M. Azedarach* populations which agrees with estimate of gene flow (Nm) obtained with POPGENE version 1.31. Nm represents historical average levels of gene flow. A $G_{ST}$ derived average genetic flow (Average Nm = 1.4381) revealed that gene flow between populations of *M. azedarach* is restricted and it is below the level (Nm>4) required to counteract genetic drift (i.e. Nm>4 according to stepping stone model) (Slatkin, 1993). But in some population pairs Nm value revealed high gene flow.

If Nm>4 (in a stepping stone model), the gene flow is sufficient to create genetic differentiation between populations balanced for migration and genetic drift. In the present study average Nm = 1.4381 which indicates limited gene flow among populations and agrees with the average $G_{ST}$ value (0.2580) because $G_{ST}$ is inversely related to Nm, the effective migration rate. A high $G_{ST}$ indicates that populations are genetically differentiated the result of a low migration of genotypes.

Maximum gene flow was found between population pairs Hanumangarh and Ganganagar (Nm = 5.9542) followed by Hanumangarh and Dabbali (4.4825) and Dabbali and Ganganagar (Nm = 4.2580). All the other population pairs had Nm values are less than 4 which indicates low gene flow (according to stepping stone model). These values agree with results of Nei's unbiased genetic identity and distance. Some of these populations are geographically closer and some are quite distant. This indicates that geographically closer populations undergo high gene flow when migrant genes arriving by pollen or seed or human interference become established in new genets (Ayres *et al.*, 1999). Pollen dispersal by wind and insects between populations may be affected by environmental conditions, geographic distances between populations and topographic conditions (Islam *et al.*, 2012). In the present study high gene flow was observed between populations which were geographically distant from each other. This may be due to human interference. Long distance seed dispersal by humans has been observed as a

source of gene flow among populations of Italian wild Cherry (*Prunus avium* L.) (Rogatis *et al.*, 2013).

Clustering of different populations of *M. azedarach* in Dendrogram based on Nei's genetic distance was not completely in congruent with their geographic locations and so it indicates that genetic diversity is not corroborating with geographical diversity. The data strongly suggests that overall genetic diversity is high and gene flow is limited but in some population pairs $G_{ST}$ value is very low that is low genetic diversity and high Nm value (Nm>4) that is high gene flow. Overall high genetic diversity may be due to geographic isolation, habitat fragmentation or mating system but high gene flow in some population pairs may be due to human interference, pollen or seed dispersal etc.

Cluster analysis in *M. azedarach* showed that genetic diversity based on morphological traits was not in accordance to geographical regions (Solouki *et al.*, 2008). There was no clear clustering pattern of geographically closer individuals. Same results were obtained in the case of *Anethum graveolens L.* (Suresh *et al.*, 2013). These observations clearly indicated that the association between genetic similarity and geographical distance was less significant. These genetic differences among populations may be due to selection, adaptation, migration, genetic drift and method of pollination. The other factor of importance is also vitally related to the environment and human interference (Solouki *et al.*, 2008). Dendrogram and cluster analysis showed that some landraces with same geographical area were clustered into different groups and no preferred relationship existed between geographical distribution and the dendrogram. Investigations related to cluster analysis have found clusters including trees from different populations which indicated that plants can be more similar to those from other populations than to those of their own population in case of *Ilex paraguariensis* (Gauer *et al.*, 2000).

Both of the original genetic stocks consisted of individuals of populations from different geographical locations i.e. the two genetic clusters identified by STRUCTURE were not according to the geographical locations of sampling. This represented that a high level of gene flow has occurred. Structure infers the highest likelihood of both the individual clusters and the admixture of genotypes

using allele frequency and linkage disequilibrium information from dataset directly. The Bayesian structure analysis, dendrogram and unrooted tree did not group the individuals according to their geographical locations. Six *M. azedarach* populations may have originated from two ancestral gene pools and the presence of admixed cluster or stock suggested the occurrence of gene flow. None of the clusters determined by STRUCTURE either original or the admixed one consisted of specified full populations which indicated high gene flow.

Gene flow at the species level was limited due to wide geographical distribution or restricted seed and pollen dispersal. Gene flow and life history traits such as population size change, habitat fragmentation, bottlenecks etc. have been considered as the main determinants of population genetic structure. Therefore different populations considered in the same genetic clusters, suggests recent common ancestry to be the important factor rather than other events leading to gene flow. In conclusion, this study has added significant knowledge and understanding of the population structure and genetic diversity of *M. Azedarach L.* between and within population. Presence of high genetic diversity within and among the populations, high polymorphism, Nei's genetic diversity, Shannon's information index, total gene diversity values and Dendrogram analysis, resulted in insignificant correlation between geographic distance and gene diversity. The lack of clear assignment of individuals to geographical regions of sampling and consideration of different populations in the same genetic cluster suggests a recent common evolutionary history and recent divergence.

# Summary

*Melia azedarach* L., a forest tree belonging to family Meliaceae is an ecologically and economically imperative species growing in Thar Desert. It's high adaptability and tolerance to a wide range of conditions makes it drought hardy and frost hardy.

RAPD profiling of 6 populations consisting of 47 individuals from Bathinda and its adjoining regions in Punjab was done using 30 primers. A total of 87 polymorphic bands were generated with an average of 2.9 bands per primer. Different parameters including number of loci or bands, number and percentage of polymorphic loci, actual or observed number of alleles (na), effective number of alleles (ne), Nei's genetic diversity (h), Shannon information index (I), genetic diversity within population (intrapopulation diversity for subdivided populations), total genetic diversity for all populations ($H_T$), genetic differentiation between subpopulations relative to total genetic diversity ($G_{ST}$), estimate of gene flow (Nm) and Nei's genetic distance and Nei's genetic identity were used to determine within and between populations genetic diversity. Ganganagar was considered as the most differentiated population as it represented the highest value of percentage of polymorphic loci and is geographically distant from other populations.

Average $G_{ST}$ and Nm values indicated overall high genetic diversity and limited gene flow, but in some population pairs including Hanumangarh and Ganganagar, Hanumangarh and Dabbali and Dabbali and Ganganagar high gene flow was observed. Results of Nei's unbiased genetic identity and distance supported high gene flow in these populations. Overall high genetic diversity may be due to geographic isolation, habitat fragmentation and mating system, but high gene flow in some populations may be due to human interference and pollen or seed dispersal etc.

Population analysis by STRUCTURE version 2.3 and Unrooted tree generated by DARwin revealed two original and one admixed genetic stocks which were not according to the geographic locales that indicated high gene flow. Gene flow and life history traits such as population size change, habitat fragmentation, bottlenecks etc. have been considered as important factors designing population

genetic structure. Different populations clustered in the same genetic stock indicated recent common ancestry to be the ruling factor leading to gene flow.

# References

Allnutt, T., Newton, A., Premoli, A. and Lara, A. (2003). Genetic variation in the threatened South American conifer *Pilgerodendron uviferum* (Cupressaceae), detected using RAPD markers. *Biological Conservation,* ***114***(2): 245-253.

Ayres, D. R. and Ryan, F. J. (1999). Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congener *W. bolanderi* (Asteraceae) using RAPD and allozyme techniques. *American Journal of Botany,* ***86***(3): 344-353.

Badr, A., El-Shazly, H. H., Helail, N. S. and El Ghanim, W. (2012). Genetic diversity of *Artemisia* populations in central and north Saudi Arabia based on morphological variation and RAPD polymorphism. *Plant Systematics and Evolution,* ***298***(5): 871-886.

Basha, S., Francis, G., Makkar, H., Becker, K. and Sujatha, M. (2009). A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries. *Plant Science,* ***176***(6): 812-823.

Basha, S. and Sujatha, M. (2007). Inter and intra-population variability of *Jatropha curcas* L. characterized by RAPD and ISSR markers and development of population specific SCAR markers. *Euphytica,* ***156***(3): 375-386.

Booy, G., Hendriks, R., Smulders, M., Groenendael, J. v. and Vosman, B. (2000). Genetic diversity and the survival of populations. *Plant Biology,* ***2***(4): 379-395.

Brockerhoff, E. G., Jactel, H., Parrotta, J. A., Quine, C. P. and Sayer, J. (2008). Plantation forests and biodiversity: oxymoron or opportunity? *Biodiversity and Conservation,* ***17***(5): 925-951.

Caiola, M. G., Caputo, P. and Zanier, R. (2004). RAPD analysis in *Crocus sativus* L. accessions and related *Crocus* species. *Biologia Plantarum,* ***48***(3): 375-380.

Campbell, C. S., Alice, L. A. and Wright, W. A. (1999). Comparisons of within-population genetic variation in sexual and agamospermous *Amelanchier* (Rosaceae) using RAPD markers. *Plant systematics and evolution,* ***215***(1-4): 157-167.

Chalmers, K., Waugh, R., Sprent, J., Simons, A. and Powell, W. (1992). Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. *Heredity,* **69:** 465-472.

Cole, C. T. and Kuchenreuther, M. A. (2001). Molecular markers reveal little genetic differentiation among *Aconitum noveboracense* and *A. columbianum* (Ranunculaceae) populations. *American Journal of Botany,* ***88***(2): 337-347.

Cooper, M. (2000). Random amplified polymorphic DNA analysis of southern brown bandicoot (*Isoodon obesulus*) populations in Western Australia reveals genetic differentiation related to environmental variables. *Molecular Ecology,* ***9***(4): 469-479.

Coria, C., Almiron, W., Valladares, G., Carpinella, C., Luduena, F., Defago, M. and Palacios, S. (2008). Larvicide and oviposition deterrent effects of fruit and leaf extracts from *Melia azedarach* L. on Aedes aegypti (L.)(Diptera: Culicidae). *Bioresource Technology,* ***99***(8): 3066-3070.

Dawson, I., Simons, A., Waugh, R. and Powell, W. (1995). Diversity and genetic differentiation among subpopulations of *Gliricidia sepium* revealed by PCR-based assays. *Heredity,* ***74***(1): 10-18.

De Nardo, E., Costa, A. and Lourencao, A. (1997). *Melia azedarach* extract as an antifeedant to *Bemisia tabaci* (Homoptera: Aleyrodidae). *The Florida entomologist,* ***80***(1): 92-94.

De Rogatis, A., Ferrazzini, D., Ducci, F., Guerri, S., Carnevale, S. and Belletti, P. (2013). Genetic variation in Italian wild cherry (*Prunus avium* L.) as characterized by nSSR markers. *Forestry,* ***86***(3): 391-400.

Díaz-Matallana, M., Schuler-García, I., Ruiz-García, M. and Hodson de Jaramillo, E. (2009). Analysis of diversity among six populations of Colombian mango (*Mangifera indica* L. cvar. Hilacha) using RAPDs markers. *Electronic Journal of Biotechnology,* ***12***(3): 1-2.

Doligez, A. and Joly, H. I. (1997). Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, *Carapa procera* (Meliaceae), in French Guiana. *Heredity,* ***79***(1): 72-82.

Doyle, J. J. (1990). Isolation of plant DNA from fresh tissue. *Focus,* **12**: 13-15.

Du, Z., Hou, R., Zhu, Y., Li, X., Zhu, H. and Wang, Z. (2011). A random amplified polymorphic DNA(RAPD) molecular marker linked to late-bolting gene in

pak-choi(*Brassica campestris* ssp. chinensis Makino L.). *African Journal of Biotechnology,* ***10***(41): 7962-7968.

Dubreuil, M., Riba, M. and Mayol, M. (2008). Genetic structure and diversity in *Ramonda myconi* (Gesneriaceae): effects of historical climate change on a preglacial relict species. *American Journal of Botany,* ***95***(5): 577-587.

Ehlers, A., Worm, B. and Reusch, T. B. (2008). Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. *Marine ecology progress series,* **355:** 1-7.

El-Juhany, L. I. (2011). Evaluation of some wood quality measures of eight-year-old *Melia azedarach* trees. *Turkish Journal of Agriculture and Forestry,* **35**: 165-171.

Ellstrand, N. C. and Elam, D. R. (1993). Population genetic consequences of small population size: implications for plant conservation. *Annual review of Ecology and Systematics,* **24**: 217-242.

Etisham-Ul-Haq, M., Allnutt, T., Smith-Ramirez, C., Gardner, M. F., Armesto, J. and Newton, A. (2001). Patterns of genetic variation in in and ex situ populations of the threatened Chilean Vine *Berberidopsis corallina*, detected using RAPD markers. *Annals of Botany,* ***87***(6): 813-821.

Evanno, G., Regnaut, S. and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* ***14***(8): 2611-2620.

Fischer, M., Husi, R., Prati, D., Peintinger, M., van Kleunen, M. and Schmid, B. (2000). RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). *American Journal of Botany,* ***87***(8): 1128-1137.

Fontaine, C., Lovett, P., Sanou, H., Maley, J. and Bouvet, J. (2004). Genetic diversity of the shea tree (*Vitellaria paradoxa* CF Gaertn), detected by RAPD and chloroplast microsatellite markers. *Heredity,* ***93***(6): 639-648.

Frankham, R. (2010). Challenges and opportunities of genetic approaches to biological conservation. *Biological Conservation,* ***143***(9): 1919-1927.

Frankham, R., Briscoe, D. A. and Ballou, J. D. (2002). *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

Gauer, L. and Cavalli-Molina, S. (2000). Genetic variation in natural populations of mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) using RAPD markers. *Heredity,* ***84***(6): 647-656.

Geburek, T. (1997). Isozymes and DNA markers in gene conservation of forest trees. *Biodiversity & Conservation,* ***6***(12): 1639-1654.

Gorji, A. M., Poczai, P., Polgar, Z. and Taller, J. (2011). Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *American Journal of Potato Research,* ***88***(3): 226-237.

Govarthanan, M., Guruchandar, A., Arunapriya, S., Selvankumar, T. and Selvam, K. (2011). Genetic variability among Coleus sp. studied by RAPD banding pattern analysis. *International Journal for Biotechnology and Molecular Biology Research,* ***2***(12): 202-208.

Hadrys, H., Balick, M. and Schierwater, B. (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology,* ***1***(1): 55-63.

Hamrick, J. and Godt, M. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* ***351***(1345): 1291-1298.

Hamza, N. B. (2010). Genetic variation within and among three invasive *Prosopis juliflora* (Leguminosae) populations in the River Nile State, Sudan. *International Journal of Genetics and Molecular Biology,* ***2***(5): 92-100.

Harris, S. A. (1995). Systematics and randomly amplified polymorphic DNA in the genus Leucaena (Leguminosae, Mimosoideae). *Plant systematics and evolution,* ***197***(1-4): 195-208.

He, F., Kang, D., Ren, Y., Qu, L., Zhen, Y. and Gu, H. (2007). Genetic diversity of the natural populations of *Arabidopsis thaliana* in China. *Heredity,* ***99***(4): 423-431.

Hill, W. and Weir, B. (2004). Moment estimation of population diversity and genetic distance from data on recessive markers. *Molecular Ecology,* ***13***(4): 895-908.

Hogbin, P. M. and Peakall, R. (1999). Evaluation of the contribution of genetic research to the management of the endangered plant *Zieria prostrata*. *Conservation Biology,* ***13***(3): 514-522.

Holsinger, K. E., Lewis, P. O. and Dey, D. K. (2002). A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology,* ***11***(7): 1157-1164.

Hördegen, P., Hertzberg, H., Heilmann, J., Langhans, W. and Maurer, V. (2003). The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. *Veterinary Parasitology,* ***117***(1): 51-60.

Hughes, A. R. and Stachowicz, J. J. (2004). Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences of the United States of America,* ***101***(24): 8998-9002.

Islam, M. S., Lian, C., Kameyama, N. and Hogetsu, T. (2012). Analyses of genetic population structure of two ecologically important mangrove tree species, *Bruguiera gymnorrhiza* and *Kandelia obovata* from different river basins of Iriomote Island of the Ryukyu Archipelago, Japan. *Tree Genetics and Genomes,* ***8***(6): 1247-1260.

Johnson, M. T., Lajeunesse, M. J. and Agrawal, A. A. (2006). Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters,* ***9***(1): 24-34.

Kettle, C. J. (2010). Ecological considerations for using dipterocarps for restoration of lowland rainforest in Southeast Asia. *Biodiversity and Conservation,* ***19***(4): 1137-1151.

Khan, M., Kihara, M. and Omoloso, A. (2001). Antimicrobial activity of *Horsfieldia helwigii* and *Melia azedarach*. *Fitoterapia,* ***72***(4): 423-427.

Khan, S. and Sharma, V. (2010). Genetic differentiation and diversity analysis of medicinal tree *Syzygium cumini* (Myrtaceae) from ecologically different regions of India. *Physiology and Molecular Biology of Plants,* ***16***(2): 149-158.

Khurana, K. V., Kachhwaha, S. and Kothari, S. L. (2012). Characterization of genetic diversity in *Jatropha curcas* L. germplasm using RAPD and ISSR markers. *Indian Journal of Biotechnology,* ***11***(1): 54-61.

Kumar, P., Gupta, V., Misra, A., Modi, D. and Pandey, B. (2009). Potential of molecular markers in plant biotechnology. *Plant Omics Journal,* ***2***(4): 141-162.

Leung, H., Zhu, Y., Revilla-Molina, I., Fan, J. X., Chen, H., Pangga, I., Cruz, C. V. and Mew, T. W. (2003). Using genetic diversity to achieve sustainable rice disease management. *Plant Disease,* ***87***(10): 1156-1169.

Loveless, M. and Hamrick, J. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics,* **15**:65-95.

Lynch, M. and Milligan, B. G. (1994). Analysis of population genetic structure with RAPD markers. *Molecular Ecology,* ***3***(2): 91-99.

Mahar, K. S., Rana, T. S. and Ranade, S. A. (2011). Molecular analyses of genetic variability in soap nut (*Sapindus mukorossi* Gaertn.). *Industrial Crops and Products,* ***34***(1): 1111-1118.

Mahar, K. S., Rana, T. S., Ranade, S. A. and Meena, B. (2011). Genetic variability and population structure in *Sapindus emarginatus* Vahl from India. *Gene,* ***485***(1): 32-39.

Narzary, D., Mahar, K. S., Rana, T. and Ranade, S. (2009). Analysis of genetic diversity among wild pomegranates in Western Himalayas, using PCR methods. *Scientia Horticulturae,* ***121***(2): 237-242.

Narzary, D., Rana, T. and Ranade, S. (2010). Genetic diversity in inter simple sequence repeat profiles across natural populations of Indian pomegranate (*Punica granatum* L.). *Plant biology,* ***12***(5): 806-813.

Nathan, S. S., Savitha, G., George, D. K., Narmadha, A., Suganya, L. and Chung, P. G. (2006). Efficacy of *Melia azedarach* L. extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Bioresource Technology,* ***97***(11): 1316-1323.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences,* ***70***(12): 3321-3323.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* ***89***(3): 583-590.

Nkongolo, K., Deverno, L. and Michael, P. (2003). Genetic validation and characterization of RAPD markers differentiating black and red spruces: molecular certification of spruce trees and hybrids. *Plant systematics and evolution,* ***236***(3-4): 151-163.

Nongrum, I., Kumar, S., Kumaria, S. and Tandon, P. (2012). Genetic variation and gene flow estimation of *Nepenthes khasiana* Hook F-A threatened

insectivorous plant of India as revealed by RAPD markers. *Journal of Crop Science and Biotechnology,* ***15***(2): 101-105.

O'Grady, J. J., Brook, B. W., Reed, D. H., Ballou, J. D., Tonkyn, D. W. and Frankham, R. (2006). Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. *Biological Conservation,* ***133***(1): 42-51.

Olmos, S., Lavia, G., Di Renzo, M., Mroginski, L. and Echenique, V. (2002). Genetic analysis of variation in micropropagated plants of *Melia azedarach* L. *In Vitro Cellular & Developmental Biology-Plant,* ***38***(6): 617-622.

Poczai, P. and Hyvonen, J. (2011). On the origin of Solanum nigrum: can networks help? *Molecular biology reports,* ***38***(2): 1171-1185.

Poczai, P., Varga, I., Bell, N. E. and Hyvonen, J. (2012). Genomics Meets Biodiversity: Advances in Molecular Marker Development and Their Applications in Plant Genetic Diversity Assessment (pp. 3-32). Helsinki: InTech.

Pomper, K., Azarenko, A., Bassil, N., Davis, J. and Mehlenbacher, S. (1998). Identification of random amplified polymorphic DNA (RAPD) markers for self-incompatibility alleles in *Corylus avellana* L. *Theoretical and Applied Genetics,* ***97***(3): 479-487.

Prevost, A. and Wilkinson, M. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics,* ***98***(1): 107-112.

Pritchard, J. K., Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics,* ***155***(2): 945-959.

Radhika, K., Sumer, S., Gopinath, S. and GM, A. P. (2012). A study on assessment of genetic diversity and relationships of medicinal plants using RAPD markers. *Global Journal of Research on Medicinal Plants & Indigenous Medicine,* ***1***(12): 678-686.

Reusch, T. B., Ehlers, A., Hämmerli, A. and Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences of the United States of America,* ***102***(8): 2826-2831.

Runo, M., Muluvi, G. and Odee, D. (2005). Analysis of genetic structure in *Melia volkensii* (Gurke.) populations using random amplified polymorphic DNA. *African Journal of Biotechnology, **3***(8): 421-425.

Saito, Y., Shiraishi, S., Tanimoto, T., Yin, L., Watanabe, S. and Ide, Y. (2002). Genetic diversity of *Populus euphratica* populations in northwestern China determined by RAPD DNA analysis. *New Forests, **23***(2): 97-103.

Samantaray, S. and Maiti, S. (2010). An assessment of genetic fidelity of micropropagated plants of *Chlorophytum borivilianum* using RAPD markers. *Biologia Plantarum, **54***(2): 334-338.

Savolainen, O. and Pyhäjärvi, T. (2007). Genomic diversity in forest trees. *Current opinion in plant biology, **10***(2): 162-167.

Schlag, E. M. and McIntosh, M. S. (2012). RAPD based assessment of genetic relationships among and within American ginseng (*Panax quinquefolius* L.) populations and their implications for a future conservation strategy. *Genetic Resources and Crop Evolution, **59***(7): 1553-1568.

Schnell, R., Ronning, C. and Knight Jr, R. (1995). Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. *Theoretical and Applied Genetics, **90***(2): 269-274.

Scocchi, A., Faloci, M., Medina, R., Olmos, S. and Mroginski, L. (2004). Plant recovery of cryopreserved apical meristem-tips of *Melia azedarach* L. using encapsulation/dehydration and assessment of their genetic stability. *Euphytica, **135***(1): 29-38.

Scocchi, A., Faloci, M., Medina, R., Olmos, S. and Mroginski, L. (2004). Plant recovery of cryopreserved apical meristem tips of *Melia azedarach* L. using encapsulation/dehydration and assessment of their genetic stability. *Euphytica, **135***(1): 29-38.

Sharma, S. K., Kumar, S., Rawat, D., Kumaria, S., Kumar, A. and Rao, S. R. (2011). Genetic diversity and gene flow estimation in *Prosopis cineraria* L. Druce: A key stone tree species of Indian Thar Desert. *Biochemical Systematics and Ecology, **39***(1): 9-13.

Sheikh, M. I. (1993). *Trees of Pakistan*. Pakistan: Pictorial Printers.

Sheng, Y., Zheng, W., Pei, K. and Ma, K. (2005). Genetic variation within and among populations of a dominant desert tree *Haloxylon ammodendron* (Amaranthaceae) in China. *Annals of Botany, **96***(2): 245-252.

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution,* ***47***(1): 264-279.

Snezana, D. M., Marija, K., Danijela, R., Milena, S. and Lidija, S. (2012). Assessment of genetic relatedness of the two *Amaranthus retroflexus* populations by protein and random amplified polymorphic DNA (RAPD) markers. *African Journal of Biotechnology,* ***11***(29): 7331-7337.

Solouki, M., Mehdikhani, H., Zeinali, H. and Emamjomeh, A. (2008). Study of genetic diversity in Chamomile (*Matricaria chamomilla*) based on morphological traits and molecular markers. *Scientia Horticulturae,* ***117***(3): 281-287.

Suresh, S., Chung, J.-W., Sung, J.-S., Cho, G.-T., Park, J.-H., Yoon, M. S., Kim, C.-K. and Baek, H.-J. (2013). Analysis of genetic diversity and population structure of 135 dill (*Anethum graveolens* L.) accessions using RAPD markers. *Genetic Resources and Crop Evolution,* **60:** 893-903.

Syamsuwida, D. (2011). Genetic variation of *Melia azedarach* in community forests of West Java assessed by RAPD. *Biodiversitas,* ***12***(2): 64-69.

Szewczuk, V. D., Mongelli, E. R. and Pomilio, A. B. (2003). Antiparasitic activity of Melia azedarach growing in Argentina. *Molecular Medicinal Chemistry,* **1:** 54-57.

Torres, E., Iriondo, J. M. and Perez, C. (2003). Genetic structure of an endangered plant, *Antirrhinum microphyllum* (Scrophulariaceae): allozyme and RAPD analysis. *American Journal of Botany,* ***90***(1): 85-92.

Varsha, K.-K., Kachhwaha, S. and Kothari, S. (2012). Characterization of genetic diversity in *Jatropha curcas* L. germplasm using RAPD and ISSR markers. *Indian Journal of Biotechnology,* ***11***(1): 54-61.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van De Lee, T., Hornes, M., Friters, A., Pot, J., Paleman, J. and Kuiper, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic acids research,* ***23***(21): 4407-4414.

Wachowiak, W., Boratyńska, K. and Cavers, S. (2013). Geographical patterns of nucleotide diversity and population differentiation in three closely related European pine species in the *Pinus mugo* complex. *Botanical Journal of the Linnean Society,* ***172***(2): 225-238.

Wagner, V., Durka, W. and Hensen, I. (2011). Increased genetic differentiation but no reduced genetic diversity in peripheral vs. central populations of a steppe grass. *American Journal of Botany,* ***98***(7): 1173-1179.

Wang, K.-J. and Li, X.-H. (2012). Genetic characterization and gene flow in different geographical distance neighbouring natural populations of wild soybean (*Glycine soja* Sieb. & Zucc.) and implications for protection from GM soybeans. *Euphytica,* ***186***(3): 817-830.

Ward, S. (2006). Genetic analysis of invasive plant populations at different spatial scales. *Biological Invasions,* ***8***(3): 541-552.

Warghat, A. R., Bajpai, P. K., Murkute, A. A., Sood, H., Chaurasia, O. P. and Srivastava, R. B. (2012). Genetic diversity and population structure of *Dactylorhiza hatagirea* (Orchidaceae) in cold desert Ladakh region of India. *Journal of Medicinal Plants Research,* ***6***(12): 2388-2395.

Wijarat, P., Keeratinijakal, V., Toojinda, T., Vanavichit, A. and Tragoonrung, S. (2012). Genetic evaluation of *Andrographis paniculata* (Burm. f.) Nees. revealed by SSR, AFLP and RAPD markers. *Journal of Medicinal Plants Research,* ***6***(14): 2777-2788.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research,* ***18***(22): 6531-6535.

Xue, X., Wang, Y., Korpelainen, H. and Li, C. (2007). Genetic diversity of *Picea asperata* populations based on RAPDs. *Plant Biology,* ***9***(1): 101-108.

Yeh, F., Yang, R. and Boyle, T. (1999). POPGENE. Microsoft Windows-Based Freeware for Population Genetic Analysis: Release 1.31. *University of Alberta, Edmonton*.

Yulianti, S. I., Wijayanto, N., Darma, I. T. and Syamsuwida, D. (2011). Genetic Variation of *Melia Azedarach* in Community Forests of West Java Assessed by RAPD. *Journal Biodiversitas,* ***12***(2): 64-69.

Zheng, D. J., Xie, L. S., Zhu, J. H. and Zhang, Z. L. (2012). Low genetic diversity and local adaptive divergence of *Dracaena cambodiana* (Liliaceae) populations associated with historical population bottlenecks and natural selection: an endangered long lived tree endemic to Hainan Island, China. *Plant biology,* ***14***(5): 828-838.

Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics,* ***20***(2): 176-183.

Zong, M., Liu, H.-L., Qiu, Y.-X., Yang, S.-Z., Zhao, M.-S. and Fu, C.-X. (2008). Genetic diversity and geographic differentiation in the threatened species *Dysosma pleiantha* in China as revealed by ISSR analysis. *Biochemical Genetics,* ***46***(3-4): 180-19

# Appendices

## APPENDIX-A

### DNA isolation protocol

DNA was isolated using the following protocol of Doyle 1990:

- 5g of plant material was homogenized in liquid nitrogen with precooled mortar and pestle.
- The powdered plant material was transferred to 25ml polypropylene centrifuge tube containing 10 ml DNA extraction buffer already kept at 65°C and the suspension was incubated at 65°C for 1 hour and cooled to room temperature after incubation.
- The suspension was mixed with an equal volume of chloroform: isoamly alcohol (24:1) for 5 minutes by gentle inversion.
- Added equal volume of Chloroform isoaml alcohol to each centrifuge tube and centrifuged at 15,000 rpm for 30 minutes.
- The upper aqueous phase was transferred to a fresh centrifuge tube with a wide bore pipette tip and mixed with 2/3 volume of isopropanol to it by gentle inversion and centrifuged at 10,000 rpm for 15 minutes.
- The DNA pellet was transferred to a fresh microcentrifuge tube and washed twice with 70% alcohol at 13,000 rpm for 5 minutes.
- The pellet was dried overnight at room temperature and then dissolved in TE buffer and kept at 4°C until use.

### DNA purification protocol

Purification of DNA is required to remove RNA, proteins and polysaccharides as they are considered as the main contaminants in DNA. RNase is used to remove RNA and proteins are removed by phenol: chloroform treatments. So to purify DNA following protocol was used as:

- 10µl RNase (1µg/µl) was added to 500µl DNA sample and incubated at 37°C for 1 hour.
- After an incubation of 1 hour equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and the micro centrifuge tube was centrifuged at 10,000 rpm for 5 minutes at room temperature. The upper aqueous was transferred to a new micro centrifuge tube.
- Aqueous phase was treated with chloroform: isoaml alcohol (24:1) and centrifuged at 10,000 rpm for 5 minutes. The aqueous phase is transferred to fresh tube and again treated with chloroform: isoamyl alcohol.
- The separated aqueous phase was treated with $1/10^{th}$ volume of 3M sodium acetate and two volumes of absolute alcohol. The DNA was pelleted by centrifuging at 13,000 rpm for 3 minutes.
- The pellet was washed twice with 70% alcohol and dried overnight. The dried DNA pellet was dissolved in TE buffer.

## Appendix-B

**Table 1.** Nei's genetic diversity analysis

| Locus | Sample size | $H_T$ | $H_S$ | $G_{ST}$ | Nm |
|---|---|---|---|---|---|
| OPA01-1 | 47 | 0.4816 | 0.2973 | 0.3827 | 0.8065 |
| OPA01-2 | 47 | 0.2434 | 0.2275 | 0.0654 | 7.1439 |
| OPA01-3 | 47 | 0.4892 | 0.3192 | 0.3475 | 0.9387 |
| OPA01-4 | 47 | 0.4348 | 0.2279 | 0.4760 | 0.5505 |
| OPA02-1 | 47 | 0.0346 | 0.0315 | 0.0896 | 5.0833 |
| OPA02-2 | 47 | 0.3009 | 0.2431 | 0.1920 | 2.1036 |
| OPA02-3 | 47 | 0.4778 | 0.3984 | 0.1662 | 2.5085 |
| OPA02-4 | 47 | 0.4213 | 0.3078 | 0.2693 | 1.3569 |
| OPA04-1 | 47 | 0.4998 | 0.4832 | 0.0332 | 14.5408 |
| OPA04-2 | 47 | 0.4997 | 0.3669 | 0.2657 | 1.3816 |
| OPA04-3 | 47 | 0.1374 | 0.1340 | 0.0243 | 20.0726 |
| OPA04-4 | 47 | 0.4983 | 0.3845 | 0.2284 | 1.6889 |
| OPA05-1 | 47 | 0.4983 | 0.3845 | 0.2284 | 1.6889 |
| OPA05-2 | 47 | 0.4678 | 0.4426 | 0.0539 | 8.7708 |
| OPA05-3 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA05-4 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA07-1 | 47 | 0.4908 | 0.1357 | 0.7235 | 0.1910 |
| OPA07-2 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA07-3 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA07-4 | 47 | 0.4350 | 0.4086 | 0.0608 | 7.7235 |
| OPA09-1 | 47 | 0.3539 | 0.2399 | 0.3220 | 1.0529 |
| OPA09-2 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA09-3 | 47 | 0.0189 | 0.0180 | 0.0481 | 9.8912 |
| OPA09-4 | 47 | 0.4591 | 0.4451 | 0.0305 | 15.8991 |
| OPA10-1 | 47 | 0.3222 | 0.2778 | 0.1377 | 3.1317 |
| OPA10-2 | 47 | 0.4848 | 0.4670 | 0.0368 | 13.0857 |
| OPA10-3 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA10-4 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPA11-1 | 47 | 0.4718 | 0.4031 | 0.1456 | 2.9331 |
| OPA11-2 | 47 | 0.0170 | 0.0162 | 11.0921 | 11.0921 |
| OPA11-3 | 47 | 0.1209 | 0.1209 | 0.0788 | 5.8451 |
| OPA11-4 | 47 | 0.1209 | 0.1209 | 0.0788 | 5.8451 |
| OPA12-1 | 47 | 0.2513 | 0.2127 | 0.1535 | 2.7578 |
| OPA12-2 | 47 | 0.1648 | 0.1648 | 0.1503 | 2.8271 |
| OPA12-3 | 47 | 0.3411 | 0.2878 | 0.1564 | 2.6977 |
| OPA12-4 | 47 | 0.0355 | 0.0342 | 0.0370 | 13.0145 |
| OPA15-1 | 47 | 0.0549 | 0.0509 | 0.0716 | 6.4787 |
| OPA15-2 | 47 | 0.4040 | 0.3733 | 0.0760 | 6.0803 |
| OPA15-3 | 47 | 0.0170 | 0.0162 | 11.0921 | 11.0921 |
| OPA15-4 | 47 | 0.0170 | 0.0162 | 0.0431 | 11.0921 |
| OPA17-1 | 47 | 0.0551 | 0.0516 | 0.0635 | 7.3795 |
| OPA17-2 | 47 | 0.0867 | 0.0867 | 0.1289 | 3.3793 |
| OPA17-3 | 47 | 0.0995 | 0.0867 | 0.1289 | 3.3793 |
| OPA17-4 | 47 | 0.1126 | 0.0969 | 0.1398 | 3.0766 |
| OPA18-1 | 47 | 0.1126 | 0.0969 | 0.1398 | 3.0766 |
| OPA18-2 | 47 | 0.1244 | 0.1244 | 0.1018 | 4.4123 |
| OPA18-3 | 47 | 0.4003 | 0.3614 | 0.0971 | 4.6470 |
| OPA18-4 | 47 | 0.1607 | 0.1478 | 0.0803 | 5.7245 |
| OPB08-1 | 47 | 0.4035 | 0.3199 | 0.2071 | 1.9146 |
| OPB08-2 | 47 | 0.4712 | 0.2398 | 0.4911 | 0.5181 |
| OPB08-3 | 47 | 0.3126 | 0.0456 | 0.8543 | 0.0853 |
| OPB08-4 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPB11-1 | 47 | 0.1251 | 0.1196 | 0.0441 | 10.8494 |
| OPB11-2 | 47 | 0.3860 | 0.2979 | 0.2281 | 1.6921 |
| OPB11-3 | 47 | 0.4838 | 0.2965 | 0.3872 | 0.7914 |

| | | | | | |
|---|---|---|---|---|---|
| OPB11-4 | 47 | 0.3495 | 0.2588 | 0.2597 | 1.4254 |
| OPB12-1 | 47 | 0.4992 | 0.2187 | 0.5618 | 0.3900 |
| OPB12-2 | 47 | 0.2988 | 0.2286 | 0.2350 | 1.6277 |
| OPB12-3 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPB15-1 | 47 | 0.4848 | 0.4070 | 0.1605 | 2.6161 |
| OPB15-2 | 47 | 0.2673 | 0.2156 | 0.1935 | 2.0835 |
| OPB15-3 | 47 | 0.2967 | 0.2691 | 0.0931 | 4.8718 |
| OPB17-1 | 47 | 0.4999 | 0.3840 | 0.2319 | 1.6561 |
| OPB17-2 | 47 | 0.4079 | 0.3894 | 0.0453 | 10.5262 |
| OPB17-3 | 47 | 0.4999 | 0.3762 | 0.2474 | 1.5211 |
| OPB18-1 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPB18-2 | 47 | 0.0170 | 0.0162 | 0.0431 | 11.0921 |
| OPB18-3 | 47 | 0.4620 | 0.3180 | 0.3117 | 1.1039 |
| OPC12-1 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPC12-2 | 47 | 0.4724 | 0.3828 | 0.1898 | 2.1344 |
| OPC12-3 | 47 | 0.2785 | 0.2223 | 0.2016 | 1.9804 |
| OPC11-1 | 47 | 0.2785 | 0.2223 | 0.2016 | 1.9804 |
| OPC11-2 | 47 | 0.0379 | 0.0364 | 0.0402 | 11.9472 |
| OPC11-3 | 47 | 0.3601 | 0.3006 | 0.1651 | 2.5285 |
| OPC18-1 | 47 | 0.0364 | 0.0364 | 0.0402 | 11.9472 |
| OPC18-2 | 47 | 0.1049 | 0.0741 | 0.2941 | 1.2000 |
| OPC18-3 | 47 | 0.2740 | 0.1461 | 0.4665 | 0.5717 |
| OPC02-1 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPC02-2 | 47 | 0.2200 | 0.1566 | 1.2345 | 1.2345 |
| OPC02-3 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPC08-1 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPC08-2 | 47 | 0.0386 | 0.0347 | 0.1004 | 4.4812 |
| OPC08-3 | 47 | 0.2263 | 0.1565 | 0.3086 | 1.1200 |
| OPC15-1 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPC15-2 | 47 | 0.4443 | 0.3254 | 0.2676 | 1.3682 |
| OPC15-3 | 47 | 0.0837 | 0.0792 | 0.0536 | 8.8346 |
| OPD02-1 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPD02-2 | 47 | 0.3834 | 0.2430 | 0.3663 | 0.8651 |
| OPD02-3 | 47 | 0.4879 | 0.2489 | 0.4900 | 0.5205 |
| OPD03-1 | 47 | 0.3693 | 0.3284 | 0.3284 | 1.0225 |
| OPD03-2 | 47 | 0.0189 | 0.0481 | 0.0481 | 9.8912 |
| OPD03-3 | 47 | 0.4994 | 0.3101 | 0.3790 | 0.8192 |
| OPD04-1 | 47 | 0.0189 | 0.0180 | 0.0481 | 9.8912 |
| OPD04-2 | 47 | 0.4378 | 0.3066 | 0.2997 | 1.1681 |
| OPD04-3 | 47 | 0.3693 | 0.2480 | 1.0225 | 1.0225 |
| OPB07-1 | 47 | 0.0386 | 0.0347 | 4.4812 | 4.4812 |
| OPB07-2 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPB07-3 | 47 | 0.4285 | 0.3312 | 0.2271 | 1.7014 |
| OPB14-1 | 47 | 0.0355 | 0.0342 | 0.0370 | 13.0145 |
| OPB14-2 | 47 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| OPB14-3 | 47 | 0.0831 | 0.0714 | 0.1410 | 3.0466 |
| OPB10-1 | 47 | 0.4751 | 0.0897 | 0.8113 | 0.1163 |
| OPB10-2 | 47 | 0.4800 | 0.2927 | 0.3902 | 0.7813 |
| OPB10-3 | 47 | 0.2228 | 0.1973 | 0.1145 | 3.8666 |
| **Mean** | 47 | 0.2381 | 0.1767 | 0.2580 | 1.4381 |

## Appendix-C

**Table 2.** Nei's unbiased measures of genetic identity and genetic distance (1978). Nei's genetic identity (above diagonal) and genetic distance (below diagonal) (POPGENE version 1.31).

| Pop ID | Bti | Han | Dab | Gng | Kes | Pdm |
|---|---|---|---|---|---|---|
| **Bti** | **** | 0.9392 | 0.9162 | 0.9024 | 0.8990 | 0.9187 |
| **Han** | 0.0627 | **** | 0.9665 | 0.9682 | 0.8671 | 0.9124 |
| **Dab** | 0.0875 | 0.0340 | **** | 0.9624 | 0.9002 | 0.9179 |
| **Gng** | 0.1027 | 0.0323 | 0.0383 | **** | 0.9279 | 0.9371 |
| **Kes** | 0.1065 | 0.1426 | 0.1052 | 0.0748 | **** | 0.9506 |
| **Pdm** | 0.0848 | 0.0917 | 0.0856 | 0.0650 | 0.0507 | **** |

Trees - Structure and Function

# Genetic diversity and population structure of Melia azedarach L. growing in Indian Thar Desert.

--Manuscript Draft--

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## Original Article

# Genetic diversity and population structure of *Melia azedarach* L. growing in Indian Thar Desert.

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**Abstract**

*Melia azedarach* L is ecologically imperative species growing in Thar Desert. Biological effects such as antiviral, anthelminthic, antibacterial, etc. makes it important, yet a little is known about its genetic diversity and structure. In this study, we employed 30 RAPD primers for DNA profiling of 91 individuals representing 14 geographically isolated populations. A total of 104 bands were scored with an average of 3.5 bands per primer. The PIC ranged from 0.1898 to 0.5000 with an average of 0.4365. Nei's genetic diversity (h) and Shannon's information index (I) ranged from 0.0112 to 0.4997 and 0.0348 to 0.6928 respectively with average Nei's genetic diversity of 0.2597. The gene flow (Nm) 0.9702 and the genetic diversity of 0.2586 at species level demonstrated overall high level of genetic diversity. Cladistics analysis using DARwin and Bayesian cluster analysis using STRUCTURE placed 91 individuals into two main clusters or original genetic stocks (K = 2) which show little or no association with the geographic origin. The lack of clear assignment of individuals to geographical regions of sampling and consideration of different populations in the same genetic cluster suggests a recent common evolutionary history.

**Key message:** *M. azedarach* is a good reforestation tree because of its fast growth and drought hardy nature. This study adds a foundation for more precise inference about the biogeography and management in the reforestation projects in the Thar Desert.

**Keywords:** Genetic diversity, Gene flow, Thar Desert, Dendrogram.

## Introduction

*Melia azedarach L*, a forest tree belongs to family Meliaceae, known as bead tree, Persian lilac, Paradise tree, Bakain, is native to north western India and China. It is grown widely in the world due to its high adaptability to a wide range of climatic conditions which naturalized it in other countries (Olmos et al. 2002; Yulianti et al. 2011). It is an important fast growing forest tree (Scocchi et al. 2004) which grows well in deep and sandy clay soils with good drainage having an alkaline pH of around 5.5-6.5. It grows at hills with elevation of 700-1400m above sea level and with a rainfall of 600-2000 mm/year and in warmer conditions (Yulianti et al. 2011). It is considered important for reforestation programms (Scocchi et al. 2004) and commercial purposes because of its good wood characteristics (Scocchi et al. 2004) and several biological effects including antifeedant (De Nardo et al. 1997), anthelminthic (Hördegen et al. 2003), antiviral (Andrei et al., 2003), antibacterial (Khan et al. 2001), and antiparasitic (Szewczuk et al. 2003) etc.

Due to restricted or limited mobility of plants, genetic structure refers to spatial structure or actual geographical distribution of plants which results from different environmental or ecological traits along with migration, dispersion, mutation, natural selection, genetic drift etc. Small, localized populations are more susceptible to genetic drift and limited gene flow as compared to widely distributed species resulting in an increased genetic diversity between populations and decreased genetic diversity within populations (Loveless et al. 1984). Limited gene flow between populations of plant species cause genetic differentiation due to increased geographic distance among populations or distant spatial pattern of populations. Alteration and fragmentation of many habitats lead to increased geographic distance which may be the cause of low level of gene flow and high genetic diversity among populations (Fischer et al. 2000).

Genetic variation in a species will allow itself to respond to environmental stress, adapt, evolve and survive for a long term. Severe environmental stress play an important role in revealing evolutionary history of forest trees (Sheng et al. 2005). Information about genetic structure of forest species of desert region not only provides information regarding significance of evolutionary forces such as gene flow, genetic drift, mutation and migration under extreme environmental conditions but also provides basic information for designing plans related to restoration and rational exploitation (Doligez et al. 1997; Fischer et al. 2000).

As many forest trees are of economic importance and their breeding is slow, so it is desirable to detect functionally important regions in the genome. Genetic diversity can be studied with a number of molecular markers, but RAPD is preferred because it is relatively easy and cheap method for detecting polymorphism within short duration and requires limited amount of DNA (Hadrys et al. 1992; Welsh et al. 1990).

In the present study RAPD technique was adopted to examine the genetic structure and diversity within and between 14 populations of *M. azedarach* in Thar desert.

## Materials and Methods

### Plant material

A total of 14 populations consisting of 91 individuals of *M. azedarach* have been collected from 14 different geographically isolated regions of Thar Desert. 6 populations were collected from Bathinda region of Punjab

and 8 populations were collected from Jodhpur, Jaisalmer and Raisinghnagar regions of Rajasthan. Within each region populations were isolated from each other at a distance of at least 50-60 Km and their size varied from 5-10 plants. Complete information regarding sample collection and location is given in Fig 1. and Table 1. Fresh leaves of each selected plant were collected and kept inside multiple layers of blotting sheets for transportation to the laboratory.

**DNA isolation and RAPD analysis**

Total DNA was extracted from leaf tissue by the CTAB method (Doyle 1990) with some modifications. The quality and concentration of the extracted DNA were estimated on 0.8% agarose gel and Nano Drop 2000 Spectrophotometer.

Genomic DNA was analysed using standard 10 mer oligonucleotide RAPD molecular markers by Williams et al method with some modifications (Williams et al. 1990). We standardized the DNA amplification conditions by assessing DNA concentration, primer concentration, different bands and concentrations of Taq polymerase as well as temperatures for PCR amplifications. Reactions were carried out in a final volume of 20ul containing 25mM $MgCl_2$ solution, 2.5mM dNTPs,10 picomoles primer, 25ng/ul of genomic DNA, 1.5 U of Taq polymerase (Banglore Genei). Amplifications were performed in a thermal cycler (Applied Biosystems Veriti 96 well) programmed for initial denaturation cycle of 5 min at 94°C followed by 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 37°C and extension for 2 min at 72°C with a final extension of 7 min at 72°C.

PCR amplified products mixed with 6X loading dye were electrophoresed on 2% agarose gel prepared in 1X TAE, stained with EtBr at 55V for 90 minutes; then visualized and photographed with the BIORAD Gel Doc X - imager.

**Data analysis**

Each fragment amplified using RAPD was treated as a binary unit character and scored 0 and 1 for absent and present bands respectively. Only intense and clearly visible bands were scored. Polymorphism information content (PIC) and percentage polymorphism were calculated for each primer which gives estimate about discrimination ability of a marker by considering number of alleles at a locus along with their relative frequencies (Table 2).

Genetic diversity was estimated using POPGENE software version 1.31 (Yeh et al. 1999) to determine different parameters which include number of polymorphic loci and their percentage (Table 3), observed number of loci, effective number of alleles ( $n_e = 1/\sum p_i^2$, where $p_i$ = frequency of the *i*th allele for the studied locus), Nei's genetic diversity ( $h= \sum h_k/r$, where $h_k$ = the value of h for the $_k$th locus and r = number of alleles studied) (Nei 1973), Shannon's information index (Table 1) and dendrograms. The phylogenetic relationship among populations was generated using software DARwin.

STRUCTURE (version 2.3) (Pritchard et al. 2000) was used to perform a Bayesian analysis to identify hidden population structure by delineating individuals into genetically distinguishable clusters on the basis of their genotypes at multiple loci. The optimal value of K was identified using both the adhoc procedure introduced by Pritchard et al (2000) (Fig 3.) and method developed by Evanno et al (2005) (Fig 4.). Population assignment test based on a Bayesian approach was conducted for all the samples using the software STRUCTURE for assigned number of populations of K=1 to 14 and with 3 replicates with a random start for each K value and 1000 runs. The number of distinct population clusters were determined using Δ K method (Evanno et al. 2005).

**Results**

Out of the 80 primers that were evaluated in 14 populations, 39 primers were polymorphic and finally 30 primers were chosen on the basis of generation of stable and reproducible bands in all the samples. A total of 104 polymorphic bands were generated with an average of 3.5 bands per primer and 0.4365 average polymorphism information content (PIC). The highest numbers of polymorphic bands were achieved with primers OPA07, OPB12, OPC15 and OPD02, while the most informative primer was OPA04 with PIC value of 0.5879 (Table 2). The number of polymorphic loci ranged from 33 (Kesrisinghpur) to 70 (New Pali road) within 14 populations with an average of 48. The percentages of polymorphic loci ranged from 31.73% (Kesrisinghpur) to 67.31% (New Pali Road) with an average of 46.22 (Table 3).

Eight parameters were used to assess the genetic variation within populations and they included number of loci or bands, number and percentage of polymorphic loci, actual or observed number of alleles (na), effective number of alleles (ne), Nei's genetic diversity (h), Shannon information index (I), expected or genetic diversity within population (intrapopulation diversity for subdivided populations). The highest number of observed alleles (na=1.6731) were present in New Pali Road population. The highest effective number of alleles (ne=1.4171), Nei's genetic diversity (h=2.375), Shannon's information index (I=0.3500) was found in Raisinghnagar population (Table 1).

Five parameters were calculated to investigate the genetic variation among populations and these included expected heterozygosity ($H_T$) or total genetic diversity for all populations, genetic differentiation between subpopulations relative to total genetic diversity ($G_{ST} = 1 – h_s/h_T$ where $h_S$= population diversity and $h_T$ = total diversity), estimate of gene flow [ Nm = 0.5(1 - Gst)/Gst ], Nei's genetic distance and Nei's genetic identity (Supplementary Figure 1). The average genetic diversity was 0.1707 within population (Hs) and 0.2568 among populations or at species level (Ht). Average estimate of $F_{ST}$ or $G_{ST}$ across all loci were significantly different from zero (average $G_{ST}$ = 0.3401) which indicated limited gene flow and genetic structuring amongst sampling locales (supplementary Table 1).

Dendrogrm was drawn by Dendrogram based on Neis's genetic distance method (UPGMA modified from Neighbor procedure of PHYLIP version 3.5) to visualize the relationships among 14 populations of *M. azedarach* L. (Figure 2). The results represented two clusters, one consisting of Gharsana and Anupgarh and the other consisting of the other 12 populations. Second cluster further consisted of two subclusters, one of which included Bathinda, Kesrisinghpur, Padampur and Jaisalmer and the other subcluster consisted of Hanumangarh, Dabbali, Ganganagar, NewPali road, Raisinghnagar, Karanpur, Mahajan and Lunkaransar.

According to Nei's unbiased measures of genetic distance (1978) data, the highest genetic distance pairs were between New Pali road and Bathinda (0.2057), New Pali road and Gharsana (0.1938), new Pali road and Kesrisinghpur (0.1923), New Pali road and Anupgarh (0.1749) and New Pali road and Jaisalmer (0.1664). Therefore, it indicates New Pali road population to be the most differentiated of all the populations. Data of Nei's (1978) Unbiased measures of genetic identity validates the data of genetic distance and the highest genetic identity pairs were between Hanumangarh and Ganganagar (0.9682), Hanumangarh and Dabbali (0.9665), Ganganagar and Dabbali (0.9624), Ganganagar and Raisinghpur (0.9608) and Hanumangarh and Raisinghpur (0.9604). Therefore it indicates that Hanumangarh, Ganganagar, Dabbali and Raisinghpur are highly similar on genetic level. Hence, these populations were showing strong relationships between them (Supplementary Figure 1). Unrooted tree constructed using DARwin determines phylogenetic relationship between the individuals on the basis of genetic distance and indicated two clusters of original genetic stocks and one cluster consisting of individuals migrated from the two original genetic stocks (Fig 6.).

STRUCTURE version 2.3 based on Bayesian approach was applied to search for hidden population structure among genotypes of different populations of *M. azedarach.* The relative distinctiveness of sampled populations was determined by assigning individuals on the basis of genetically homogeneous groups rather than on geographical locations of sampling. The highest value of ΔK identified 2 clusters or genetic stocks that were present among 14 sampled populations. This grouping is consistent with UPGMA dendrogram. Graphical method used to detect true number of populations i.e. K indicated mostly the number of genetic stocks at K=2 considering ΔK distribution (Fig 4.). Out of 91 individuals, 47 individuals were observed in the admixed stock and 40 and 4 individuals were in the two original genetic stocks (Fig. 3). Of the 91individuals sampled, first smaller genetic cluster occupied 4.39% (4 individuals from 3 populations), second larger genetic cluster occupied 43.95% (40 individuals from 13 populations) and the admixed cluster occupied 51.65% (47 individuals from 13 populations) (Fig 5.).

**Discussion**

Genetic diversity, a basic source of biodiversity is the total number of traits leading to variation within or among populations of a species (Poczai et al.). Maintenance of genetic diversity and population distinctiveness plays an important role in biodiversity and conservation of species as it helps to understand the processes or factors involved in genetic variation in populations and species (Nongrum et al. 2012).

RAPD has been used to detect genetic diversity within and between populations of *Populus euphratica* (Saito et al. 2002), *Vitellaria paradoxa* (Fontaine et al. 2004), *Gliricidia sepium* (Dawson et al. 1995), *Mangifera indica* (Díaz-Matallana et al. 2009) and many others. High values of percentage polymorphism for all the primers indicated that the main cause for high genetic diversity may be geographic isolation which regulates the process of genetic diversity and variation. High level of genetic diversity due to geographic isolation in *Anethum graveolens* populations and RAPD was used to reveal its genetic structure (Suresh et al. 2013). Population wise highest value of percentage of polymorphic loci is of New Pali road population (67.31%) which is geographically distant from other populations and dendrogram agrees with this data. This suggested that New Pali road population was the most distant and differentiated population. Present investigation using RAPD marker revealed that in *M. azedarach* highest genetic variation was observed among populations ($H_T$ = 0.2586)

as compared to within population ($H_S$ = 0.1707). Till now investigations regarding genetic diversity revealed low levels of genetic diversity within populations in case of populations of geographically restricted plant species but high levels of genetic diversity within population was observed in case of out-crossing species (Torres et al. 2003; Wagner et al. 2011).When genetic diversity is classified into within and among population genetic diversities, selfing species exhibit low levels of genetic diversity within population, but a considerable high genetic diversity among populations (He et al. 2007).

Genetic diversity among the populations and geographic range are highly related (Warghat et al. 2012). Several factors like geographical isolation, population fragmentation, breeding system and genetic drifts may be responsible for high population differentiation (Hogbin et al. 1999; Zong et al. 2008). $F_{ST}$ values ranging from 0 to 0.05 represent little or very little genetic diversity, from 0.05 to 0.15 represent considerable genetic diversity and that >0.25 represent very high genetic diversity. The results of average $F_{ST}$ (0.3401) indicated very high genetic diversity among populations. If populations are small and isolated from one another, the genetic drift could be capable of influencing the genetic structure and increasing differentiation among populations (Ellstrand et al. 1993). High genetic diversity indicated limited gene flow among *M. azedarach* populations which agrees with estimate of gene flow (Nm) obtained with POPGENE (version 1.31). Nm represents historical average levels of gene flow. A $G_{ST}$ derived average genetic flow (Average Nm = 0.9702) revealed that gene flow between populations of *M. azedarach* is restricted and it is below the level (Nm>4) required to counteract genetic drift (i.e. Nm>4 according to stepping stone model) (Slatkin 1993). But in some population pairs Nm value revealed high gene flow.

If Nm>1 (in an infinite island model) or Nm>4 (in a stepping stone model), the gene flow is quite sufficient to create genetic differentiation between populations balanced for migration and genetic drift. But according to infinite island model, if 0.5>Nm>1, genetic differentiation among populations is small but considerable in case of stepping stone model (Díaz-Matallana et al. 2009). In the present study average Nm = 0.9702 which indicates limited gene flow among populations and agrees with the average $F_{ST}$ value (0.3401) because $F_{ST}$ is inversely related to Nm, the effective migration rate. A high $F_{ST}$ indicates that populations are genetically differentiated the result of a low migration of genotypes.

Maximum gene flow was found between population pairs Hanumangarh and Ganganagar (Nm = 5.9542) followed by Ganganagar and Raisinghnagar (Nm = 5.3863), Hanumangarh and Raisinghnagar (Nm = 5.2081), Hanumangarh and Dabbali (4.4825) and Dabbali and Ganaganagar (Nm = 4.2580) (Table 4). All the other population pairs had Nm values <4 which indicates low gene flow (according to stepping stone model). These values agree with results of Nei's unbiased genetic identity and distance. Some of these populations are geographically closer and some are quite distant. This indicates that geographically closer populations undergo high gene flow when migrant genes arriving by pollen or seed or human interference become established in new genets (Ayres et al. 1999). Pollen dispersal by wind and insects between populations may be affected by environmental conditions, geographic distances between populations and topographic conditions (Islam et al. 2012). In the present study high gene flow was observed between populations which were geographically distant from each other. This may be due to human interference. Long distance seed dispersal by humans has been observed as a source of gene flow among populations of Italian wild Cherry (*Prunus avium* L.) (De Rogatis et al. 2013).

Clustering of different populations of *M. azedarach* in dendrogram based on Nei's genetic distance was not completely in congruent with their geographic locations and so it indicates that genetic diversity is not corroborating with geographical diversity. The data strongly suggests that overall genetic diversity is high and gene flow is limited but in some population pairs $G_{ST}$ value is very low that is low genetic diversity and high Nm value (Nm>4) that is high gene flow. Overall high genetic diversity may be due to geographic isolation, habitat fragmentation or mating system but high gene flow in some population pairs may be due to human interference, pollen or seed dispersal etc.

Cluster analysis in *M. azedarach* showed that genetic diversity based on morphological traits was not in accordance to geographical regions (Solouki et al. 2008b). There was no clear clustering pattern of geographically closer individuals. Same results were obtained in the case of *Anethum graveolens L.* (Suresh et al. 2013). These observations clearly indicated that the association between genetic similarity and geographical distance was less significant. These genetic differences among populations may be due to selection, adaptation, migration, genetic drift and method of pollination. The other factor of importance is also vitally related to the environment and human interference (Solouki et al. 2008a). Dendrogram and cluster analysis showed that some landraces with same geographical area were clustered into different groups and no preferred relationship existed between geographical distribution and the dendrogram. Investigations related to cluster analysis have found clusters including trees from different populations which indicated that plants can be more similar to those from other populations than to those of their own population in case of *Ilex paraguariensis* (Gauer et al. 2000).

Both of the original genetic stocks consisted of individuals of populations from different geographical locations i.e. the two genetic clusters identified by STRUCTURE were not according to the geographical locations of sampling. This represented that a high level of gene flow has occurred. Structure infers the highest likelihood of both the individual clusters and the admixture of genotypes using allele frequency and linkage disequilibrium information from dataset directly. The Bayesian structure analysis, dendrogram and unrooted tree (Fig 6.) did not group. 14 *M. azedarach* populations may have originated from two ancestral gene pools and the presence of admixed cluster or stock suggested the occurrence of gene flow. None of the clusters determined by STRUCTURE either original or the admixed one consisted of specified full populations which indicated high gene flow.

Gene flow at the species level was limited due to wide geographical distribution or restricted seed and pollen dispersal. Gene flow and life history traits such as population size change, habitat fragmentation, bottlenecks etc. have been considered as the main determinants of population genetic structure. Therefore different populations considered in the same genetic clusters, suggests recent common ancestry to be the important factor rather than other events leading to gene flow.

**Conclusion**

This study has added significant knowledge and understanding to the genetic diversity and structure of *M. Azedarach* between and within the populations. Presence of high level of genetic diversity within and among the populations, Nei's genetic diversity, Shannon's information index and total gene diversity resulted in insignificant correlation between geographic distance and genetic diversity. The lack of clear assignment of

individuals to geographical regions of sampling and consideration of different populations in the same genetic cluster suggests a recent common evolutionary history and recent divergence.

**Authors' contributions**

NP, KA and GS collected samples and isolated DNA. NP and KA carried out DNA profiling and statistical analysis. RGS helped in drafting the manuscript. PB conceived the study, participated in designing, coordination, data analysis, interpretation, reviewed and improved the manuscript. All authors have read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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**References:**

Ayres DR,Ryan FJ (1999) Genetic diversity and structure of the narrow endemic Wyethia reticulata and its congener W. bolanderi (Asteraceae) using RAPD and allozyme techniques. Am J Bot 86(3):344-353

Dawson I, Simons A, Waugh R,Powell W (1995) Diversity and genetic differentiation among subpopulations of Gliricidia sepium revealed by PCR-based assays. Heredity 74(1):10-18

De Nardo E, Costa A,Lourencao A (1997) Melia azedarach extract as an antifeedant to Bemisia tabaci (Homoptera: Aleyrodidae). Fla Entomol 80(1):92-94

De Rogatis A, Ferrazzini D, Ducci F, Guerri S, Carnevale S,Belletti P (2013) Genetic variation in Italian wild cherry (Prunus avium L.) as characterized by nSSR markers. Forestry 86(3):391-400

Díaz-Matallana M, Schuler-García I, Ruiz-García M,Hodson de Jaramillo E (2009) Analysis of diversity among six populations of Colombian mango (Mangifera indica L. cvar. Hilacha) using RAPDs markers. Electron J Biotechnol 12(3):1-2

Doligez A,Joly HI (1997) Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, Carapa procera (Meliaceae), in French Guiana. Heredity 79(1):72-82

Doyle JJ (1990) Isolation of plant DNA from fresh tissue. Focus 12:13-15

Ellstrand NC,Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annual review of Ecology and Systematics:217-242

Evanno G, Regnaut S,Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14(8):2611-2620

Fischer M, Husi R, Prati D, Peintinger M, van Kleunen M,Schmid B (2000) RAPD variation among and within small and large populations of the rare clonal plant Ranunculus reptans (Ranunculaceae). Am J Bot 87(8):1128-1137

Fontaine C, Lovett P, Sanou H, Maley J,Bouvet J (2004) Genetic diversity of the shea tree (Vitellaria paradoxa CF Gaertn), detected by RAPD and chloroplast microsatellite markers. Heredity 93(6):639-648

Gauer L,Cavalli-Molina S (2000) Genetic variation in natural populations of maté (Ilex paraguariensis A. St.-Hil., Aquifoliaceae) using RAPD markers. Heredity 84(6):647-656
Hadrys H, Balick M,Schierwater B (1992) Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol Ecol 1(1):55-63
He F, Kang D, Ren Y, Qu L, Zhen Y,Gu H (2007) Genetic diversity of the natural populations of Arabidopsis thaliana in China. Heredity 99(4):423-431
Hogbin PM,Peakall R (1999) Evaluation of the contribution of genetic research to the management of the endangered plant Zieria prostrata. Conservation Biology 13(3):514-522
Hördegen P, Hertzberg H, Heilmann J, Langhans W,Maurer V (2003) The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. Vet Parasitol 117(1):51-60
Islam MS, Lian C, Kameyama N,Hogetsu T (2012) Analyses of genetic population structure of two ecologically important mangrove tree species, Bruguiera gymnorrhiza and Kandelia obovata from different river basins of Iriomote Island of the Ryukyu Archipelago, Japan. Tree Genet Genom 8(6):1247-1260
Khan M, Kihara M,Omoloso A (2001) Antimicrobial activity of Horsfieldia helwigii and Melia azedarach. Fitoterapia 72(4):423-427
Loveless M,Hamrick J (1984) Ecological determinants of genetic structure in plant populations. Annu Rev Ecol Syst 15:65-95
Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci 70(12):3321-3323
Nongrum I, Kumar S, Kumaria S,Tandon P (2012) Genetic variation and gene flow estimation of Nepenthes khasiana Hook. F-A threatened insectivorous plant of India as revealed by RAPD markers. J Crop Sci Biotech 15(2):101-105
Olmos S, Lavia G, Di Renzo M, Mroginski L,Echenique V (2002) Genetic analysis of variation in micropropagated plants of Melia azedarach L. In Vitro Cellular & Developmental Biology-Plant 38(6):617-622
Poczai P, Varga I, Bell NE,Hyvönen J. Genomics Meets Biodiversity: Advances in Molecular Marker Development and Their Applications in Plant Genetic Diversity Assessment. In Mahmut Çalışkan (Ed.), (InTech Helsinki 3-32
Pritchard JK, Stephens M,Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155(2):945-959
Saito Y, Shiraishi S, Tanimoto T, Yin L, Watanabe S,Ide Y (2002) Genetic diversity of Populus euphratica populations in northwestern China determined by RAPD DNA analysis. New forests 23(2):97-103
Scocchi A, Faloci M, Medina R, Olmos S,Mroginski L (2004) Plant recovery of cryopreserved apical meristem-tips of Melia azedarach L. using encapsulation/dehydration and assessment of their genetic stability. Euphytica 135(1):29-38
Sheng Y, Zheng W, Pei K,Ma K (2005) Genetic variation within and among populations of a dominant desert tree Haloxylon ammodendron (Amaranthaceae) in China. Ann Bot 96(2):245-252
Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. Evolution:264-279
Solouki M, Mehdikhani H, Zeinali H,Emamjomeh A (2008a) Study of genetic diversity in Chamomile ( Matricaria chamomilla) based on morphological traits and molecular markers. Scientia Horticulturae 117(3):281-287
Solouki M, Mehdikhani H, Zeinali H,Emamjomeh A (2008b) Study of genetic diversity in Chamomile (Matricaria chamomilla) based on morphological traits and molecular markers. Sci Hortic 117(3):281-287
Suresh S, Chung J-W, Sung J-S *et al* (2013) Analysis of genetic diversity and population structure of 135 dill (Anethum graveolens L.) accessions using RAPD markers. Genet Resour Crop Evol:1-11
Szewczuk VD, Mongelli ER,Pomilio AB (2003) Antiparasitic activity of Melia azedarach growing in Argentina. Mol Med Chem 1:54-57
Torres E, Iriondo JM,Pérez C (2003) Genetic structure of an endangered plant, Antirrhinum microphyllum (Scrophulariaceae): allozyme and RAPD analysis. Am J Bot 90(1):85-92
Wagner V, Durka W,Hensen I (2011) Increased genetic differentiation but no reduced genetic diversity in peripheral vs. central populations of a steppe grass. Am J Bot 98(7):1173-1179
Warghat AR, Bajpai PK, Murkute AA, Sood H, Chaurasia OP,Srivastava RB (2012) Genetic diversity and population structure of Dactylorhiza hatagirea (Orchidaceae) in cold desert Ladakh region of India. J Med Plants Res 6:2388-2395
Welsh J,McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18(24):7213-7218
Williams JG, Kubelik AR, Livak KJ, Rafalski JA,Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18(22):6531-6535

Yeh F, Yang R,Boyle T (1999) POPGENE. Microsoft Windows-Based Freeware for Population Genetic Analysis: Release 1.31. University of Alberta, Edmonton

Yulianti SI, Wijayanto N, Darma IT,Syamsuwida D (2011) Genetic Variation of Melia Azedarach in Community Forests of West Java Assessed by RAPD. J Biodiversitas 12(2)

Zong M, Liu H-L, Qiu Y-X, Yang S-Z, Zhao M-S,Fu C-X (2008) Genetic diversity and geographic differentiation in the threatened species Dysosma pleiantha in China as revealed by ISSR analysis. Biochemical genetics 46(3-4):180-196

Figure

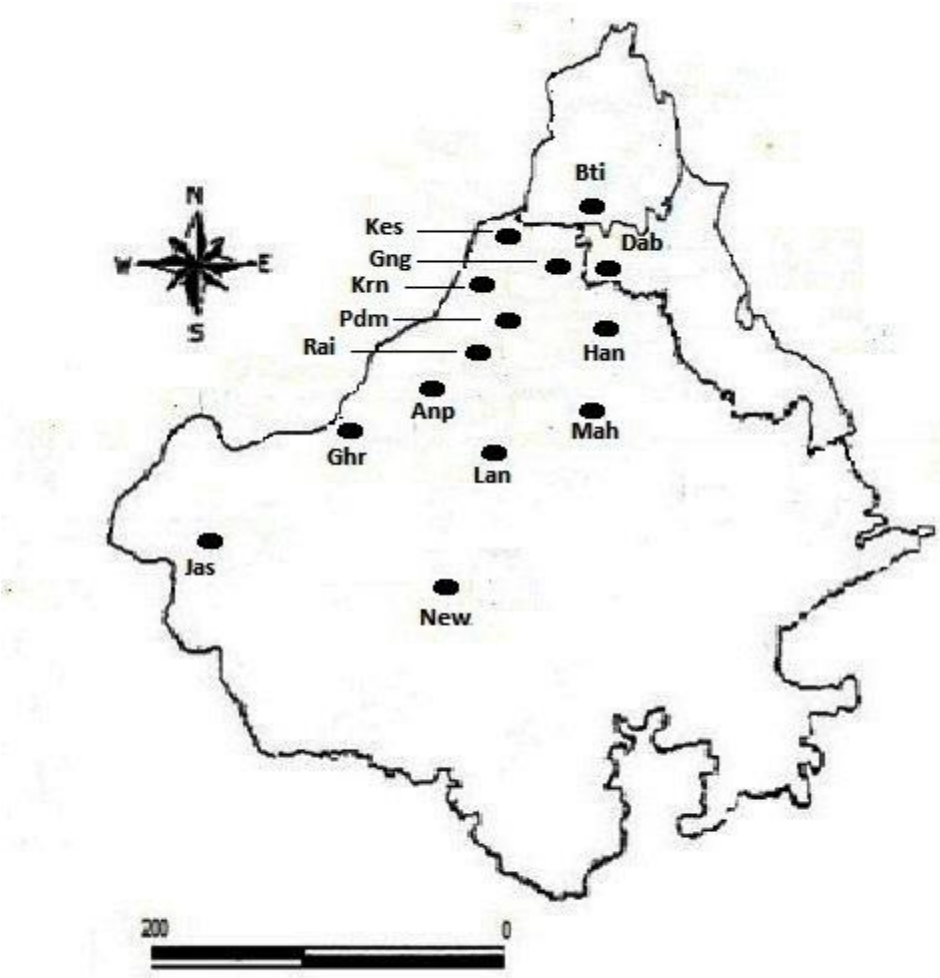


**Fig 1:** Sites of sample collection in North-western part (Thar Desert) of India.

Figure

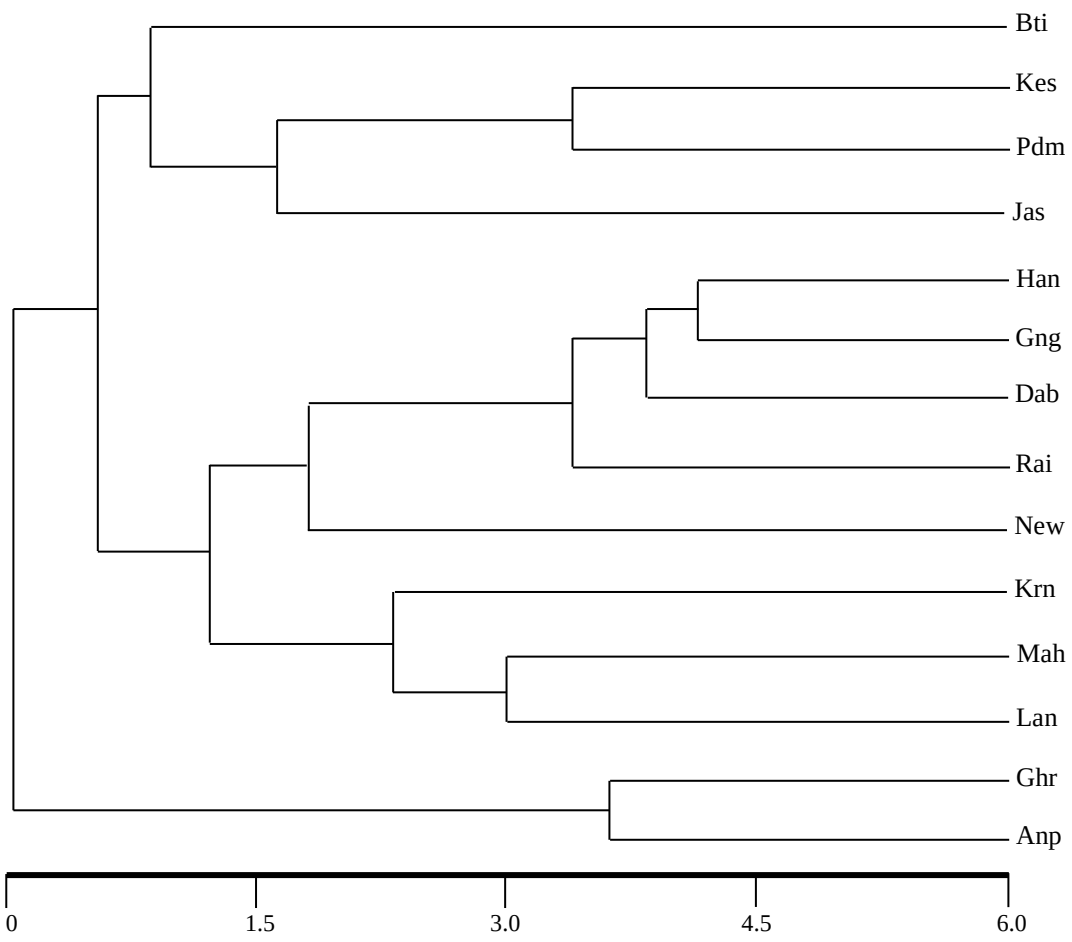


**Fig 2.** Dendrogram based on Nei's (1978) genetic distance for 14 popuations of *Melia azedarach L*.

Figure

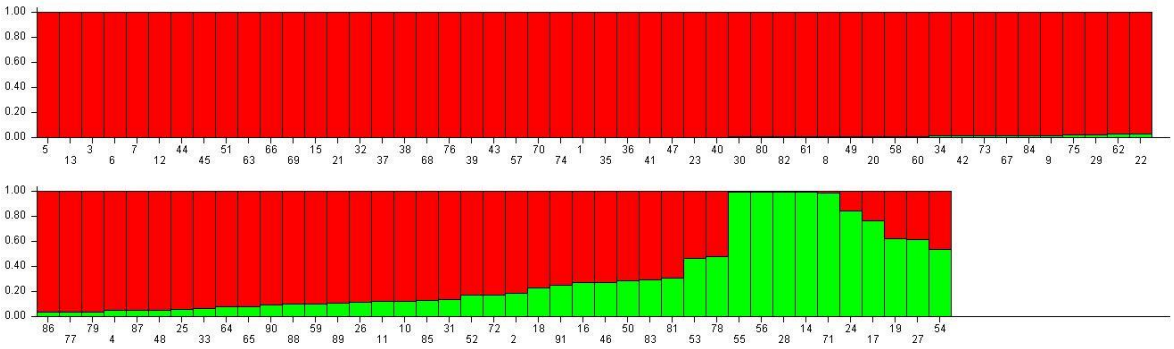


**Fig 3.** Structure plot with membership coefficient of individuals on y-axis. Assignment of all 91 individuals into two clusters. Membership coefficient of an individual for a subgroup represents fraction of its genome that has ancestry in subgroup. Two clusters inferred by Structure in combined clustering of all individuals. Each colour represents different genetic stock.

Figure

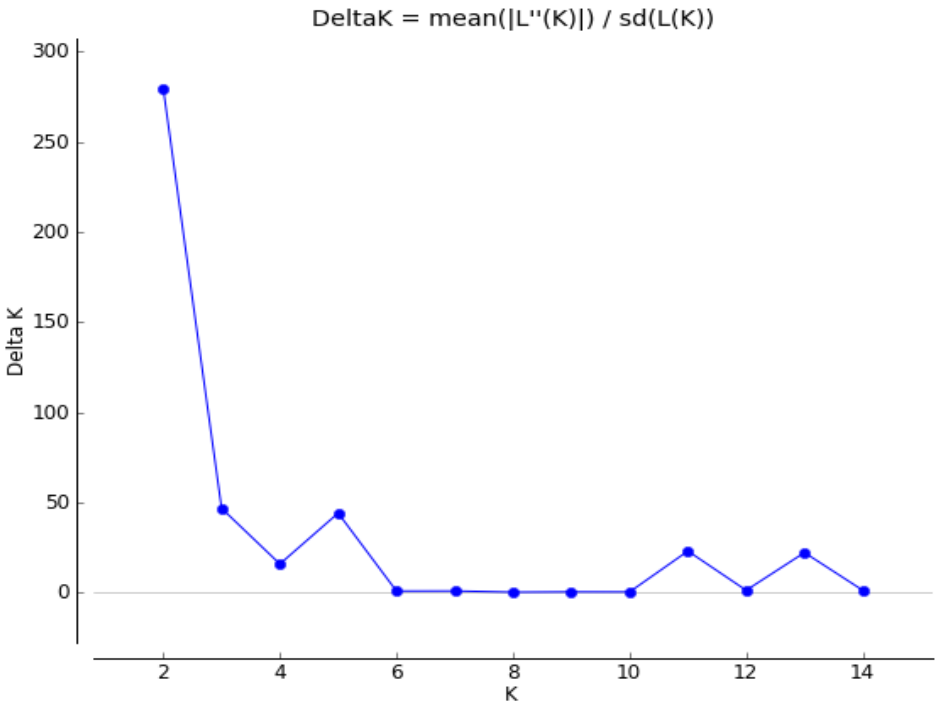


**Fig 4.** Most appropriate value of K from a range of 1-14 populations using the second order statistics (Δ K) given by Evanno et al. (2005)

Figure

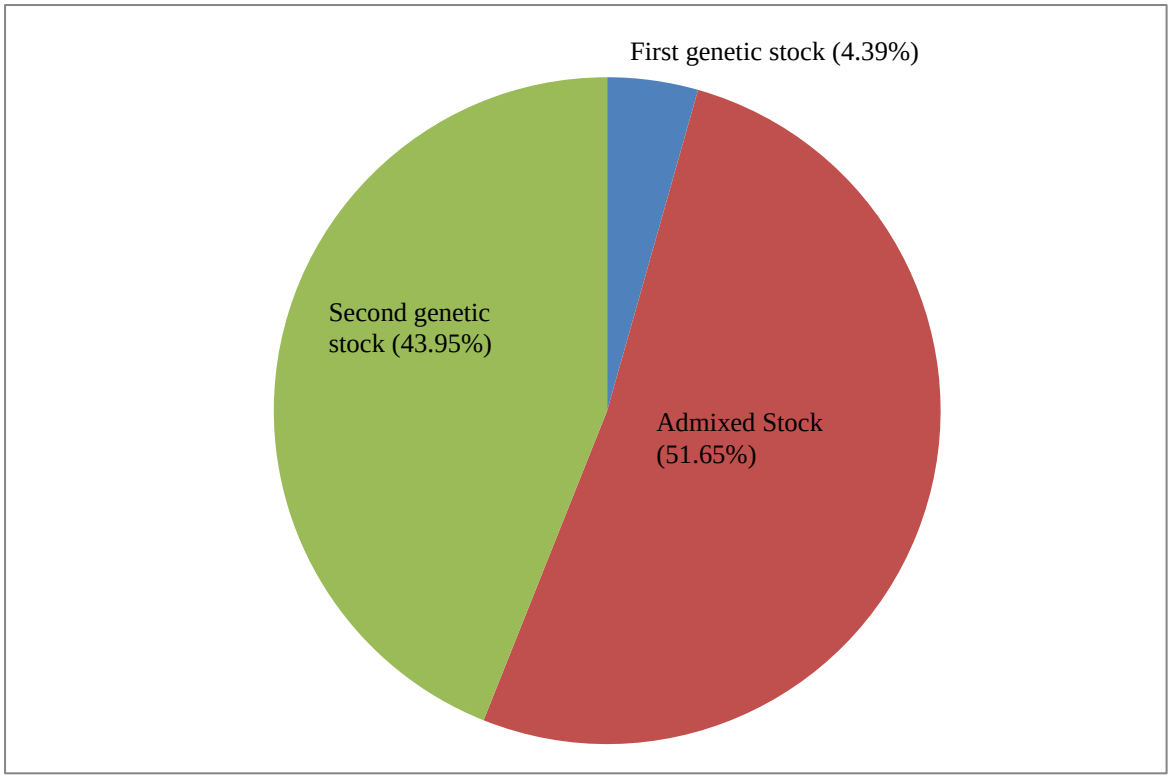


**Fig. 5.** Pie chart representing two original and one admixed genetic stock revealed by software STRUCTURE version 2.3.

Figure

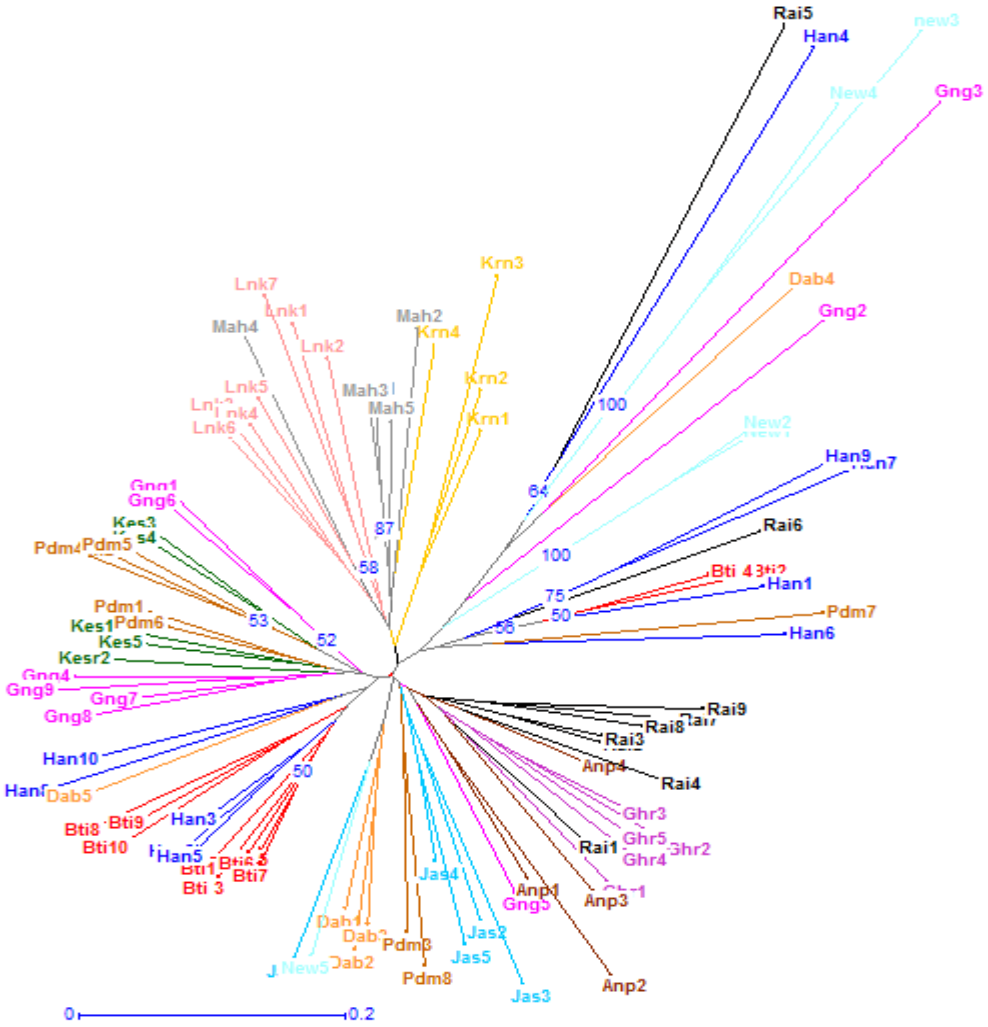


**Fig 6.** Unrooted tree construted by DARwin. Each coloured branch represents one individual collected from corresponding inferred population.

Table

**Table1:** *Melia azedarach* populations from Thar Desert and their genetic variability detected by RAPD analysis.

| Population | Sample Size | Longitude (E) | Latitude (N) | Na | Ne | h | I |
|---|---|---|---|---|---|---|---|
| Bathinda (Bti) | 10 | 74°47’29.52”E | 30°08’26.96”N | 1.4519 | 1.2788 | 0.1584 | 0.2356 |
| Hanumangarh (Ham) | 10 | 74°17’32.04”E | 29°36’48.33”N | 1.5962 | 1.3621 | 0.2094 | 0.3119 |
| Dabbali (Dab) | 5 | 74°38’46.09”E | 29°55’28.72”N | 1.3942 | 1.2984 | 0.1631 | 0.2358 |
| Ganga nagar (Gng) | 9 | 73°51’32.55”E | 29°55’12.69”N | 1.6346 | 1.4047 | 0.2286 | 0.3384 |
| Kesrisinghpur (Kes) | 5 | 73°37’16.18”E | 29°56’51.68”N | 1.3173 | 1.1934 | 0.1125 | 0.1684 |
| Padampur (Pdm) | 8 | 73°37’34.83”E | 29°42’25.86”N | 1.5096 | 1.3318 | 0.1881 | 0.2779 |
| Jaisalmer (Jas) | 5 | 70°21’48.26”E | 26°52’23.50”N | 1.4135 | 1.3021 | 0.1680 | 0.2443 |
| New Pali Road (New) | 5 | 72°59’57.12”E | 26°13’17.32”N | 1.6731 | 1.3609 | 0.2222 | 0.3398 |
| Gharsana (Ghr) | 5 | 73°04’43.72”E | 29°01’18.57”N | 1.3462 | 1.2158 | 0.1237 | 0.1845 |
| Anupgarh (Anp) | 4 | 73°12’34.18”E | 29°11’21.07”N | 1.3558 | 1.2757 | 0.1489 | 0.2148 |
| Raisinghnagar (Rai) | 9 | 73°26’56.75”E | 29°32’09.01”N | 1.6346 | 1.4171 | 0.2375 | 0.3500 |
| Karanpur (Krn) | 4 | 76°58’14.85”E | 26°10’55.44”N | 1.3750 | 1.2479 | 0.1405 | 0.2079 |
| Mahajan (Mah) | 5 | 73°50’12.67”E | 28°47’16.13”N | 1.3462 | 1.2479 | 0.1384 | 0.2019 |
| Lunkaransar (Lnk) | 7 | 73°45’18.23”E | 28°30’01.75”N | 1.4231 | 1.2533 | 0.1499 | 0.2247 |

na = Observed number of alleles

ne = Effective number of alleles

h = Nei’s gene diversity

I = Shannon’s Information index

Table

**Table 2:** Data of RAPD primers used in the present study and the extent of polymorphism.

| S.No. | Primer Name | Primer Sequence(5'-3') | Amplified Bands | Polymorphic Bands | % Polymorphism | PIC |
|---|---|---|---|---|---|---|
| 1. | OPA 01 | CAGGCCCTTC | 1 | 1 | 100 | 0.4680 |
| 2. | OPA 02 | TGCCGAGCTG | 4 | 4 | 100 | 0.4882 |
| 3. | OPA 04 | AATCGGGCTG | 4 | 4 | 100 | 0.5000 |
| 4. | OPA 05 | AGGGGTCTTG | 4 | 4 | 100 | 0.4996 |
| 5. | OPA 07 | GAAACGGGTG | 5 | 5 | 100 | 0.4082 |
| 6. | OPA 09 | GGGTAACGCC | 4 | 4 | 100 | 0.4652 |
| 7. | OPA 10 | GTGATCGCAG | 4 | 4 | 100 | 0.4933 |
| 8. | OPA 11 | CAATCGCCGT | 4 | 4 | 100 | 0.2753 |
| 9. | OPA 12 | TCGGCGATAG | 3 | 3 | 100 | 0.4232 |
| 10. | OPA 15 | TTCCGAACCC | 4 | 4 | 100 | 0.2789 |
| 11. | OPA 17 | GACCGCTTGT | 3 | 3 | 100 | 0.2898 |
| 12. | OPA 18 | AGGTGACCGT | 3 | 3 | 100 | 0.1898 |
| 13. | OPB 07 | GGTGACGCAG | 3 | 3 | 100 | 0.4419 |
| 14. | OPB 08 | GTCCACACGG | 4 | 4 | 100 | 0.2615 |
| 15. | OPB 10 | CTGCTGGGAC | 2 | 2 | 100 | 0.4990 |
| 16. | OPB 11 | GTAGACCCGT | 2 | 2 | 100 | 0.4970 |
| 17. | OPB 12 | CCTTGACGCA | 5 | 5 | 100 | 0.4374 |
| 18. | OPB 14 | TCCGCTCTGG | 3 | 3 | 100 | 0.4994 |
| 19. | OPB 15 | GGAGGGTGTT | 2 | 2 | 100 | 0.4707 |
| 20. | OPB 17 | AGGGAACGAG | 3 | 3 | 100 | 0.4991 |
| 21. | OPB 18 | CCACAGCAGT | 4 | 4 | 100 | 0.4815 |
| 22. | OPC 02 | GTGAGGCGTC | 3 | 3 | 100 | 0.4394 |
| 23. | OPC 08 | TGGACCGGTG | 3 | 3 | 100 | 0.4602 |
| 24. | OPC 11 | AAAGCTGCGG | 2 | 2 | 100 | 0.4864 |
| 25. | OPC 12 | TGTCATCCCC | 3 | 3 | 100 | 0.4875 |
| 26. | OPC 15 | GACGGATCAG | 5 | 5 | 100 | 0.3666 |
| 27. | OPC 18 | CACACTCCAG | 4 | 4 | 100 | 0.4623 |
| 28. | OPD 02 | GGACCCAACC | 5 | 5 | 100 | 0.4585 |
| 29. | OPD 03 | GTCGCCGTCA | 4 | 4 | 100 | 0.5000 |
| 30. | OPD 04 | TCTGGTGAGG | 4 | 4 | 100 | 0.4815 |

PIC = Polymorphism Information Content

Table

**Table 3:** Different populations with Number of polymorphic loci and percentage polymorphic loci.

| **Population** | **Number of polymorphic loci** | **Percentage of polymorphic loci** |
|---|---|---|
| Bathinda | 47 | 45.19 % |
| Hanumangarh | 62 | 59.62 % |
| Dabbali | 41 | 39.42 % |
| Ganga nagar | 66 | 63.46 % |
| Kesrisinghpur | 33 | 31.73 % |
| Padampur | 53 | 50.96 % |
| Jaisalmer | 43 | 41.35 % |
| New Pali Road | 70 | 67.31 % |
| Gharsana | 36 | 34.62 % |
| Anupgarh | 37 | 35.58 % |
| Raisinghnagar | 66 | 63.46 % |
| Karanpur | 39 | 37.50 % |
| Mahajan | 36 | 34.62 % |
| lunkaransar | 44 | 42.31 % |

Table

**Table 4.** Populations with maximum gene flow.

| Population pairs | $H_T$ | $H_S$ | $G_{ST}$ | Nm |
|---|---|---|---|---|
| Hanumangarh & Ganganagar | 0.2374 | 0.2190 | 0.0775 | 5.9542 |
| Ganganagar & Raisinghnagar | 0.2546 | 0.2330 | 0.0849 | 5.3863 |
| Hanumangarh & Raisinghnagar | 0.2449 | 0.2234 | 0.0876 | 5.2081 |
| Hanumangarh & Dabbali | 0.2070 | 0.1862 | 0.1004 | 4.4825 |
| Dabbali & Ganganagar | 0.2188 | 0.1958 | 0.1051 | 4.2580 |

$H_T$ = Total genetic diversity

$H_S$ = Subpopulation genetic diversity

$G_{ST}$ = Genetic differentiation between subpopulations relative to the genetic diversity of the total population.

Nm = Estimate of gene flow from $G_{ST}$

| | Bathinda | Hanumangarh | Dabbali | Ganga nagar | Kesrisinghpur | Padampur | Jaisalmer | New Pali Road | Gharsana | Anupgarh | Raisinghnagar | Karanpur | Mahajan | Lunkaransar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bathinda | **** | 0.9392 | 0.9162 | 0.9024 | 0.8990 | 0.9187 | 0.8764 | 0.8141 | 0.8861 | 0.8901 | 0.9181 | 0.8691 | 0.8751 | 0.8864 |
| Hnumangarh | 0.0627 | **** | 0.9665 | 0.9682 | 0.8671 | 0.9124 | 0.8993 | 0.9249 | 0.8962 | 0.8919 | 0.9604 | 0.9057 | 0.8798 | 0.9119 |
| Dabbali | 0.0875 | 0.0340 | **** | 0.9624 | 0.9002 | 0.9179 | 0.9042 | 0.9011 | 0.9023 | 0.9161 | 0.9477 | 0.8941 | 0.9126 | 0.8999 |
| Ganga nagar | 0.1027 | 0.0323 | 0.0383 | **** | 0.9279 | 0.9371 | 0.9075 | 0.9486 | 0.8884 | 0.9007 | 0.9608 | 0.9314 | 0.9217 | 0.9252 |
| Kesrisinghpur | 0.1065 | 0.1426 | 0.1052 | 0.0748 | **** | 0.9506 | 0.9042 | 0.8251 | 0.8607 | 0.8693 | 0.8897 | 0.8934 | 0.9061 | 0.8744 |
| Padampur | 0.0848 | 0.0917 | 0.0856 | 0.0650 | 0.0507 | **** | 0.9201 | 0.8634 | 0.8926 | 0.9080 | 0.9414 | 0.9045 | 0.9064 | 0.9090 |
| Jaisalmer | 0.1320 | 0.1062 | 0.1007 | 0.0970 | 0.1007 | 0.0833 | **** | 0.8467 | 0.8763 | 0.9061 | 0.9143 | 0.8903 | 0.8981 | 0.8939 |
| New Pali Road | 0.2057 | 0.0781 | 0.1042 | 0.0527 | 0.1923 | 0.1469 | 0.1664 | **** | 0.8238 | 0.8395 | 0.9152 | 0.9092 | 0.8780 | 0.8903 |
| Gharsana | 0.1209 | 0.1096 | 0.1028 | 0.1184 | 0.1500 | 0.1136 | 0.1321 | 0.1938 | **** | 0.9584 | 0.9369 | 0.8773 | 0.8561 | 0.8604 |
| Anupgarh | 0.1164 | 0.1144 | 0.0877 | 0.1046 | 0.1401 | 0.0965 | 0.0986 | 0.1749 | 0.0424 | **** | 0.9437 | 0.8697 | 0.8832 | 0.8711 |
| Raisinghnagar | 0.0855 | 0.0404 | 0.0537 | 0.0400 | 0.1169 | 0.0604 | 0.0896 | 0.0886 | 0.0652 | 0.0579 | **** | 0.9205 | 0.9164 | 0.9272 |
| Karanpur | 0.1403 | 0.0991 | 0.1119 | 0.0711 | 0.1128 | 0.1004 | 0.1162 | 0.0952 | 0.1309 | 0.1396 | 0.0828 | **** | 0.9343 | 0.9305 |
| Mahajan | 0.1334 | 0.1281 | 0.0914 | 0.0815 | 0.0986 | 0.0982 | 0.1074 | 0.1301 | 0.1554 | 0.1242 | 0.0874 | 0.0680 | **** | 0.9465 |
| Lunkaransar | 0.1206 | 0.0922 | 0.1055 | 0.0778 | 0.1342 | 0.0954 | 0.1121 | 0.1162 | 0.1504 | 0.1380 | 0.0756 | 0.0721 | 0.0550 | **** |

**Figure 1.**Nei's unbiased measures of genetic identity and genetic distance (1978). Nei's genetic identity (above diagonal) and genetic distance (below diagonal). (POPGENE version 1.31).

**Table 1.** Nei's Genetic Variation Analysis

| Locus | Sample Size | $H_t$ | $H_s$ | Gst | Nm |
|---|---|---|---|---|---|
| OPA01-1 | 91 | 0.5000 | 0.3276 | 0.3448 | 0.9499 |
| OPA01-2 | 91 | 0.3031 | 0.1855 | 0.3879 | 0.7890 |
| OPA01-3 | 91 | 0.4989 | 0.3040 | 0.3906 | 0.7801 |
| OPA01-4 | 91 | 0.4897 | 0.2547 | 0.4800 | 0.5418 |
| OPA02-1 | 91 | 0.1019 | 0.0918 | 0.0993 | 4.5344 |
| OPA02-2 | 91 | 0.2145 | 0.1721 | 0.1978 | 2.0282 |
| OPA02-3 | 91 | 0.4807 | 0.3601 | 0.2509 | 1.4929 |
| OPA02-4 | 91 | 0.4479 | 0.3317 | 0.2594 | 1.4272 |
| OPA04-1 | 91 | 0.4952 | 0.4038 | 0.1846 | 2.2084 |
| OPA04-2 | 91 | 0.4821 | 0.3826 | 0.2063 | 1.9234 |
| OPA04-3 | 91 | 0.1734 | 0.1512 | 0.1278 | 3.4130 |
| OPA04-4 | 91 | 0.4872 | 0.3247 | 0.3335 | 0.9991 |
| OPA05-1 | 91 | 0.4877 | 0.3298 | 0.3238 | 1.0439 |
| OPA05-2 | 91 | 0.5000 | 0.3359 | 0.3282 | 1.0235 |
| OPA05-3 | 91 | 0.4969 | 0.0920 | 0.8148 | 0.1136 |
| OPA05-4 | 91 | 0.0317 | 0.0249 | 0.2127 | 1.8504 |
| OPA07-1 | 91 | 0.3484 | 0.0935 | 0.7317 | 0.1833 |
| OPA07-2 | 91 | 0.0317 | 0.0249 | 0.2127 | 1.8504 |
| OPA07-3 | 91 | 0.1702 | 0.1409 | 0.1719 | 2.4086 |
| OPA07-4 | 91 | 0.4800 | 0.3191 | 0.3352 | 0.9918 |
| OPA09-1 | 91 | 0.4954 | 0.2637 | 0.4677 | 0.5691 |
| OPA09-2 | 91 | 0.0500 | 0.0415 | 0.1700 | 2.4409 |
| OPA09-3 | 91 | 0.0230 | 0.0212 | 0.0779 | 5.9225 |
| OPA09-4 | 91 | 0.4999 | 0.3851 | 0.2297 | 1.6772 |
| OPA10-1 | 91 | 0.3019 | 0.2463 | 0.1841 | 2.2160 |
| OPA10-2 | 91 | 0.4935 | 0.3645 | 0.2613 | 1.4137 |
| OPA10-3 | 91 | 0.0081 | 0.0077 | 0.0533 | 8.8767 |
| OPA10-4 | 91 | 0.0150 | 0.0135 | 0.0988 | 4.5619 |
| OPA11-1 | 91 | 0.4918 | 0.3922 | 0.2024 | 1.9698 |
| OPA11-2 | 91 | 0.0261 | 0.0235 | 0.0992 | 4.5424 |
| OPA11-3 | 91 | 0.2145 | 0.1744 | 0.1870 | 2.1745 |
| OPA11-4 | 91 | 0.2683 | 0.1994 | 0.2566 | 1.4486 |
| OPA12-1 | 91 | 0.4585 | 0.1830 | 0.6009 | 0.3320 |
| OPA12-2 | 91 | 0.1721 | 0.1523 | 0.1151 | 3.8427 |
| OPA12-3 | 91 | 0.3375 | 0.2857 | 0.1533 | 2.7611 |
| OPA12-4 | 91 | 0.0485 | 0.0447 | 0.0776 | 5.9412 |
| OPA15-1 | 91 | 0.0847 | 0.0717 | 0.1530 | 2.7670 |
| OPA15-2 | 91 | 0.4303 | 0.3268 | 0.2405 | 1.5786 |
| OPA15-3 | 91 | 0.0073 | 0.0070 | 0.0478 | 9.9544 |
| OPA15-4 | 91 | 0.0531 | 0.0454 | 0.1456 | 2.9338 |
| OPA17-1 | 91 | 0.0403 | 0.0370 | 0.0826 | 5.5513 |
| OPA17-2 | 91 | 0.1168 | 0.1071 | 0.0833 | 5.4991 |
| OPA17-3 | 91 | 0.1478 | 0.1244 | 0.1582 | 2.6604 |
| OPA17-4 | 91 | 0.0956 | 0.0851 | 0.1099 | 4.0486 |
| OPA18-1 | 91 | 0.0500 | 0.0415 | 0.1700 | 2.4409 |
| OPA18-2 | 91 | 0.2930 | 0.1455 | 0.5034 | 0.4933 |
| OPA18-3 | 91 | 0.3273 | 0.2831 | 0.1351 | 3.2019 |
| OPA18-4 | 91 | 0.1431 | 0.1068 | 0.2539 | 1.4691 |
| OPB08-1 | 91 | 0.4716 | 0.3178 | 0.3261 | 1.0333 |
| OPB08-2 | 91 | 0.4997 | 0.3488 | 0.3020 | 1.1558 |
| OPB08-3 | 91 | 0.1524 | 0.0195 | 0.8719 | 0.0735 |
| OPB08-4 | 91 | 0.0317 | 0.0249 | 0.2127 | 1.8504 |
| OPB11-1 | 91 | 0.1517 | 0.1306 | 0.1394 | 3.0878 |
| OPB11-2 | 91 | 0.4940 | 0.2651 | 0.4635 | 0.5789 |

| | | | | | |
|---|---|---|---|---|---|
| OPB11-3 | 91 | 0.4491 | 0.2357 | 0.4752 | 0.5521 |
| OPB11-4 | 91 | 0.2473 | 0.1894 | 0.2340 | 1.6367 |
| OPB12-1 | 91 | 0.5000 | 0.2628 | 0.4743 | 0.5542 |
| OPB12-2 | 91 | 0.3949 | 0.2121 | 0.4628 | 0.5803 |
| OPB12-3 | 91 | 0.0105 | 0.0098 | 0.0692 | 6.7204 |
| OPB15-1 | 91 | 0.4986 | 0.4002 | 0.1973 | 2.0341 |
| OPB15-2 | 91 | 0.1909 | 0.1553 | 0.1865 | 2.1813 |
| OPB15-3 | 91 | 0.1559 | 0.1288 | 0.1736 | 2.3809 |
| OPB17-1 | 91 | 0.4865 | 0.3229 | 0.3364 | 0.9864 |
| OPB17-2 | 91 | 0.4724 | 0.3830 | 0.1894 | 2.1403 |
| OPB17-3 | 91 | 0.4808 | 0.2756 | 0.4269 | 0.6713 |
| OPB18-1 | 91 | 0.0317 | 0.0249 | 0.2127 | 1.8504 |
| OPB18-2 | 91 | 0.0073 | 0.0070 | 0.0478 | 9.9544 |
| OPB18-3 | 91 | 0.4075 | 0.2226 | 0.4538 | 0.6019 |
| OPC12-1 | 91 | 0.0150 | 0.0135 | 0.0988 | 4.5619 |
| OPC12-2 | 91 | 0.4765 | 0.3607 | 0.2431 | 1.5568 |
| OPC12-3 | 91 | 0.3996 | 0.2452 | 0.3865 | 0.7937 |
| OPC11-1 | 91 | 0.3874 | 0.2473 | 0.3617 | 0.8823 |
| OPC11-2 | 91 | 0.0244 | 0.0233 | 0.0464 | 10.2715 |
| OPC11-3 | 91 | 0.2694 | 0.1963 | 0.2714 | 1.3425 |
| OPC18-1 | 91 | 0.0164 | 0.0156 | 0.0509 | 9.3323 |
| OPC18-2 | 91 | 0.1465 | 0.0988 | 0.3254 | 1.0367 |
| OPC18-3 | 91 | 0.1704 | 0.0944 | 0.4460 | 0.6212 |
| OPC02-1 | 91 | 0.0317 | 0.0249 | 0.2127 | 1.8504 |
| OPC02-2 | 91 | 0.2636 | 0.2157 | 0.1819 | 2.2490 |
| OPC02-3 | 91 | 0.1050 | 0.0603 | 0.4261 | 0.6736 |
| OPC08-1 | 91 | 0.0317 | 0.0249 | 0.2127 | 1.8504 |
| OPC08-2 | 91 | 0.0167 | 0.0149 | 0.1106 | 4.0216 |
| OPC08-3 | 91 | 0.3536 | 0.2009 | 0.4317 | 0.6582 |
| OPC15-1 | 91 | 0.0230 | 0.0212 | 0.0779 | 5.9225 |
| OPC15-2 | 91 | 0.4639 | 0.2863 | 0.3829 | 0.8059 |
| OPC15-3 | 91 | 0.0670 | 0.0623 | 0.0706 | 6.5808 |
| OPD02-1 | 91 | 0.0317 | 0.0249 | 0.2127 | 1.8504 |
| OPD02-2 | 91 | 0.3280 | 0.2027 | 0.3822 | 0.8082 |
| OPD02-3 | 91 | 0.4840 | 0.2723 | 0.4374 | 0.6431 |
| OPD03-1 | 91 | 0.4141 | 0.2757 | 0.3342 | 0.9961 |
| OPD03-2 | 91 | 0.0230 | 0.0212 | 0.0779 | 5.9225 |
| OPD03-3 | 91 | 0.4996 | 0.1896 | 0.6204 | 0.3059 |
| OPD04-1 | 91 | 0.0081 | 0.0077 | 0.0533 | 8.8767 |
| OPD04-2 | 91 | 0.4838 | 0.2844 | 0.4122 | 0.7130 |
| OPD04-3 | 91 | 0.4061 | 0.2558 | 0.3702 | 0.8506 |
| OPB07-1 | 91 | 0.0167 | 0.0149 | 0.1106 | 4.0216 |
| OPB07-2 | 91 | 0.1487 | 0.1022 | 0.3128 | 1.0983 |
| OPB07-3 | 91 | 0.4320 | 0.2918 | 0.3246 | 1.0405 |
| OPB14-1 | 91 | 0.0154 | 0.0147 | 0.0470 | 10.1322 |
| OPB14-2 | 91 | 0.0618 | 0.0353 | 0.4290 | 0.6656 |
| OPB14-3 | 91 | 0.0366 | 0.0306 | 0.1627 | 2.5728 |
| OPB10-1 | 91 | 0.4578 | 0.2266 | 0.5050 | 0.4902 |
| OPB10-2 | 91 | 0.4873 | 0.3120 | 0.3597 | 0.8902 |
| OPB10-3 | 91 | 0.2496 | 0.2120 | 0.1506 | 2.8198 |
| Mean | 91 | 0.2586 | 0.1707 | 0.3401 | 0.9702 |

**$H_T$** = Total genetic diversity

**$H_S$** = Subpopulation genetic diversity

**$G_{ST}$** = Genetic differentiation between subpopulations relative to the genetic diversity of the total population.

**Nm** = Estimate of gene flow from $G_{ST}$