

Genetic Diversity Analysis of *Salvadora Oleoides* Decne: An Evergreen Dry Land Fruit Tree of Rajasthan, India

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Abstract

Genetic diversity of *Salvadora oleoides* Decne is analyzed by cumulative data of 10 Random Amplified Polymorphic DNA (RAPD), 10 Inter Simple Sequence Repeats (ISSR) and 7 Intron Splice Junction (ISJ) markers. The plant is an evergreen fruit tree and well distributed in semi-arid and sub-humid climatic conditions of Rajasthan, India. RAPD, ISSR and ISJ primers accounted for 84.4%, 85.3%, 85.9% polymorphism. Average 0.23 PIC is accounted for RAPD, ISSR and ISJ primers. The genetic similarity ranged between 0.42-0.89. Analysis of molecular variance (AMOVA) revealed higher variation (73%) at intra-population than inter-population (27%) level. Genetic distances based on Un-weighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram and Principal Coordinate Analysis (PCoA) is correlated with physical distances or climatic conditions of *Salvadora oleoides* Decne in a semi-arid and sub-humid environment of Rajasthan. The present investigation may help in the understanding of gene flow systems between physical distances and environmental heterogeneity of the populations for better management of *Salvadora oleoides* Decne in the region.

1. Introduction

Plant resources are the major source of traditional food supplements, medicines, fuels and fodder worldwide and the genetic structure of the plant is a valuable asset for characterization and conservation of these species (Mantel et. al., 2005). Understanding of the genetic composition can be a positive feature for assessment of mating at inter and intra-population level and the prediction of genetic makeup in the future generation. Genetic diversity of a population provides a pool for genomic flexibility of a species in changing climatic conditions for adaptation. Higher genetic variability increase adaptive flexibility of a species in ever-changing climatic conditions and habitat disturbances (Kimatu, 2012). In-breeding systems are characterized by genetic variation among the population whereas the out-breeding system is characterized by the genetic diversity within the population (Hamrick, 1990). The plants of Indian arid and semi-arid regions needed characterization, conservation, propagation for sustainable utilization and maintenance of the ecosystem (Dagla et. al., 2014). *Salvadora oleoides* Decne belongs to family Salvadoraceae and locally known as “Jaal”. It is an important tree species of arid environment and distributed in India, Asia and African deserts (Singh, 1991). The fruits (Peeloo) of *Salvadora oleoides* Decne are edible and leaves possess analgesic, anti-inflammatory and anti-ulcer activities. They are used for the treatment of fever, enlarged spleen and to relieve cough (Anonymous, 1972).

Molecular markers are valuable tools for population genetics to reveal different types of variations at a genetic level (Ebrahimi et. al., 2012). It also helps in understanding the affinities between and within populations of a species in a geographical area. The development and effective utilization of molecular markers for detection and identification of genetic variation proved noteworthy in the exploration of genetic diversity, population analyses, and gene mapping in plants. The molecular markers show polymorphic differences which make it possible to identify genetic differences between the individual organism(s) or species which is consistent and reliable since the markers are not affected by pleiotropic, epistatic, or environmental factors (Agarwal et. al., 2008). Molecular marker-based genetic diversity

analysis has been used successfully for many tree species like *Capparis decidua* (Vyas et. al., 2009), *Prosopis cineraria* (Sharma et. al., 2010; 2011), *Sapindus trifoliatus* (Mahar et. al., 2013), *Morinda tomentosa* (Arya et. al., 2014), *Pithecellobium dulce* (Goyal et. al., 2015) and *Monoon tirunelveliense* (Viswanathan et. al., 2015). Yadav et. al., (2014) reported genetic diversity analysis in *Salvadora oleoides* Decne using RAPD marker. Phulwaria et. al., (2014) analysed genetic stability on micro-propagated *Salvadora oleoides* Decne using RAPD and ISSR molecular markers. Usually, more than one type of molecular markers is considered best for estimation of genome-wide variability in a species. The present investigation was aimed at standardization of RAPD, ISSR and ISJ molecular markers for genetic diversity analysis of *Salvadora oleoides* Decne from semi-arid and sub-humid environments of Rajasthan, India. These markers are the best choice to study intraspecific variation among different accessions of *S. oleoides* due to low cost, high speed, greater efficiency and most importantly they do not require any pre-sequence information for designing primers (Muhammad et. al., 2017). Molecular marker-based DNA analysis will help in the understanding of gene flow systems within and between the population of different geographical locations and climatic conditions. Relationship between physical distances and climatic conditions on the genetic makeup of *Salvadora oleoides* Decne was established in present investigation using RAPD, ISSR and ISJ markers. The quantification of genetic variability among the genotypes of *S. oleoides* and the analysis pattern of relatedness and gene flow within the species will be crucial in determining levels of species integrity, diversity and subdivision, and were, the extent of genetic variation within the population is influenced by a large number of biotic and anthropogenic habitat heterogeneity (Nybom et. al., 2014). Thus, the genetic diversity data may help in future breeding programs and conservation of genetic resources of *Salvadora oleoides* Decne in the region.

2. Materials And Methods

2.1. Plant material

Juvenile leaves of *Salvadora oleoides* Decne were collected from semi-arid (Jodhpur, Lohawat, Pachpadra) and sub-humid (Pali, Jalore) environment of Rajasthan, India (Fig. 1, Table 1). Leaves were preserved in liquid nitrogen during collection and stored at -20°C.

2.2. Genomic DNA extraction

The preserved leaves were cleaned with 70% alcohol and powdered in liquid nitrogen. The DNA was extracted by Murray and Thompson (1980) method with some minor modifications. Quality and quantity of DNA were confirmed by appropriate methods viz. gel electrophoresis with 0.85% (w/v) agarose (Merk-GeNei, India) in 1X TAE buffer and UV-spectrophotometer respectively.

2.3. Primers screening and DNA amplification

Initially, 60 RAPD primers of OPC, OPH and OPX series (Eurofins Genomics India Pvt. Ltd., Bangalore, India), 20 ISSR and 26 ISJ primers (Sigma Aldrich Chemicals Pvt. Ltd., India) were screened for amplification. The DNA amplification was carried out in thermal cycler (BIO-RAD T100™ Berkeley,

California, USA) in 25 µl reaction mixture containing 50 ng genomic DNA, 2 µl of primer (10 µM/µl), 4 µl of dNTP's mix (2 mM/µl) (GeNei™, Bangalore, India), 2.5 µl of 10X assay buffer (10 mM Tris HCl, pH 8.0 with 1.5 mM MgCl₂), 1U of Taq DNA polymerase (3 U/µl) (Genei™, Bangalore, India) and double distilled water for selected primers. Reaction mixture contents were subjected to thermal cycle at 94 °C for 5 min followed by 35 cycles at 94 °C for 1 min, annealing at 37 °C (RAPD), 45 to 55 °C (ISSR and ISJ) for 1 min and at 72 °C for 1min 30 sec, with a final extension at 72 °C for 7 min for a polymerase chain reaction. The amplified DNA was resolved on 1.5% (w/v) agarose in 1X TAE buffer with EtBr (1%) at 100 V for 1.30 hr. Gel images were analysed by BIO-RAD Gel Doc™ XR + system (Fig. 3).

2.4. Data analysis

Data were scored based on the presence (1) or absence (0) of the amplified bands. Pair-wise similarity matrix between populations was generated using the Jaccard coefficient (Jaccard, 1901) by the DARwin program, ver. 5.0.158 (Perrier et. al., 2003; Perrier and Jacquemoud, 2006). This similarity matrix was used for the construction of UPGMA dendrograms using the DARwin program ver. 5.0.158. The principal coordinate analysis (PCoA) and analyses of molecular variance (AMOVA) were carried out by GenAlEx (Ver. 6.501) program (Peakall and Smouse, 2012).

3. Results

3.1. Primer screening and genetic diversity analysis

Among 106 primers (60 RAPD, 20 ISSR and 26 ISJ) used for preliminary screening, a total of 27 primers (10 RAPD, 10 ISSR and 7 ISJ) were selected based on reproducible distinct bands (Fig. 2a,b,c). RAPD, ISSR and ISJ primers accounted for 84.4%, 85.3%, 85.9% polymorphism respectively. Average 0.23 PIC is accounted for RAPD, ISSR and ISJ primers (Table 2). The genetic similarity ranged between 0.42–0.89. Populations of *Salvadora oleoides* Decne are distributed mainly in two major clusters in UPGMA dendrogram (Fig. 3). Closely related samples are joined at a node and separated in branches due to genetic dissimilarity. Individual samples both in UPGMA dendrogram (Fig. 4) and PCoA (Fig. 5) form a similar pattern of cluster and coordinates as in case of population dendrogram. Seventy-three (73%) and 27% molecular variance (AMOVA) were observed respectively at intrapopulation and inter populations levels.

4. Discussion

Genetic diversity of insect and air pollinated species known to be influenced by the physical distance between and within the population. A heritable epigenetic variation in plants due to environmental conditions has been known (Zhang et. al., 2009; Kimatu and Liu, 2010). Relationship between genetic diversity and inbreeding have been reported by Schoen and Brown (1991), Hamrick and Godt (1996). Intra-population polymorphism studies help in the understanding of adaptive modifications of species during temporal and spatial self-maintenance (Tikhonova, 2009). Genetic similarity and dissimilarity

based on UPGMA dendrogram (Fig. 3, 4) and PCoA (Fig. 5) are closely related with physical distances and climatic conditions (Fig. 1) of *Salvadora oleoides* Decne in the present investigation. Jodhpur, Lohawat and Pachpadra populations of the semi-arid region form a major cluster and Pali, Jalore of sub-humid climatic conditions form another major cluster. Physical distance may influence cross-pollination either through insect or wind within and between populations in a region. Zhang et. al., (2009) correlated the gene flow frequencies between the population pairs and the geographical distances of *Leymus chinensis*. The gene flow change either through seed dispersal or pollen transfers in cross-pollination can impose a significant impact on intra and inter-population genome variations.

A close relationship between genetic diversity and inbreeding system has also been reported by Schoen and Brown (1991), Hamrick and Godt (1996). Effect of physical distances and climatic conditions on genetic variability of *Salvadora persica* Linn of Rajasthan, India has also been reported by Upendra et. al., (2017). Harish et. al., (2014) reported separate lineages of *Commiphora wightii* in dry and humid climatic conditions of Jaisalmer and Barmer regions respectively of Indian Thar Desert. Heritable epigenetic variations in plants of abiotic stress conditions have also been observed by Sharma et. al., (2010) in *Prosopis cineraria* and Haque et. al., (2010) in *Commiphora wightii*. Analysis of molecular variance (AMOVA) revealed higher variation (73%) at intrapopulation than inter populations (27%) levels. Inter population genetic variation > 50% considered as inbreeding species by Bussell (1999). High per cent molecular variance within the population was also observed in *Sapindus trifoliatu*s L. (Mahar et. al., 2013) and *Morinda tomentosa* Heyne (Arya et. al., 2014). Tikhonova (2009) demonstrated that intra-population polymorphism studies are more informative for the understanding of adaptive modifications of species for temporal and spatial self-maintenance. Based on present investigations it can be concluded that the genetic similarity and dissimilarity among and within populations of *Salvadora oleoides* Decne is influenced by physical distances and climatic conditions and is evidenced in UPGMA dendrogram, PCoA and AMOVA of genetic analysis. The present study may help in the understanding of gene flow systems between physical distances of the populations and environmental heterogeneity for better breeding programs and genetic conservation of *Salvadora oleoides* Decne in the region.

5. Conclusion

Cumulative data of RAPD, ISSR and ISJ markers are more appropriate for genetic diversity assessment of *Salvadora oleoides* Decne. Genetic similarity and dissimilarity of *Salvadora oleoides* Decne populations are correlated with physical distances and climatic conditions of the region in present investigations. A comparison of the level of polymorphism and discrimination efficacy of RAPD, ISSR and ISJ showed that the three markers are capable of detecting genetic variability and gene flow in *Salvadora oleoides* Decne and have collectively provided a comprehensive description of nature and the extent of variation that exist in the 25 genotypes used in the present study.

Declarations

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Author contribution

JMU designed, performed experiments, contributed to data interpretation and prepared the manuscript; SN helped in data interpretation; SRR, and HRD planned, supervised research and edited the final draft of the manuscript.

Conflict of interest

The authors contributed equally and declare that they have no conflict of interest.

Consent to Participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Due to technical limitations, table 1 & 2 is only available as a download in the Supplemental Files section.

Figures

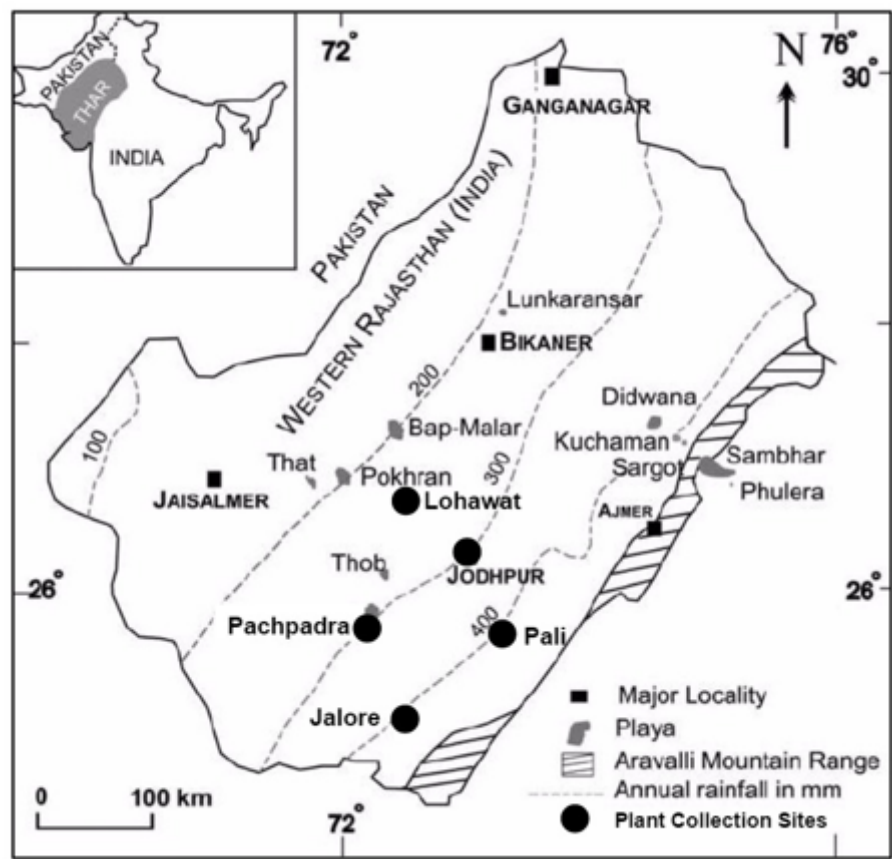


Figure 1

Collection sites of *Salvadora oleoides* Decne populations from semi-arid and sub-humid climatic conditions of Rajasthan, India. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

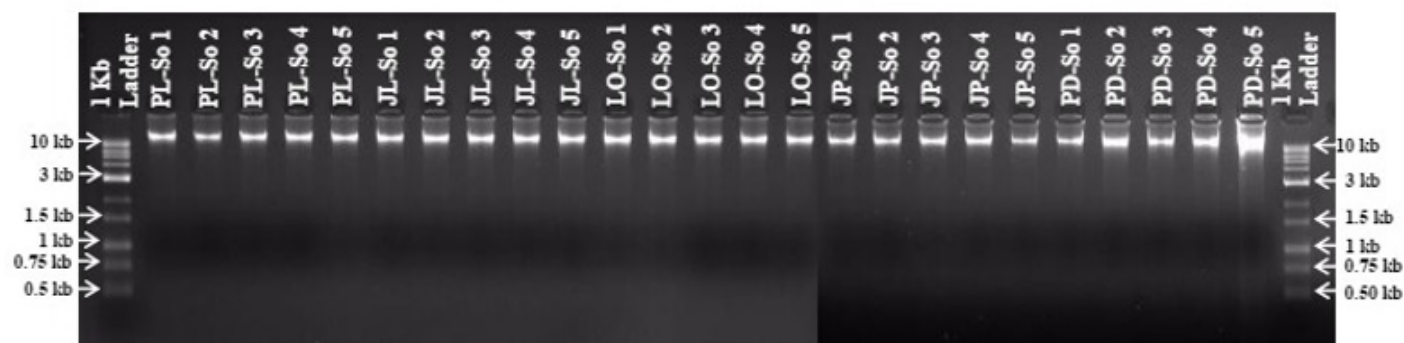


Figure 2

Gel profile for genomic DNA of *Salvadora oleoides* from semi-arid and sub-humid climatic conditions of Rajasthan, India.

1 Kb Ladder; PLSo = 01-05; JLSo = 01-05; LOSo = 01-05; JPSo = 01-05; PDSo = 01-05; 1 Kb Ladder

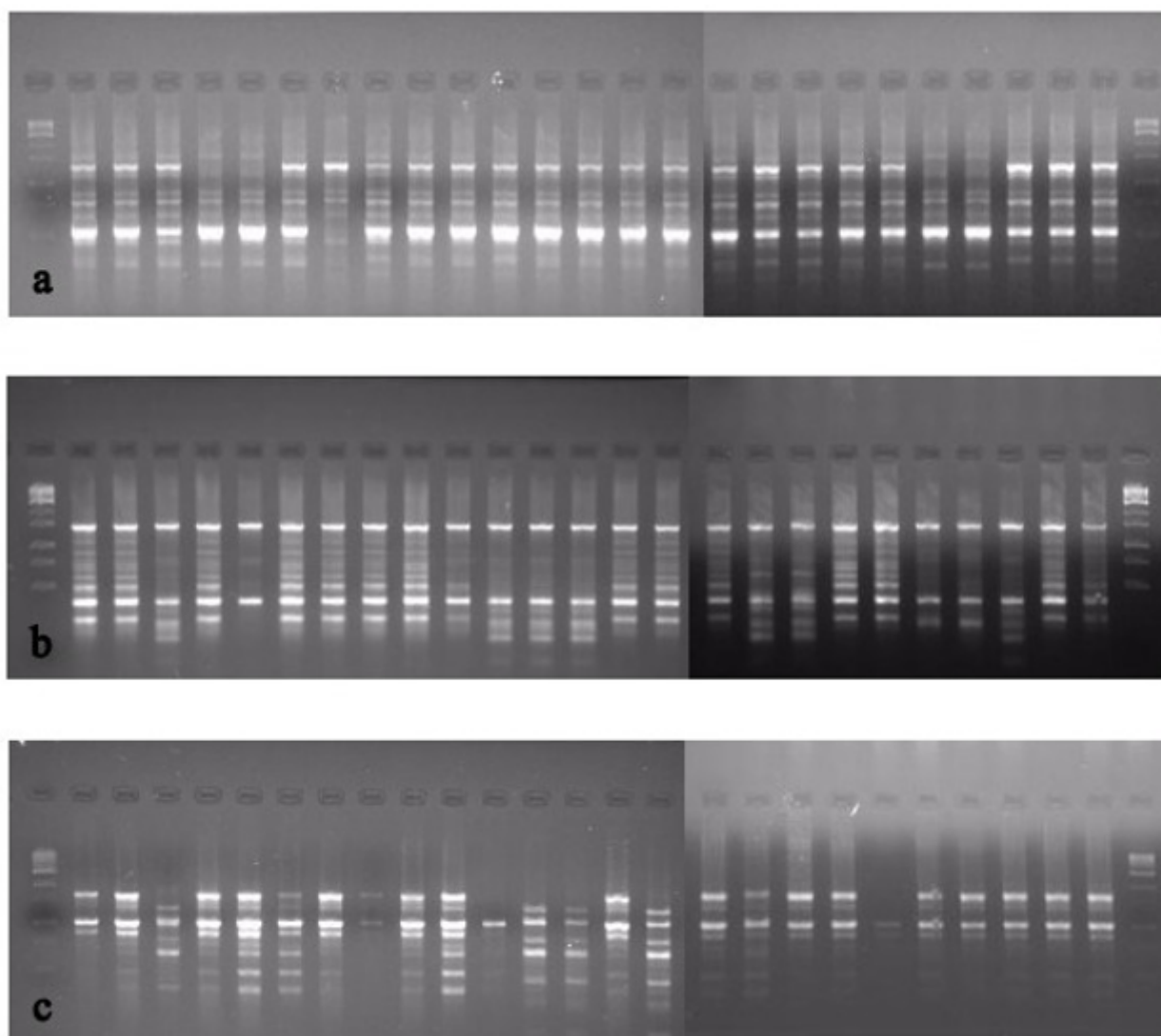


Figure 3

Gel profiles of (a) RAPD (OPC-6), (b) ISSR (840) and (c) ISJ (H-10) primers, from *Salvadora oleoides* Decne populations collected from semi-arid and sub-humid climatic conditions of Rajasthan, India. The size of the amplified products varied from 100 to 3,000 bp.

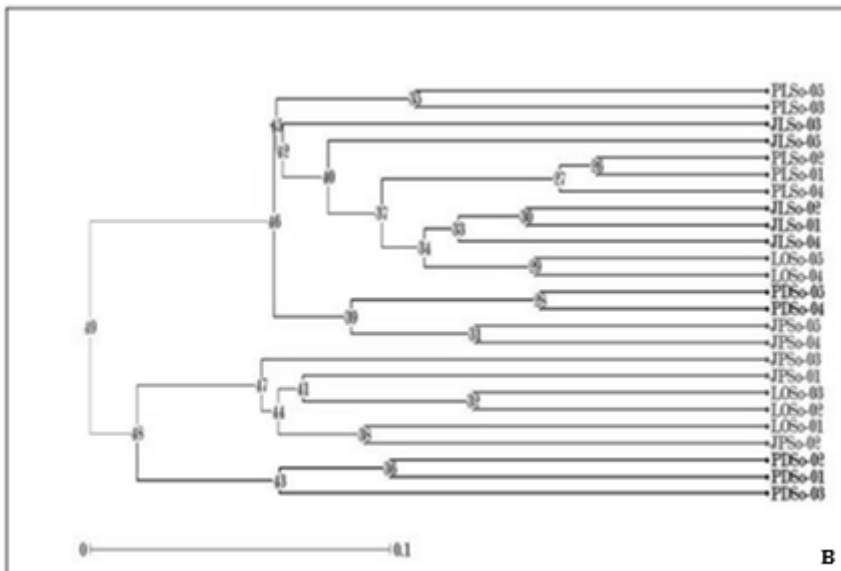
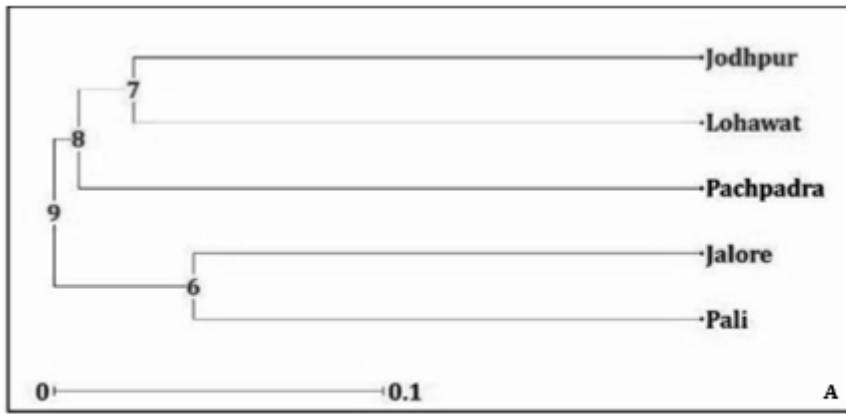


Figure 4

UPGMA dendrograms based on (A) populations and (B) individual samples of *Salvadora oleoides* Decne from semi-arid and sub-humid climatic conditions of Rajasthan, India.

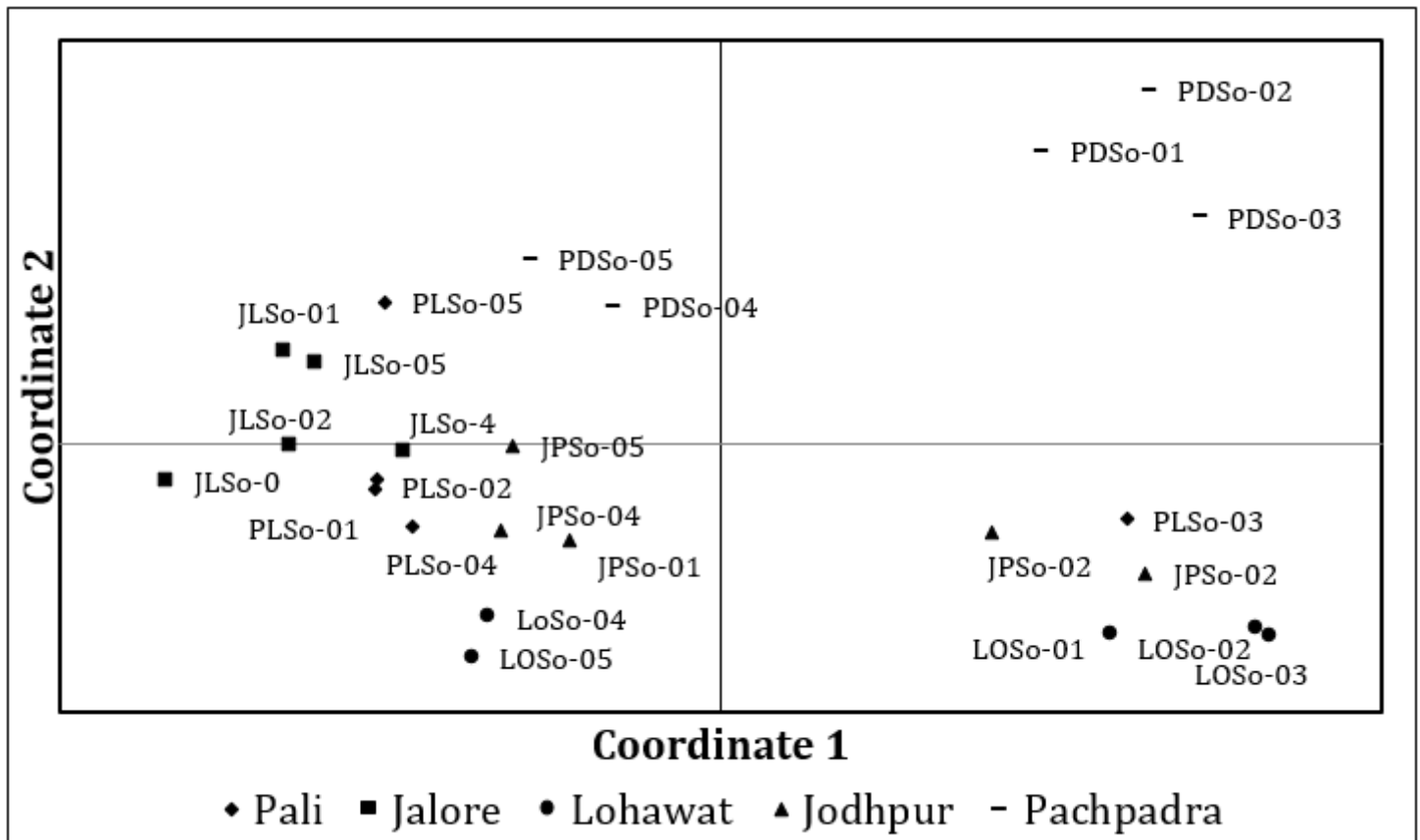


Figure 5

PCo Analysis based on individual samples of *Salvadora oleoides* Decne from semi-arid and sub-humid climatic conditions of Rajasthan, India.

Supplementary Files

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