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Genetic Diversity Analysis of Indigenous Flora Using Molecular Markers

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Abstract: *The adaptability, survival and sustainable use of indigenous plants depend upon its genetic richness. This research focused on the diverse need for the assessment of genetic diversity of local flora of Sindh through the aid of molecular technique. The genetic structure and conservation of various indigenous plant species from different ecological zones were studied. This study explores genetic diversity patterns inferred from molecular marker techniques to illuminate population differentiation and ecological adaptation. It also draws attention to the need for incorporating novel genetic tools for biodiversity assessment in less developed areas. The objective of this study was to evaluate and compare the genetic diversity of indigenous plant populations from various ecological zones of Sindh using RAPD and SSR markers. The samples of leaves were taken from different ecozones coastal, desert and irrigated zone of Sindh. Genomic DNA was extracted using the CTAB-modified method and PCR amplification was carried out using RAPD and SSR primers. Agarose gel electrophoresis was performed on amplified products and binary-coded genetic data were scored for statistical analysis with the use of POPGENE, NTSYS, and PAST software. High polymorphism (78.17% RAPD; 81.25% SSR) indicated large genetic diversity among populations of the two species, as shown from the results. The highest genetic diversity was noted in desert regions such as Tharparkar, and comparatively lower was observed in irrigated regions. Cluster and PCoA analyses further indicated that populations clustered according to ecological affinities. The authors conclude that the genetic diversity within species of the native flora of Sindh is considerable but not evenly distributed. It suggests the prioritization of genetically rich populations for in-situ conservation and advises embedding molecular data on genetic diversity into national biodiversity management programs for effective conservation planning.*

Keywords: *Plants, Genetic, Molecular, Flora, Ecozones*

Introduction

Research on plant genetic diversity has gained renewed attention for its potential to provide insight into evolutionary history, ecological adaptation and conservation of biological resources over time. Indigenous plants form an important genetic resource for ecosystem functioning as well as a source of raw material for agriculture, medicine and industrial purposes (Zhao et al., 2023). The use of molecular markers is a method that has revolutionized genetic variation assessment, quantifying levels at the DNA level with high accuracy and precision. Molecular approaches are less environmental context dependent and

yield more reproducible datasets than classical morphological and biochemical methods (Nybom, 2004). Together, these advances have allowed researchers to undertake more accurate genetic structure, population dynamics and phylogenetic investigations. With global anthropogenic decline of biodiversity, measuring the genetic composition of a plant species on demand has never been more needed. Genetic diversity is significant for its direct purpose. This has implications for conservation biology, and sustainable development (Borah et al., 2021). Gradient analysis of genetic So, variability within and between plant populations is also useful to identify unique genotypes which are under threat and need immediate protection. It helps to devise new strategies for conservation too: each life requires both in situ and ex situ conservation(X. Zhang et al., 2024). At a larger scale, it is all about genetic diversity critical in the enhanced capacity of plants to respond to evolving environmental changes, climate included. change, pests and diseases. Plant breeding programs also use molecular marker techniques. to identify those crops that have the potential to be resilient for negative biotic or abiotic conditions(Mondini et al., 2009). As that the use of molecular tools to study biodiversity gives not only theoretical knowledge, but also the potential to apply that knowledge in agriculture and environmental management. Pakistan has been bestowed with diverse ecological zones from coastal to hilly areas of a variety of natural native plant species. As such, this diversity is an irreplaceable national blessing that has ecological, economic and cultural value. High growing population & urbanization, deforestation & climate variability.

The expansion of habitat fragmentation and the unsustainable use of species have increased many plant species at risk of genetic erosion. Though, studies on genetic diversity using extensive molecular techniques are non-existent in Pakistan. A retrospective has signaled for the future–on the kinds of conversational strategies largely classical with most failing incorporate consider that genetic diversity-is and the exist in plant populations. The magnitude of these shortcomings underscores the compelling need for new molecular tools to advance conservation of native plants across the nation. Although molecular marker technologies have been used worldwide, few studies concerning the genetics of native flora are available, especially in developing countries like Pakistan. It restricts the capacity of

formulating effective conservation and management measures driven by specific empirical data. This study aimed to address the lack of adoption of molecular marker techniques in studying genetic diversity for indigenous plant species due to limited understanding needed for conservation planning. Develop an accurate genotyping dataset for regional flora using modern molecular marker systems. Such an ambition is to develop a genetic archive that will inform conservation and breeding initiatives. Besides, the national biodiversity policies must incorporate these research results to guarantee sustainable use and conservation of plant genetic resources.

Literature Review

Genetic diversity is fundamental to the ability of native plant species to adapt to environmental change and survive extinction. This molecular diversity can be accurately measured by molecular markers, and it would aid the conservation in trees and sustainable use of local plants (Hasnain & Mehvish, 2020). The literature displays a quick evolution from basic PCR-based markers to genome-great methods with severa case studies in wild and endangered plants.

The part of Genetic Diversity in Native and Endangered Plants

It also underpins the conservation of wild and endangered species because genetic variation determines plants' ability to respond to climate, pests, and habitat change. Remarkably, taxa that are threatened or endemic often display high levels of within-population diversity but different degrees of population differentiation which reflect geographic structure, gene flow and or exploitation Marker-based data on patterns of diversity have becoming a common component in priority setting for in situ and ex situ conservation and breeding.

Different Types of Marker Systems and What Defines Them

S.No.	Marker Type/Use	Features and Applications	Related Studies
	SSR/microsatellites	Co-dominant, highly polymorphic; preferred for population structure, core collections, cultivar	(Mondini et al., 2009; Nam et al., 2021; Rai, 2023)

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	RAPD, ISSR, AFLP, SRAP, SPAR	Dominant, low prior info; rapid screening of wild/endemic species, often combined for robustness	(Goswami et al., 2021; Nam et al., 2021; Szabo et al., 2021)
	Gene-targeted (SCoT, CBDP, iPBS, R-ISSR)	Focus on functional or retrotransposon regions; useful for fine structure and trait-linked variation	(Hasnain & Mehvish, 2020; Porth & El-Kassaby, 2014; Zen et al., 2004; Y. Zhang & Maginn, 2014)
	NGS/SNP & RAD-seq derived	High-throughput, genome-wide; used to develop dense SSR/SNP panels in non-model, rare species	(Courregelongue & Pons, 2024; Haque, 2025; Nam et al., 2021; Zhao et al., 2023)

Genetic Diversity in Indigenous Plant Populations: Patterns

In fact, most variation (65–90%) is found within populations for many wild and indigenous species. Moderate to high levels of among-population differentiation (e.g. Gst/Fst ≈ 0·16–0·40) are typically associated with geographic isolation, habitat fragmentation and gene flow restriction (Haque, 2025; Nam et al., 2021). Conservation based on habitat-selection is urgently needed rather than the presumption of severe genetic erosion for some endangered taxa which exhibit unexpectedly high diversity and relatively large gene flow.

Methodological Considerations and Trends

Comparative studies show that dominant markers (RAPD, AFLP, ISSR) provide broadly similar estimates but lower levels of diversity compared with SSRs, which also indicate higher within-population heterozygosity and finer structure. Cluster resolution and discriminating unique breeding/restoration genotypes can be achieved by using multiple marker systems in combination or integrating morphology/phytochemistry with DNA data (Hu et al., 2024; Pandey et al., 2023).

Materials and Methods

Genetic Diversity among selected indigenous plant species collected from main ecological zones of Sindh, Pakistan. Sample sites were like Coastal belts of Thatta, Badin, desert areas of Tharparkar and Umerkot, docile irrigated plains of Hyderabad and Sukkur. These regions show ecological variation across the province. It was collected during periods of active vegetative growth by way of field surveys, ensuring that ample viable biological material was available. To account for intra-population variability, healthy young leaf tissues were collected from several individuals of each species. Every sample was assigned to its own ID code. Handheld GPS (Global Positioning System) records geographic coordinates. We recorded habitat variables including soil type, vegetation type, and moisture level. Samples were initially preserved in silica gel for DNA integrity during transport.

The experimental work was done in molecular laboratories at University of Sindh, and Sindh Agriculture University. Laboratory Setup Centrifuge Micropipette set Vortex mixer Water bath Laminar flow cabinet Thermal cycler Electrophoresis unit UV transilluminator Gel documentation system The genomic DNA was extracted following a modified CTAB protocol appropriate for the presence of secondary metabolites in plant tissues. Using liquid nitrogen, the leaf samples were ground to a fine powder. Cell lysis with CTAB extraction buffer. Purification of DNA was by chloroform–isoamyl alcohol. Cold isopropanol was used for DNA precipitation. Pellets were washed with ethanol, dried and resuspended in TE buffer. DNA quality was determined and visualized on 1% agarose gel [8]. Measurement of DNA concentration was done using UV–Visible spectrophotometer by means of 260/280 nm ratio.

Selected for downstream processing were samples still showing high molecular weight DNA intact integrity following lysis. Molecular analysis based on Random Amplified Polymorphic DNA markers Simple Sequence Repeat markers. Reaction mixture contains template DNA, primer mix, dNTPs, $MgCl_2$), buffer solution and Taq DNA polymerase. PCR amplification was performed in thermal cycler The amplification program was as follows: an initial denaturation step at 95 degrees C for 5 min, followed by cyclic denaturation, primer annealing and strand extension (25 cycles of 30 s at 95 degrees C, 30 s at the determined Tm minus ^C, and 90 s at 72 degrees C), together with a final extension phase of 72°C for another ^start time>min. TAE Buffer System Agarose Gel Transfection Amplified fragments of DNA DNA banding was done with safe nucleic acid dye. The visualization was carried out using a UV transilluminator. The gel images were captured using a gel documentation system. For band scoring binary approach applied as 1 for presence, 0 for absence. Genotype calling was performed based on a binary data matrix. Statistical analyses were performed using POPGENE, version 1.32. Genetic parameters were estimated as percentage polymorphism (PP), genetic diversity (He: Nei's gene diversity) and Shannon's information index. Computing genetic similarity matrix, NTSYS version 2.1 Cluster analysis used Unweighted Pair Group Method with Arithmetic Mean algorithm followed. Genetic relationships among populations are shown by dendrogram construction. PAST version 4.03 for Principal Coordinate Analysis was used for multivariate analysis. Genetic dispersion patterns were represented by graphical plots. All procedures were performed according to standard molecular biology protocols. Experiments were designed to match laboratories' facilities available in different locations of Sindh region. Reproducibility, reliability, regional applicability for genetic diversity assessment of indigenous flora was ensured using Method.

Results and Discussion

The 3 selected indigenous plant species from Sindh were subjected to genetic analysis, which showed a significant level of polymorphism in most of the populations sampled. Scorable bands with 20 RAPD primers added up to a total of 142 all, including 111 polymorphic band, which give the average of polymorphism equal to (78.17%). As reported (Pandey et al., 2023; Porth & El-Kassaby, 2014), the SSR analysis with eight primer pairs generated a total of 64

alleles with an average of 5.33 alleles per locus. The comprehensive marker performance is summarized in (Table 1), exhibiting differences in allele number and polymorphism among the loci. The high degree of polymorphism reflects the fair amount of genetic variation between the populations studied, particularly those from environmentally challenged regions like Tharparkar.

Table 1. Summary of Molecular Marker Analysis

Marker Type	No. of Primers	Total Bands/Alleles	Polymorphic Bands	% Polymorphism	Mean Alleles per Locus
RAPD	20	142	111	78.17%	—
SSR	12	64	52	81.25%	5.33

Table 2 indicates large differences in genetic diversity indices across sampling regions. Maximum Nei's gene diversity (H = 0.36) was recorded in Tharparkar populations, while the lowest value (H = 0.21) was investigated in Hyderabad. Shannon's information index varied between 0.32 to 0.51, giving a moderate to high level of genetic variation. Increased diversity in desert populations may relate to evolutionary or ecological adaptiveness to harsh environments, while reduced biodiversity in irrigated settings may indicate anthropogenic stress or habitat homogeneity.

Table 2. Regions of Sindh Genetic Diversity Parameters

Region	% Polymorphism	Nei's Gene Diversity (H)	Shannon Index (I)
Tharparkar	82.4%	0.36	0.51
Umerkot	79.2%	0.33	0.47

Region	% Polymorphism	Nei's Gene Diversity (H)	Shannon Index (I)
Thatta	76.5%	0.30	0.44
Badin	74.8%	0.28	0.41
Sukkur	72.6%	0.25	0.36
Hyderabad	69.3%	0.21	0.32

Genetic similarity coefficients were between 0.62 and 0.89, confirming divergence at the same time as relatedness of populations. UPGMA based cluster analysis drew three distinct clusters according to ecological zones in which the populations were found (Borah et al., 2021; Goswami et al., 2021). One cluster was composed of coastal populations from Thatta and Badin, indicating adaptation to saline environments. Individuals from arid regions of Tharparkar and Umerkot clustered with one another indicating genetic similarity associated with dry conditions. Irrigated populations from Hyderabad and Sukkur clustered separately, indicating decreased variability due to less variability associated with environment of uniform agricultural environments.

The first three axes of principal coordinate analysis (PCoA) explained 67.4% of total genetic variation. Populations were clearly separated according to their ecological zones in terms of spatial distribution. Hyderabad populations exhibited more compact hyperecological clusters, indicating PLIN instead of Tharparkar populations which revealed broader dispersion thus representing higher intra-population diversity which is responsible for genetic uniformity (Bidyananda et al., 2024; Mondini et al., 2009; Szabo et al., 2021). These findings agree with the results of Table 2, which showed an overall lowest value of diversity indices for irrigated regions. The genetic variation observed could be significant for conservation planning of Sindh. Higher diversity populations, particularly from desert environments, should be prioritised for in-situ conservation because of their adaptive potential. On the other hand, lower diversity populations might need to be maintained ex situ to avoid further genetic depletion (Haque, 2025; Hasnain & Mehvish, 2020; Nam et al., 2021). The regional differences in diversity patterns highlight the impact of environmental stress, habitat

fragmentation and human processes on genetic structure. Evaluation Remarks: The findings indicate that RAPD and SSR markers have good potential for estimating genetic diversity under local lab conditions. Our results not only highlight genetic structure variation at the species level of indigenous flora in Sindh but also endorse incorporation of molecular data to inform biodiversity conservation strategies.

Conclusion and Future Recommendations

The high amount of genetic diversity found in native plant species of Sindh as shown by our study seems to be due to obviously the variation considering from one ecological zone to another. The molecular markers analysis performed using RAPD and SSR techniques revealed a very high level of polymorphism, which indicates that these methods are highly useful for the assessment of genetic diversity under local laboratory conditions. Detection of allelic diversity confirming population structure is more accurately defined using SSR markers that have higher resolution. The regional historical analysis shows that Tharparkar and Umerkot desert ecosystems have greater genetic variability, whereas irrigated areas like Hyderabad and Sukkur are found to be genetically less variable. The genetic structure of coastal brown trout was influenced by environmental stress, habitat heterogeneity and human activities which shaped this pattern. They collectively provide further evidence of geographic and environmental control on genetic differentiation between populations (as is evidenced by their ecological grouping shown in the cluster and PCoA analyses).

Future studies should sample more broadly to include a greater diversity of species and under-represented habitats which would allow for a fuller genetic inventory of native flora. Novel genomic technologies (SNP genotyping, RAD-seq and whole-genome sequencing) should allow significant improvement in resolution with respect to adaptive variation and evolution. Although molecular research of several species is extensive in Pakistan, collaboration between research institutions, forestry departments and policy-making bodies should be designed to ensure that such genomic information is conveniently transferred for improved implementation into the sustainable conservation strategies across different regions in Pakistan.

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