Chapter 1

Genetic analysis of Indigenous Tribal crops

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Abstract:

Genetic diversity is essential for agricultural sustainability, food security, and climate resilience, especially among indigenous crops grown in the tribal areas of Kerala. This study investigates the genetic variability of Dioscorea (yams), Manihot esculenta (cassava), and Musa spp. (banana) through the use of Inter-Simple Sequence Repeat (ISSR) markers. By employing ISSR-based genotyping along with fluorescently labeled primers and capillary electrophoresis, we gain detailed insights into genetic relationships, population structures, and adaptation strategies. The results underscore the importance of traditional farming systems as vital sources of genetic diversity, highlighting the necessity for conservation and breeding efforts. By merging molecular techniques with ethnobotanical knowledge, this research promotes sustainable agricultural practices, enhances biodiversity conservation, and protects the genetic heritage of tribal communities. Future efforts will focus on broadening molecular studies to include more indigenous crops and establishing participatory breeding programs aimed at developing climate-resilient cultivars.

1. Introduction

Genetic diversity is the foundation of agricultural sustainability and is critical in ensuring food security, resilience to climate change, and adaptation to biotic and abiotic stresses. Traditional agrarian systems, particularly those maintained by indigenous communities, serve as reservoirs of genetic diversity, where tribal farmers have cultivated and preserved unique crop varieties over generations. These varieties often possess valuable traits such as drought resistance, pest tolerance, and high nutritional content, making them essential for crop improvement and conservation efforts.

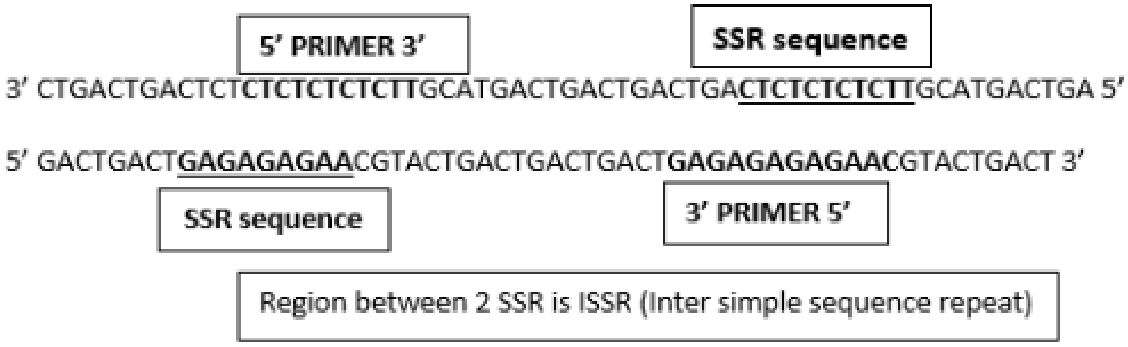
Advancements in molecular marker technologies have become indispensable for documenting, conserving, and utilizing this genetic wealth. These markers enable precise characterization of genetic diversity at the DNA level, providing insights into evolutionary history, genetic differentiation, and population structure [1]. Unlike morphological and physiological traits, which environmental factors can influence, molecular markers offer a more reliable assessment of genetic variation [2].

Among molecular markers, Inter-Simple Sequence Repeats (ISSRs) have gained prominence in genetic diversity studies due to their ability to amplify multiple loci without prior genomic information [3]. ISSR markers are particularly valuable for analyzing the genetic variability of landraces and wild relatives, making them ideal for studying traditional crop varieties maintained by tribal communities [4]. Their high reproducibility and capacity to detect polymorphisms in non-coding regions facilitate studying evolutionary relationships, genetic distinctiveness, and adaptation patterns. They require no prior knowledge of genome sequences while producing highly polymorphic patterns, making them especially suitable for diversity studies in non-model organisms like traditional crop varieties.ISSR markers provide significant information unique to each variety, and the diversity in the crop population can be analyzed well. Other marker systems, such as Simple Sequence Repeats (SSR) [5] and Amplified Fragment Length Polymorphism (AFLP) [6], also contribute to genetic analysis. Still, ISSRs stand out for their cost-effectiveness and broad applicability in assessing indigenous crop diversity.

Genetic diversity plays a crucial role in the adaptation and survival of species, particularly in response to environmental changes, diseases, and human interventions. Our study focused on the genetic diversity of indigenous crops, including Dioscorea (yams), Manihot esculenta (cassava), and Musa spp. (banana), cultivated in Kerala’s tribal regions of Thiruvananthapuram, Wayanad, and Idukki. ISSR markers were used to assess genetic variation among these species, offering valuable insights into their genetic architecture and conservation potential. By understanding the genetic basis of these traditional crops, we can guide conservation efforts and develop climate-resilient cultivars while preserving the genetic heritage of tribal agricultural systems [7].

A comprehensive approach integrating traditional ecological knowledge with modern scientific methodologies is essential for studying the genetic diversity of wild tubers and fruits in Kerala's tribal areas. Ethnobotanical surveys document traditional plant uses and aid in species identification for further genetic analysis. Molecular markers such as ISSRs and SSRs reveal genetic variability, while Geographic Information Systems (GIS) help map plant distributions and analyze genetic diversity spatially [8]. Community-based conservation initiatives actively involve indigenous communities in protecting genetic resources, while biotechnological tools, such as tissue culture and genetic engineering, support conservation and crop improvement [9]. Collaborative research involving scientists, local communities, and government agencies is vital for implementing effective conservation strategies.

A novel approach in this study involves using ISSR markers coupled with fluorescently labeled primers and fragment analysis to assess genetic diversity. ISSR markers amplify regions between microsatellite sequences, producing highly polymorphic DNA fragments that provide detailed genetic profiles [10]. Fluorescently labeled primers enhance the detection of amplified DNA fragments, which are analyzed using capillary electrophoresis to separate and visualize genetic variations. This method generates high-resolution genetic data, facilitating the identification of unique genetic markers and population structures. Such information is invaluable for conservation, as it helps identify genetically distinct populations requiring targeted conservation strategies.



Diagrammatic picture showing fragment amplification between two SSRs is ISSR (inter simple sequence repeat) fig courtesy: Mondal A et al., (2018)

Future research will focus on assessing and preserving the genetic diversity of tribal crops in Kerala using advanced molecular marker techniques. The study will expand to include diverse indigenous crops such as wild tubers, millets, pulses, and medicinal plants. Tribal regions of Kerala will remain focal points due to their rich reservoir of indigenous crop varieties. Investigating genetic variation and population structures within these crops will be critical for evaluating adaptive traits and resilience. By integrating traditional agricultural knowledge with cutting-edge molecular analyses, this research will contribute to safeguarding

Kerala’s tribal agricultural heritage, promoting sustainable farming practices, and enhancing biodiversity conservation efforts in the region.

2. Genetic Diversity of Cassava Varieties in Kerala's Tribal Areas

The genetic diversity of crop plants represents a fundamental resource for agricultural sustainability, food security, and adaptation to changing environmental conditions. This chapter comprehensively analyzes cassava (Manihot esculenta Crantz) varieties cultivated in tribal areas of Kerala, India, using fluorescently labeled Inter Simple Sequence Repeat (ISSR) markers [11]. The study documents significant genetic variation among locally maintained cassava landraces, providing scientific validation of traditional agricultural knowledge and establishing a foundation for both conservation efforts and crop improvement programs.

3. The Global and Local Significance of Cassava

Cassava is one of the most crucial staple food crops globally, supporting approximately 800 million people in tropical and subtropical regions. This remarkably adaptable crop has gained prominence due to its drought tolerance and capacity to produce substantial yields even under challenging growing conditions. The storage roots of cassava serve as a significant source of carbohydrates for human consumption and animal feed, while its starch has found extensive applications across various industries [12]. Cassava's ability to thrive in poor soils with low fertility, often outperforming other tuber crops in yield, has solidified its position as a cornerstone of food security in many regions facing environmental challenges.

In Kerala, India, cassava cultivation has profound cultural and economic significance, particularly within tribal communities. These communities have maintained diverse cassava varieties through generations of cultivation, developing an intimate knowledge of variety characteristics and their adaptation to local conditions. Each landrace represents an agricultural resource and embodies cultural heritage and ecological wisdom accumulated over centuries. Understanding the genetic makeup of these traditionally maintained varieties offers valuable insights into adaptation mechanisms and potential traits that could contribute to broader agricultural development.

The genetic diversity present in landraces serves multiple functions within traditional farming systems. Different varieties may be cultivated for specific culinary applications, varying maturation periods, adaptation to microenvironmental conditions, or cultural significance. This diversity also functions as a risk management strategy, with different varieties exhibiting varying responses to environmental stressors, pest pressures, and disease challenges [13].

However, this agrobiodiversity faces increasing threats from agricultural commercialization, changing socioeconomic dynamics, and climate change impacts, underscoring the urgency of comprehensive documentation and conservation efforts. For cassava, which demonstrates considerable phenotypic plasticity, molecular approaches are particularly valuable for clarifying genetic relationships among varieties that may share phenotypic similarities despite genetic differences.

4. Understanding Genetic Diversity Through Molecular Markers

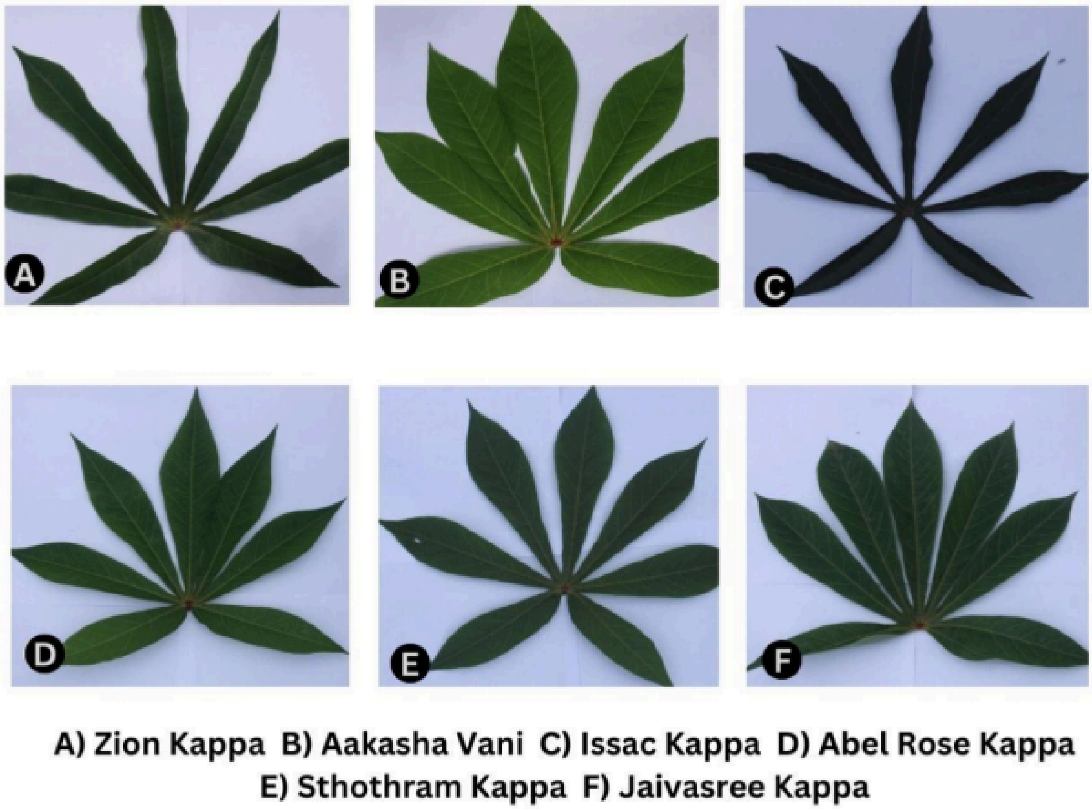
The assessment of genetic diversity has evolved significantly with advances in molecular biology techniques. Molecular markers offer distinct advantages over traditional morphological assessments by detecting variation at the DNA level, providing insights independent of environmental conditions or developmental stages.

The innovation of fluorescently labeled ISSR markers has further enhanced the sensitivity and resolution of genetic analysis. Incorporating fluorescent dyes such as 6-carboxyfluorescein into primers can detect the resulting PCR products with high precision using automated DNA analyzers [14]. This approach allows for identifying more polymorphic bands than conventional gel electrophoresis, thereby improving the discriminatory power of genetic analysis. For cassava varieties maintained by tribal communities, this technology offers a powerful means of documenting genetic relationships with unprecedented detail.

5. The Cassava Varieties of Kerala's Tribal Areas

Tribal communities in Kerala, particularly those residing in the Idukki district, have maintained a remarkable diversity of cassava varieties through traditional farming practices. Our study documented 23 distinct farmer varieties, each known by local names that often reflect their physical characteristics, origin, or particular uses. These varieties represent a living genetic repository that has evolved through generations of selection for traits suited to local environmental conditions, culinary preferences, and cultural practices.

The morphological characterization conducted as part of our research revealed substantial phenotypic diversity across the evaluated traits. Leaf characteristics showed exceptionally high variation, with differences in lobe shape, number, dimensions, margin, and coloration. Stem characteristics and growth habits also varied considerably among the varieties, reflecting adaptation to different microenvironments within the agricultural landscape. These morphological distinctions serve as practical markers for farmers to identify and manage different varieties, though they do not always correspond perfectly with genetic relationships.



A) Zion Kappa B) Aakasha Vani C) Issac Kappa D) Abel Rose Kappa
E) Sthothram Kappa F) Jaivasree Kappa

Figure :1 showing morphologically distinct cassava landraces

The varieties documented in our study include traditional landraces with long histories of cultivation and farmer-developed varieties that may represent more recent selections or introductions. Traditional varieties such as 'Kariveppu kappa,' 'Ambakkadan kappa,' 'Pathinettu kappa,' and 'Etha kappa' often hold special cultural significance and may be associated with specific culinary applications or ceremonial uses. Farmer-developed varieties, meanwhile, may reflect adaptation to emerging challenges or changing preferences, demonstrating the dynamic nature of agricultural biodiversity management within these communities.

6. Methodology: Combining Molecular and Morphological Approaches

Our research methodology integrated molecular analysis with traditional morphological characterization to comprehensively assess cassava diversity. This dual approach acknowledges the complementary nature of these different perspectives on diversity, with molecular analysis revealing genetic relationships while morphological characterization documents the phenotypic expressions that farmers directly observe and select.

For morphological characterization, we evaluated 23 farmer varieties based on internationally recognized descriptors for cassava. The assessed traits included leaf morphology (pubescence on apical leaves, color of the first fully expanded leaf, shape of the central leaf lobe, number of lobes, dimensions, margin), stem characteristics (color, prominence of foliage scars, distance between leaf scars), and growth habit. These data were recorded through field observations during the vegetative growth phase, following standardized protocols to ensure consistency and comparability.

The genetic analysis began with collecting tender leaves from cassava plants growing in tribal areas of the Idukki district. Genomic DNA was isolated using a modified CTAB method, with quality and quantity assessed through both agarose gel electrophoresis and spectrophotometric analysis. Working solutions of DNA were prepared at a uniform concentration of 100 ng/µl for PCR amplification. A total of 22 ISSR primers, labeled with 6-FAM at the 5' end, were employed for the molecular analysis, with selection based on previous studies demonstrating their effectiveness for cassava genotyping [15].

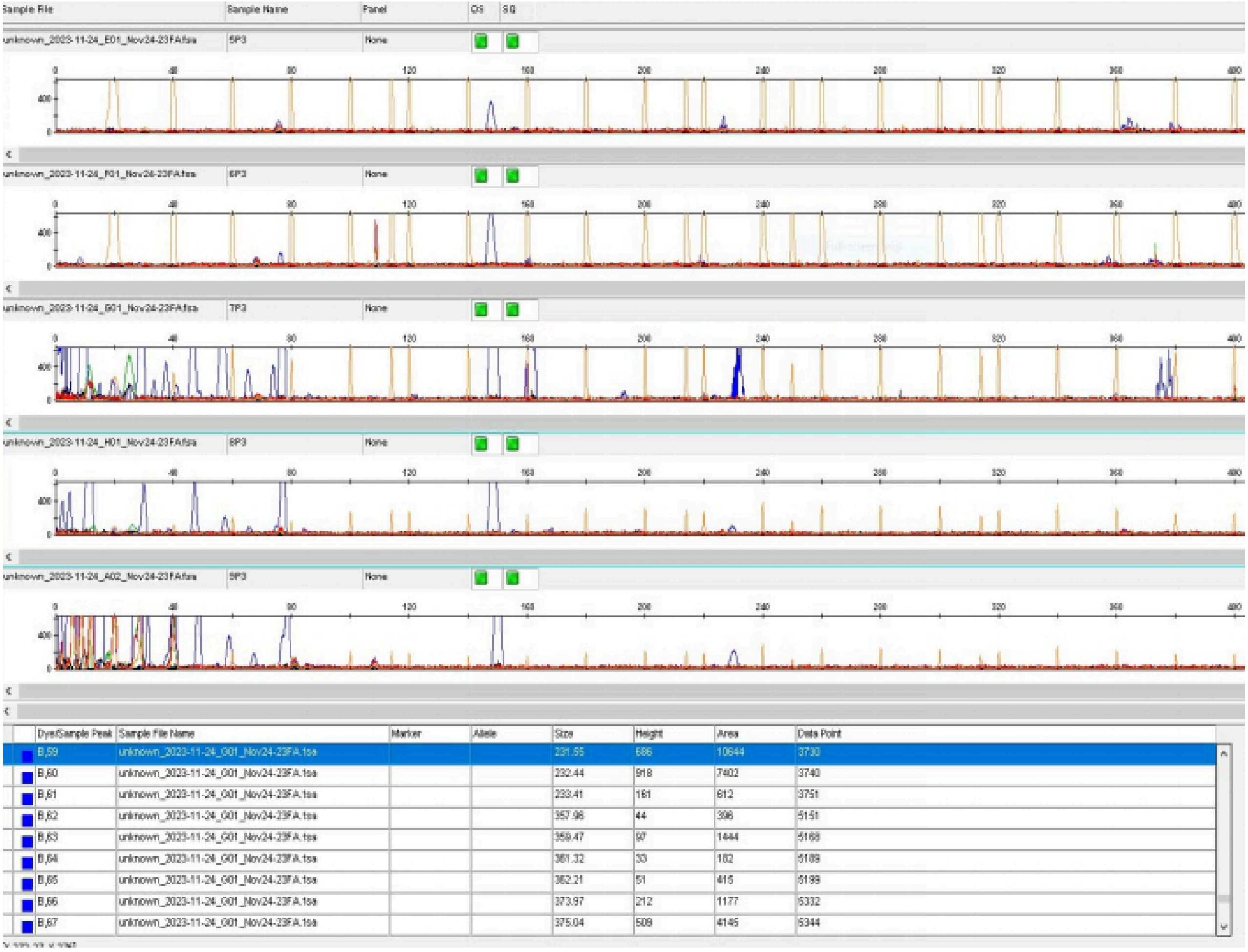
PCR products were subjected to fragment analysis using an automated DNA analyzer, with data analyzed using Genemapper 6 software. The resulting electropherograms showed the size and intensity of amplified fragments, allowing for precise scoring of fragments between 80 bp and 600 bp. Given the dominant nature of ISSR markers, data were recorded in binary format, with '1' indicating the presence of a fragment and '0' indicating its absence. This binary data matrix formed the basis for subsequent genetic analysis using PopGene32 software, including calculations of polymorphism, genetic distance, and cluster analysis.

7. Revealing the Genetic Landscape of Cassava Diversity

The application of fluorescently labeled ISSR markers revealed substantial genetic diversity among the cassava varieties cultivated in tribal areas of Kerala. Our analysis generated 72 scorable bands across the 23 cassava varieties examined, of which 49 (68.06%) were polymorphic. This level of polymorphism indicates considerable genetic diversity within the studied collection despite cultivation within a relatively confined geographical area. Each ISSR primer produced an average of 17 polymorphic bands, demonstrating the high discriminatory power of the selected markers for cassava genotyping.

The capillary gel electropherograms illustrated the polymorphic profiles generated by the fluorescently labeled primers, with distinct patterns observed for different cassava varieties. The high resolution achieved through fluorescent detection allowed for the precise identification of fragments, including those with minor size differences that might be indistinguishable in conventional gel electrophoresis. This technological advantage enabled a more detailed assessment of genetic relationships than would be possible with traditional molecular techniques.

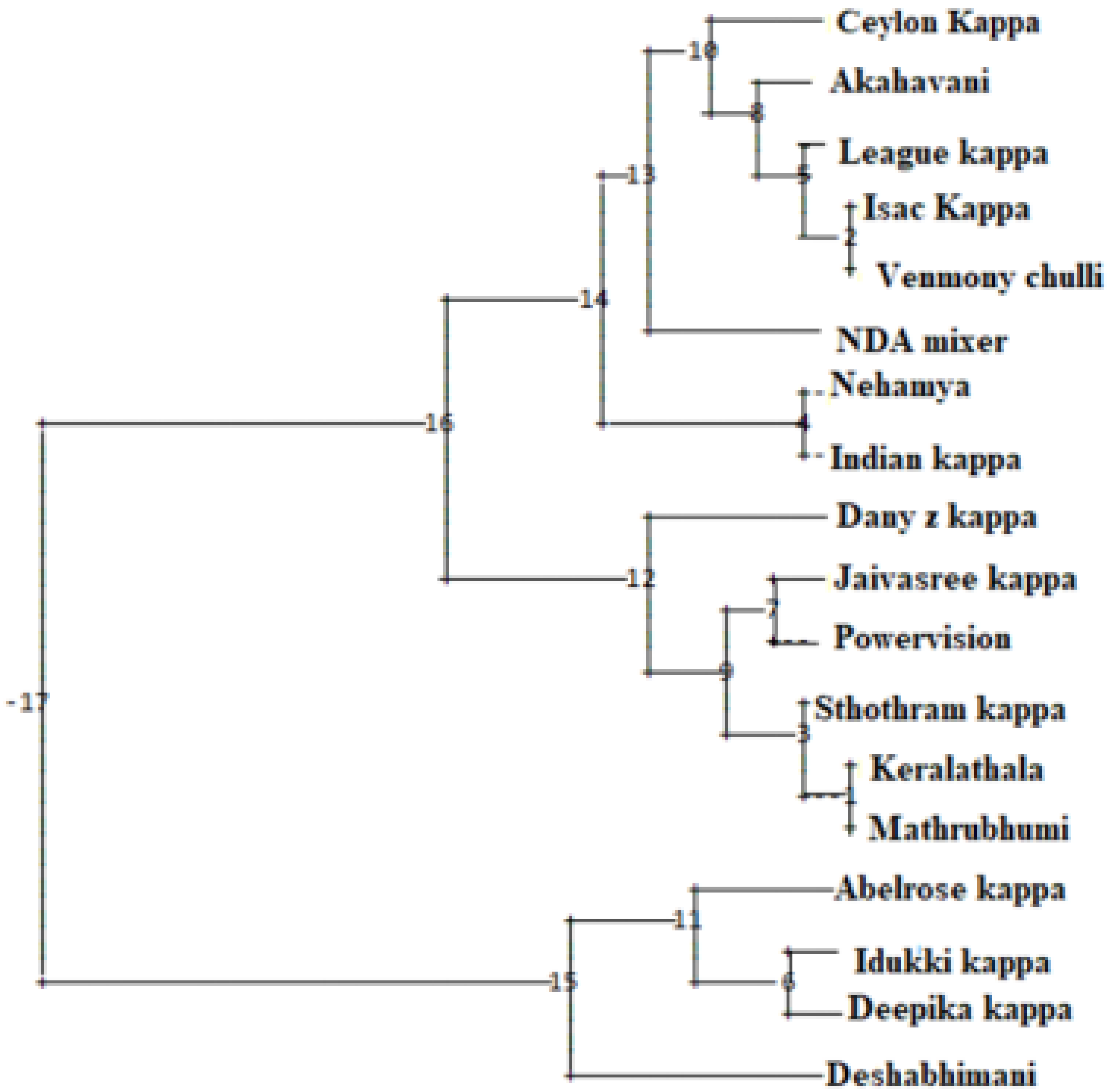
Genetic similarity coefficients ranged from 0.69 to 0.99 among the 23 cassava varieties, indicating varying degrees of genetic relatedness within the collection. Some varieties showed very high similarity, suggesting possible duplication or close genetic relationship, while others demonstrated substantial genetic divergence. The resulting dendrogram revealed several distinct clusters, generally corresponding with the classification of varieties as either traditional landraces or farmer-developed varieties, although some intermixing was observed [15].



Dye/Sample Peak	Sample File Name	Marker	Allele	Size	Height	Area	Data Point
B,59	unknown_2023-11-24_G01_Nov24-23FA.fsa			231.55	686	10644	3730
B,60	unknown_2023-11-24_G01_Nov24-23FA.fsa			232.44	918	7402	3740
B,61	unknown_2023-11-24_G01_Nov24-23FA.fsa			233.41	161	612	3751
B,62	unknown_2023-11-24_G01_Nov24-23FA.fsa			357.98	44	398	5151
B,63	unknown_2023-11-24_G01_Nov24-23FA.fsa			359.47	97	1444	5168
B,64	unknown_2023-11-24_G01_Nov24-23FA.fsa			361.32	33	162	5189
B,65	unknown_2023-11-24_G01_Nov24-23FA.fsa			362.21	51	415	5199
B,66	unknown_2023-11-24_G01_Nov24-23FA.fsa			373.97	212	1177	5332
B,67	unknown_2023-11-24_G01_Nov24-23FA.fsa			375.04	509	4145	5344

A capillary gel electrophorogram showing polymorphism of the PCR products amplified using fluorescent primers (5’ FAM labeled) showed a difference in gel profiles. Orange-coloured products are size standard.

Interestingly, certain varieties with similar local names did not always cluster together genetically, indicating that nomenclature based on morphological characteristics may not consistently reflect genetic relationships. This finding highlights the value of molecular characterization as a complement to traditional classification systems, providing insights that may not be apparent through morphological observation alone. Such discrepancies between naming and genetic clustering suggest complex germplasm exchange and selection patterns within and between communities, potentially reflecting historical migration patterns, trade relationships, or cultural exchanges.



The dendrogram showing 17 farmers varieties and one traditional variety being grouped in 5 clusters (3 major +2) based on the similarity distance matrix data

8. Implications for Conservation and Agriculture

The substantial genetic diversity documented among cassava varieties from tribal areas of Kerala has significant implications for biodiversity conservation and agricultural development. From a conservation perspective, these findings emphasize the importance of tribal farming systems as repositories of crop genetic diversity that may not be adequately represented in formal gene banks or improved varieties. The genetic distinctiveness of specific landraces suggests they may contain unique allelic combinations with potential value for broader agricultural applications.

Conservation strategies should encompass ex-situ approaches (preservation in gene banks) and in-situ methods (continued cultivation in traditional farming systems). In the study, molecular characterization facilitates rational sampling for ex-situ conservation to ensure that genetic diversity is adequately represented in germplasm repositories. In situ conservation, meanwhile, depends on supporting tribal farmers as custodians of agrobiodiversity through recognition of their knowledge, ensuring their rights over genetic resources, and developing market opportunities for traditional varieties.

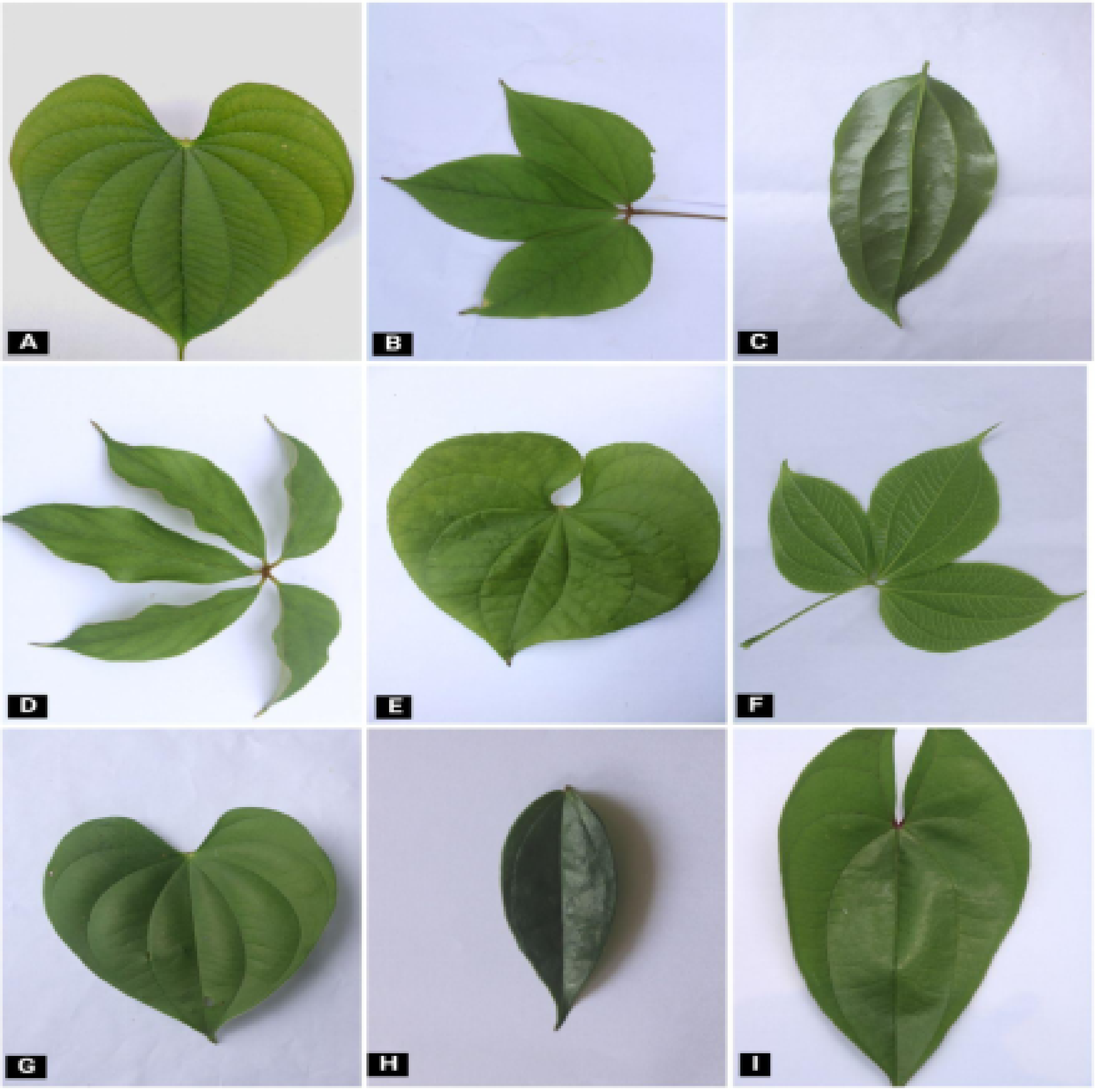
From an agricultural development perspective, the genetic diversity present in tribal farming systems represents a valuable resource for crop improvement programs. Identifying genetically distinct varieties with desirable traits provides potential parental material for breeding efforts targeting yield enhancement, drought tolerance, pest and disease resistance, or nutritional quality. The genetic similarity/distance matrix generated through our analysis can guide the selection of parental combinations for hybridization, optimizing breeding strategies to maximize genetic gain.

The scientific validation of diversity in tribal farming systems also provides a foundation for community-based agrobiodiversity management initiatives. By integrating traditional knowledge with scientific characterization, such programs can enhance both conservation outcomes and agricultural livelihoods. Approaches might include participatory variety selection, community seed banks, documentation of traditional knowledge, and the development of geographical indication systems that recognize the unique properties of cassava varieties from particular tribal areas.

9. Genetic Diversity and Polymorphism Analysis of Diascorea

The analysis of 44 Dioscorea accessions revealed substantial genetic variability. The eight polymorphic primers generated a total of 36 polymorphic bands, with primer (GA)6CC exhibiting the highest polymorphism (six bands) and primer (CA)6AC yielding the lowest (two bands). The percentage of polymorphic loci varied from 26% to 99% across species.

Dioscorea oppositifolia exhibited the highest genetic diversity (0.2892), while D. alata showed the highest percentage of polymorphism (99%) [16].



A) Dioscorea bulbifera B) Dioscorea tomentosa C) Dioscorea oppositifolia D) Dioscorea pentaphylla E) Dioscorea esculenta F) Dioscorea hispida G) Dioscorea wallichi H) Dioscorea sps. I) Dioscorea alata

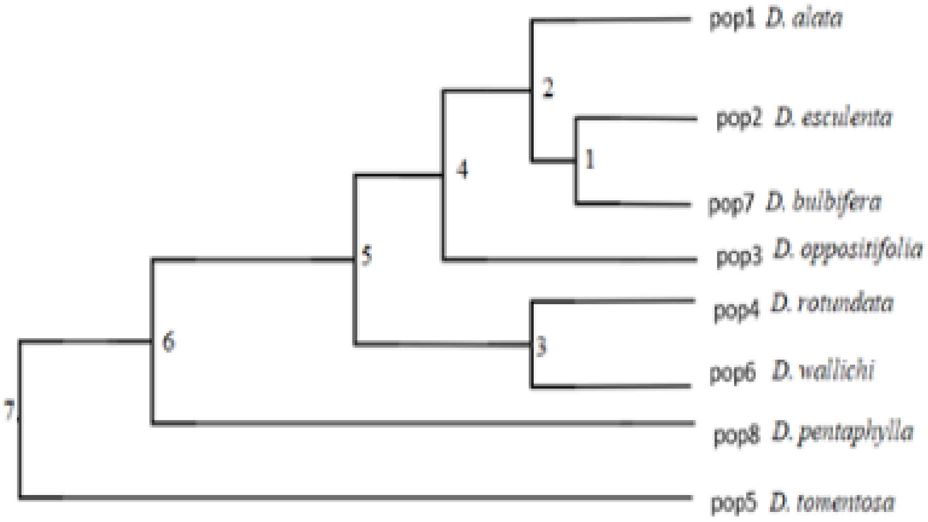
Figure :2 showing morphologically distinct Dioscorea spp

10. Significance of ISSR Markers in Dioscorea Genotyping

ISSR markers offer a high-resolution approach to assessing genetic diversity. Fluorescent labeling enhances marker sensitivity, enabling more accurate detection of polymorphisms. Compared to conventional ISSR, fluorescent ISSR (F-ISSR) markers produce more bands and provide precise size discrimination. The findings of this study demonstrate that ISSR-assisted genotyping can effectively resolve folk taxonomic ambiguities, particularly in cases where different names among tribal communities know the same landrace [17].

11.Phylogenetic Relationships and Cluster Analysis

Nei's genetic distance analysis indicated the highest genetic divergence between *D. tomentosa* and *D. rotundata* (0.5379). UPGMA dendrogram clustering grouped *D. oppositifolia* and *D. wallichi* into one cluster, whereas *D. esculenta* and *D. pentaphylla* formed another. The exotic species *D. rotundata* was the most genetically distinct, creating a separate cluster. This clustering aligns with prior reports on *Dioscorea* genetic variability.



Sl No.	**Population**	**N**	**Na**	**Ne**	**I**	**h**	**P%**
1	*D. alata*	17	1.909	1.1286	0.4394	0.2686	99
2	*D. esculenta*	9	1.8571	1.4040	0.3914	0.1078	85.71
3	*D. bulbifera*	4	1.4286	1.1295	0.1688	0.2321	42.86
4	*D. oppositifolia*	4	1.8571	1.3022	0.3939	0.2892	84.57
5	*D. rotundata*	4	1.5223	1.3030	0.2592	0.1775	43.02
6	*D. wallichi*	3	1.2857	1.1223	0.1362	0.0856	28.57
7	*D. pentaphylla*	3	1.7143	1.4007	0.3989	0.2679	71.43
8	*D. tomentosa*	2	1.100	1.111	0.1262	0.0875	26.3

Genetic diversity parameters of Dioscorea spp. were N – Sample size; Na – Observed number of alleles; Ne – Effective number of alleles; I – Shannon's information index; h – Gene diversity; P% - Percentage of polymorphic loci.

Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA --Modified from NEIGHBOR procedure of PHYLIP Version 3.5

Molecular marker-assisted studies are pivotal in conservation strategies for underutilized crops like *Dioscorea*. The genetic data generated can guide the selection of superior genotypes for breeding programs, ensuring the preservation of valuable genetic traits such as disease resistance and high nutritional content. Future studies integrating genome-wide markers and phenotypic trait analysis will further elucidate the adaptive potential of wild *Dioscorea* species.

12. Conclusion and Future Directions

Our study has demonstrated the efficacy of fluorescently labeled ISSR markers for characterizing the genetic diversity of crop varieties cultivated in tribal areas of Kerala. The results reveal substantial genetic variation among the studied varieties, confirming the vital role of tribal farming systems in maintaining agrobiodiversity. Integrating molecular analysis with morphological characterization provides a comprehensive assessment of diversity, with implications for scientific understanding and practical applications in agriculture and conservation.

The research represents a scientific validation of traditional knowledge regarding food crop diversity in tribal communities. By documenting the genetic distinctiveness of farmer varieties, the study protects farmers' rights over the landraces they cultivate and maintain. This aspect is particularly relevant in the increasing commercialization of agriculture and potential biopiracy concerns. The technique employed represents a time-saving approach compared to conventional breeding programs, offering an efficient means of assessing genetic relationships and potential breeding value.

Looking forward, several avenues for future research emerge from this work. These include expansion of genetic characterization to additional crop varieties from diverse tribal areas, integration of next-generation sequencing approaches for higher resolution analysis, investigation of the functional significance of genetic diversity through agronomic evaluation, development of participatory breeding initiatives, and exploration of market-based conservation incentives that recognize the unique properties of tribal cassava varieties.

In conclusion, the genetic diversity of wild tubers and fruit varieties in tribal areas of Kerala represents not merely a scientific interest but a living heritage embodying a century of agricultural knowledge and adaptation. The conservation and utilization of this diversity hold significant potential for addressing contemporary challenges in food security, climate resilience, and sustainable agriculture. Through continued research, community engagement, and supportive policies, these genetic resources can contribute to agricultural systems that are both productive and ecologically sound, honoring the contributions of tribal communities to agrobiodiversity while supporting their livelihoods and food sovereignty.

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