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Unveiling Genetic Diversity of Sri Lankan Avocados using SSR Markers and Morphological Traits

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Abstract

Avocado (*Persea americana* Mill.), a member of the family Lauraceae, is widely recognised as a health-promoting fruit due to its high content of monounsaturated and polyunsaturated fats, which help lower LDL cholesterol levels. Although avocados were introduced to Sri Lanka nearly 200 years ago, the local avocado accessions remain largely uncharacterised. The present study was conducted to characterise 15 avocado accessions maintained in the ex-situ germplasm collection at the Fruit Research and Development Institute, Horana, using both molecular and morphological markers. Molecular characterisation was performed using four avocado-specific Short Sequence Repeat (SSR) markers. A total of 20 distinct amplification fragments were detected, with each locus showing four to six alleles. A dendrogram was constructed using a binary matrix of SSR data in NTSYS-pc 2.10e software, followed by cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA). Results revealed that the avocado accessions were grouped into five distinct clusters. Morphological characterisation was conducted using 15 qualitative morphological traits based on the descriptors developed by the International Plant Genetic Resources Institute (IPGRI). The Shannon-Weiner diversity index ranged from 0.2449 for leaf shape to 1.459 for tree shape, indicating varying levels of phenotypic diversity. This inaugural approach of molecular and morphological characterisation of selected avocado accessions in Sri Lanka provides valuable insights into their genetic diversity, supporting effective germplasm management, breeding programmes, and conservation efforts.

Keywords: Avocado, genetic diversity, IPGRI descriptors, morphological traits, SSR markers

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Introduction

Avocado (*Persea americana* Mill.), an evergreen species of the Lauraceae family, is valued for its nutritionally rich fruit, which is high in monounsaturated and polyunsaturated fats, essential vitamins, minerals, fiber, and antioxidants (DoA). Although native to southern Mexico (Bhuyan, 2019), avocado has been cultivated in Sri Lanka for centuries (FAO), predominantly in home gardens and small-scale orchards. Despite its nutritional and economic significance, sufficient studies on the genetic diversity and morphological variation of local avocado accessions remain largely uncharacterized. Morphological characterization based on descriptors for Avocado (*Persea* spp.) published by the International Plant Genetic Resources (IPGRI), provides essential information on phenotypic traits. However, these traits can be significantly influenced by environmental conditions, limiting their reliability in genetic assessments. To address this, the present study combined both morphological and molecular characterisation using species-specific Simple Sequence Repeat (SSR) markers to enhance the accuracy of genetic diversity assessments. All local accessions conserved in the ex-situ germplasm at the Fruit Research and Development Institute (FRDI) in Horana were analysed to identify genetically distinct and agronomically superior genotypes to support germplasm conservation, future breeding programmes, and the sustainable improvement of avocado cultivation in Sri Lanka.

Materials and Methods

Planting materials

A total of 15 Sri Lankan avocado accessions were selected for this study. All accessions were obtained from the ex-situ germplasm maintained at the Fruit Research and Development Institute at Horana, Sri Lanka, based on their availability. The selected accessions were; X_{22}, X_{21}, NG_1, GP_1, KA_3, HA_1, D_1, MU_1, TT_1, Y_1, GL_1, BR_1, RC_1, P_1, and BW_1.

Morphological characterization

A total of 18 qualitative morphological characteristics were initially recorded for the 15 selected accessions according to the descriptors for avocado published by the IPGRI (1995). However, only 15 characteristics were considered in the subsequent analysis, as three characteristics, leaf base shape, crotch angle of main branches, and crotch angle of leaf petiole were monomorphic across all accessions. All of the considered morphological characteristics were overall tree and leaf characteristics. The inflorescence characteristics were excluded from the analysis because not all of the accessions were flowering, and fruit data could not be collected due to the off-season.

The recorded values for each selected morphological characteristic were subjected to further analysis using the Shannon-Weiner Diversity Index. These values were calculated by considering the natural log value of the proportion of the frequency of each characteristic among the accessions.

Molecular characterization

Isolation of DNA and SSR analysis

Genomic DNA was extracted from young avocado leaves using the modified CTAB method with slight modifications to the protocol described by Hormaza (2002). The extracted DNA was then diluted in 40 µL of nuclease-free water and stored at -20 °C.

Four species-specific SSR markers (Table 1) were selected based on their high polymorphism as reported by Sharon et al. (1997) and Ashworth et al. (2003). PCR amplifications were performed in 12.5 µL volume containing 6.25 µL of 5X GoTaq® Green Master Mix (Promega Corporation), 4.75 µL of nuclease-free water, 0.5 µL of 10 µM forward and reverse primers of each microsatellite marker, and 0.5 µL of 50 ng template DNA from each accession. Reactions were carried out on a thermal cycler (LifeECO Thermal Cycler Bioer) using the following temperature profile: initial denaturation at 95°C for 05 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing for 1 min at respective temperatures for each primer (Table 1), extension at 72°C, with final extension at 72°C for 7 min. The PCR products were analysed using agarose gel electrophoresis. A 2.5% agarose gel in 0.5× TBE buffer was used for markers AVD001, AVD006, and AVAG22, while a 3% agarose gel in 1× TBE buffer was used for AVAG05. Electrophoresis was conducted at 50V for 80 min. The optimal annealing temperatures for each primer were determined.

Table 1: *Selected SSR Markers of Avocado used in this study*

No	SSR marker	Reference	Primer sequences	Repeat motif	Annealing Temperature (^{0}C)	Expected size range (bp)
1	AVD001	Ashworth et al., (2003)	F: 5′- GTT TCC AAG CGA CTC ACG AG -3′ R: 5′- GAT TCC ATG CTG AAT TGC CG -3′	(CT)12	56	226
2	AVD 006	Ashworth et al., (2003)	F: 5′- GGG AGA GAT GTA TTG AGC A-3′ R: 5′- ACT TGG TCG TAG ATT GTA AAT -3′	(TC)9	52	314
3	AVAG 05	Sharon et al., (1997)	F: 5′- GGA TCT GAT GTG TGG GGG AG -3′ R: 5′- CCT GTC GGA AAA GAC TAT GCG -3′	(AG)10	57	94-122
4	AVAG 22	Sharon et al., (1997)	F: 5′- GAT CAT CAA GTC CTC CTT GG -3′ R: 5′- GAT CTC ATA GTC CAA ATA ATG C -3′	(GA)15	57	104-124

Data analysis

The total number of alleles was determined for each SSR locus, with putative alleles identified based on their estimated fragment size in base pairs. Genetic diversity was estimated using the following statistics: number of alleles per locus, observed heterozygosity (H_o), and expected heterozygosity ($H_e = 1 - \sum p_i^2$), where p_i is the frequency of the i^{th} allele (Nei, 1973).

Genetic relationships among the selected accessions were calculated using the UPGMA cluster analysis of the similarity matrix obtained from the binary matrices for each marker with the SAHN program of the NTSYSpc 2.10e software.

Results

Morphological characterization

Based on the Descriptors for avocado (*Persea* spp.) published by the IPGRI (1995), 15 qualitative tree and leaf characteristics were recorded for each accession. Based on the values obtained for each descriptor, the Shannon-Weiner Diversity Index was calculated for each trait.

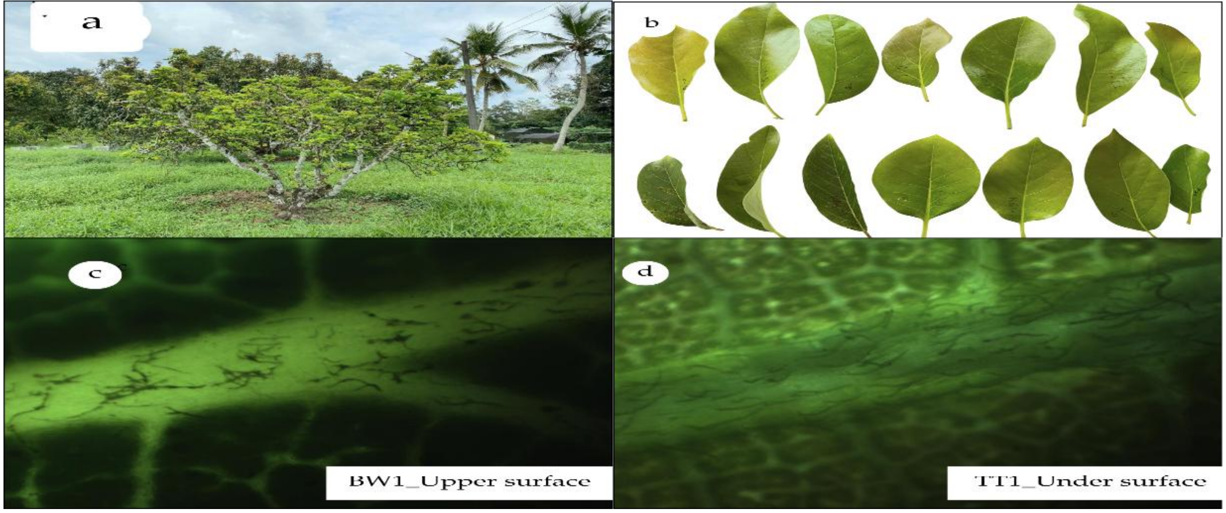

Figure 1: *Observations of some of the morphological traits: a) Tree shape of X_{22} accession. b) Leaf shape of 14 accessions. c) Pubescence of leaf upper surface of BW_1 accession. d) Pubescence of leaf under surface of TT_1 accession*

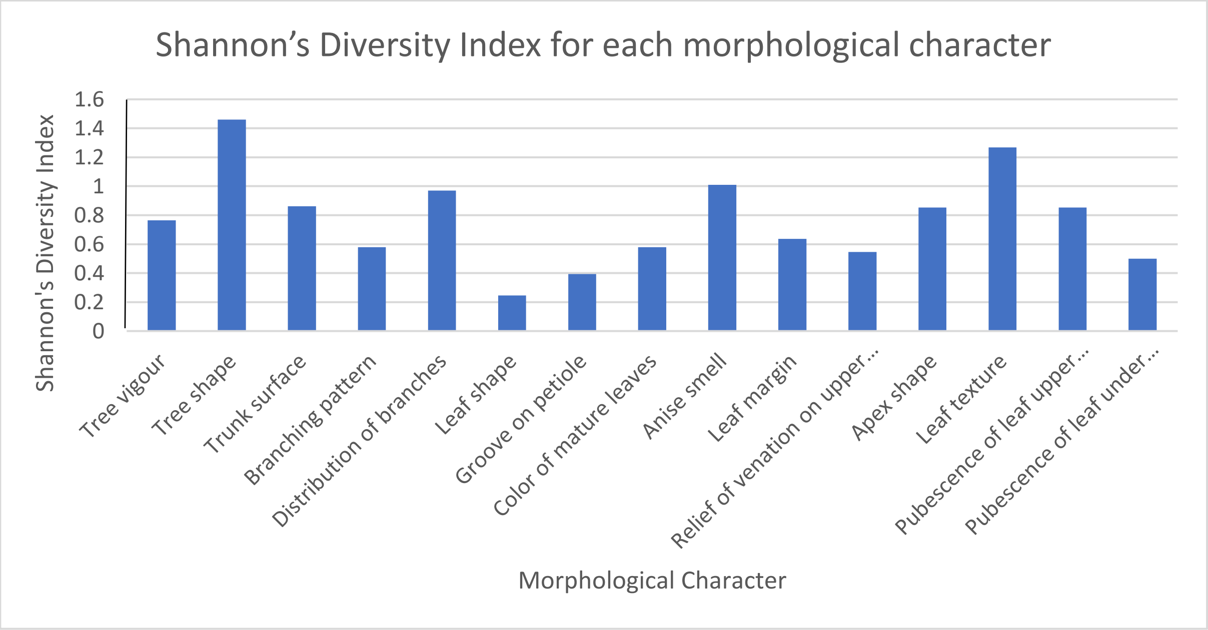

Figure 2: *Shannon's diversity index for each morphological trait*

Molecular characterisation

From the agarose gel electrophoresis of the PCR products for each SSR marker, 20 DNA fragments were observed in total. The markers AVD006 and AVAG05 each revealed four alleles, while AVD001 and AVAG22 each exhibited six alleles (Table 2).

Table 2: Expected heterozygosity of all selected SSR markers

SSR marker	Number of alleles	Range of allele frequencies	Expected heterozygosity (H_e)
AVD001	06	0.067-0.167	0.8067
AVD006	04	0.167-0.333	0.7311
AVAG05	04	0.067-0.4	0.6844
AVAG22	06	0.033-0.267	0.7333
Average Expected Heterozygosity (H_e)			0.738875

A dendrogram was constructed based on the UPGMA analysis using the similarity matrix generated from the binary matrices of the four SSR markers.

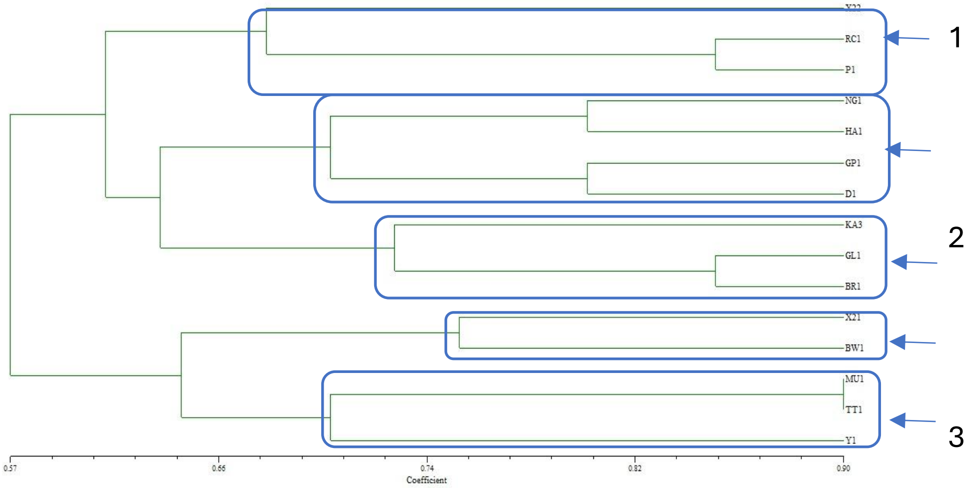

Figure 3: *Dendrogram of the 15 Sri Lankan avocado accessions based on the UPGMA analysis Based on the constructed dendrogram, 05 major clusters of the accessions could be identified. The largest cluster contained 04 accessions:* NG_1, HA_1, GP_1, *and* D_1.

Discussion

Shannon's diversity index values for the morphological traits ranged from 0.2449 for leaf shape to 1.4595 for tree shape. The Shannon diversity index has been widely used in population genetics studies. With this index, lower values indicate lower variations among the accessions for a given trait, while higher values reflect higher diversity (Nolan and Callahan, 2006). Therefore, the tree shape of the accessions varied greatly, while the leaf shapes indicate minimal variation. Other traits, such as leaf texture (1.2681) and anise smell (1.0096), also exhibited relatively higher variations among the accessions as they had values larger than 1.0 for Shannon's diversity index. The accessions expressing greater variation in tree shape indicate greater adaptation and structural integrity, and highly varied leaf texture indicates pest and disease resistance, water use efficiency, while higher variations in anise smell indicate biochemical diversity, which is useful in determining the breeding potential of local accessions (Konwar, 2025).

In the present study, the number of alleles per locus ranged from 04-06, whereas a total of 143-130 bands were observed in a study done for avocado accessions in Cuba using 16 SSR markers by Ramirez et. al, (2005). The difference in the results obtained in our study and previous studies could be due to the number and levels of polymorphism of the used markers, the selected sample size, and the diversity of the selected germplasm. The size range of the alleles for each marker was within the expected size ranges (bp) as seen in Table 1. The average expected heterozygosity of the present study was 0.7389, according to Table 2. This value is greater than 0.65, the value obtained by Juma et al., (2020), thereby indicating a greater genetic diversity in the local germplasm or different levels of polymorphisms in the SSR markers selected. The expected heterozygosity for each microsatellite marker was greater than 0.5, which indicated their effectiveness in differentiating the selected accessions.

Expected and observed heterozygosity, and Shannon's diversity index are indicators of the range of genetic diversity within a population (Juma et al., 2020). This diversity analysis at the intra-population level indicates a great genetic diversity among the accessions. According to the constructed dendrogram as seen in Figure 3, these accessions can be predicted into 05 major clusters. Selection of distantly related accessions for future breeding purposes can be done by using this dendrogram. By crossing genetically distant accessions as parental lines, maximized genetic variability of offspring can be obtained (Würschum, 2023). All the selected markers in the present study were polymorphic for the selected Sri Lankan avocado accessions, indicating their repeatability in future studies regarding avocado.

Conclusion

The 15 selected Sri Lankan avocado accessions showed considerable morphological diversity, according to Shannon's diversity index, which revealed high variations in traits such as tree shape and leaf texture. The local accessions showed higher genetic diversity among the germplasm with a mean expected heterozygosity of 0.7389. The 04 SSR markers of the present study demonstrated effective polymorphism with high expected heterozygosity. UPGMA clustering grouped the local accessions into five major clusters, indicating the genetic relationships among them.

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References

Fruit Crops – Avacado-S – Department of Agriculture Sri Lanka. (n.d.). https://doa.gov.lk/fruit crops-avacado-s/

Ashworth, V. E. T. M., & Clegg, M. T. (2003). Microsatellite markers in avocado (*Persea americana* Mill.): genealogical relationships among cultivated avocado genotypes. *Journal of Heredity*, 94(5), 407-415. https://doi.org/10.1093/jhered/esg076

Sharon, D., Cregan, P. B., S. Mhameed, M. Kusharska, Hillel, J., E. Lahav, & Lavi, U. (1997). An integrated genetic linkage map of avocado. *Theoretical and Applied Genetics*, 95(5-6), 911–921. https://doi.org/10.1007/s001220050642

Ramírez, I. M., Fuentes, J. L., Rodríguez, N. N., Coto, O., Cueto, J., Becker, D., & Rohde, W. (2005). Diversity analysis of Cuban avocado varieties based on agro-morphological traits and DNA polymorphisms. *Journal of Genetics and Breeding*, 59(3/4), 241.

Hormaza, J. I. (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theoretical and Applied Genetics*, 104(2), 321-328. https://doi.org/10.1007/s001220100684

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the national academy of sciences*, 70(12), 3321-3323. https://doi.org/10.1073/pnas.70.12.3321

Nolan, K. A., & Callahan, J. E. (2006, July). Beachcomber biology: The Shannon–Weiner species diversity index. In *Proceedings of Workshop Table 27* (pp. 334–338).

Würschum, T., Zhu, X., Zhao, Y., Jiang, Y., Reif, J. C., & Maurer, H. P. (2023). Maximization through optimization? On the relationship between hybrid performance and parental genetic distance. *Theoretical and Applied Genetics, 136*(9), 1863–1880.

Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., Devi, O. A., Barooah, M. S., Li, C. G., & Papoutsis, K. (2019). The Odyssey of Bioactive Compounds in Avocado (*Persea americana*) and their Health Benefits. *Antioxidants,* 8(10), 426. https://doi.org/10.3390/antiox8100426

Konwar, T., Sarma, R. N., & Sarmah, B. K. (2025). Genome-wide association analysis of flavour-related metabolites in tea germplasm [*Camellia sinensis* (L.) Kuntze] from Assam using a genotyping by sequencing strategy. *BMC Plant Biology, 25*(1), 1–16. https://doi.org/10.1186/s12870-025-06889-w

Juma, I., Geleta, M., Nyomora, A., Ganapathi Varma Saripella, Hovmalm, H. P., Carlsson, A. S., Moneim Fatih, & Ortiz, R. (2020). Genetic diversity of avocado from the southern highlands of Tanzania as revealed by microsatellite markers. *Hereditas*, 157(1), 1–11.https://doi.org/10.1186/s41065-020-00150-0

Descriptors for Avocado. (n.d.). Retrieved September 14, 2025, from https://www.avocadosource.com/papers/misc/ipgri_1995.pdf

Dionysius, M**.** (n.d.). *Avocado production in Sri Lanka.* Food and Agriculture Organization of the United Nations. https://www.fao.org/4/x6902e/x6902e0a.htm