

Genetic diversity and population structure of Australian-descent and open-pollinated macadamia accessions in Indonesia revealed by SSR markers

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ABSTRACT

Macadamia (*Macadamia integrifolia* Maiden & Betcher) is a highly prized global commodity that thrives in Indonesia's agroclimatic condition. To support germplasm characterization and breeding strategies, the genetic diversity and population structure of 80 macadamia accessions were evaluated using simple sequence repeat (SSR) markers. Of the 20 SSR markers tested, 18 were polymorphic and selected for further analysis. A total of 117 alleles were detected, with an average of 6.5 alleles per locus, indicating moderate to high genetic variability. The mean observed heterozygosity ($H_o = 0.67$) slightly exceeded the expected heterozygosity ($H_e = 0.63$), suggesting a heterozygote excess likely associated with outcrossing and admixture. Polymorphic information content (PIC) values ranged from 0.10 to 0.87, with an average of 0.59, confirming that most markers were highly informative. Cluster analysis based on unweighted pair group method with arithmetic mean (UPGMA) grouped the accessions into three main clusters, largely reflecting their geographic origin and breeding background. Australian-descent accessions predominantly clustered together, while open-pollinated accessions exhibited broader genetic distribution, indicating greater variability. Population structure ($K = 3$) supported these groupings and revealed admixed genotypes, highlighting gene flow and shared ancestry among accessions. Genetic distance analysis identified several highly divergent genotype combinations, which are promising candidates for parental selection in breeding programs. Overall, the results demonstrate substantial genetic variation within populations and relatively weak differentiation among populations, consistent with the biology of outcrossing perennial species. The SSR markers used proved effective for genetic characterization, providing valuable insights for the conservation and improvement of macadamia germplasm.

Key words: Breeding strategy, genetic characterization, genetic distance analysis, germplasm, heterozygosity, *Macadamia* sp.

INTRODUCTION

Macadamia (*Macadamia integrifolia* Maiden & Betcher), a perennial tree species belonging to the family Proteaceae, is native to the subtropical rainforest of eastern Australia (O'Connor et al., 2019; Wang et al., 2022). Among the eight recognized species of macadamia, *M. integrifolia* and *M. tetraphylla* L.A.S. Johnson are the only two that are commercially cultivated for their edible, non-toxic nuts. Macadamia nuts are considered among the most valuable in the global market (Li et al., 2024). In Indonesia, macadamia has demonstrated

adaptability to wide range of environmental conditions, including temperatures between 10-30 °C, low soil nutrient availability, high light intensity, and low humidity. The species thrives in highland areas situated at elevations above 1000 m a.s.l. (Widiastuti et al., 2021). Unlike their counterparts in their native Australian habitat, which flower and fruit once annually, macadamia trees grown in Indonesia are capable of flowering and fruiting throughout the year. These adaptive traits highlight the significant potential for macadamia cultivation and commercial development in Indonesia.

Despite its promise, macadamia cultivation in Indonesia remains limited, currently confined to government and private plantations. A major constraint to broader development is the lack of certified superior seedlings. Additionally, the limited genetic information and the narrow germplasm collection present significant challenges, as both are essential for enhancing plant quality through effective breeding and selection programs. Addressing these limitations requires the establishment of targeted germplasm collection gardens and comprehensive genetic studies of existing materials. Understanding the extent and structure of genetic diversity is particularly important for the genetic improvement of clonally propagated crops like macadamia. Furthermore, insight into population structure and natural genetic variation will be instrumental for the development of high-yielding, well-adapted varieties (Nock et al., 2019).

Extensive studies on the genetic diversity of macadamia have been conducted across multiple continents. Knowledge of genetic diversity is important for assessing population variability, guiding breeding strategies, enhancing yield potential, and supporting conservation initiatives (Govindaraj et al., 2015; Swarup et al., 2021). Additionally, insights into cultivar relatedness, genetic structure, and diversity play a critical role in improving breeding programs by preventing inbreeding and ensuring the maintenance of genetically diverse breeding materials (Collard and Mackill, 2008; Ranketse et al., 2022). In China, whole-genome resequencing of macadamia germplasm revealed that Australian accessions exhibited the highest genetic diversity, followed by Chinese accessions, while those introduced from USA showed the lowest levels of genetic diversity (Li et al., 2024). Similarly, Ranketse et al. (2022) investigated the genetic diversity of macadamia accessions in South Africa, which included both introduced materials and locally selected materials. In the present study, we assessed macadamia accessions introduced from Australia that have adapted to the Indonesian environment, along with their progenies derived through open pollination, using simple sequence repeat (SSR) markers.

Molecular marker genotyping has become the most widely used method for characterizing genetic diversity in plant breeding populations (O'Connor et al., 2019). The DNA molecular markers enable the direct detection of genetic variation at the DNA level among different varieties. This approach is rapid, efficient, and unaffected by environmental influences, eliminating the need for field evaluation (Yin et al., 2023). Additionally, DNA markers serve as a powerful tool to assess the genetic fidelity of breeding materials (Kho et al., 2026). Among the various options, SSR markers are widely favored for genetic identification and diversity assessment due to their codominant inheritance, high reproducibility, rich polymorphism, and cost-effectiveness (Wang et al., 2022). This study aimed to characterize the genetic relationships and population structure of Australian-descent macadamia accessions and their open-pollinated progenies using SSR markers.

MATERIALS AND METHODS

Plant materials and DNA extraction

A total of 80 macadamia (*Macadamia integrifolia* Maiden & Betcher) accessions were collected from the PT Mitra Kerinci plantation in Solok Regency, West Sumatra, Indonesia. Among these, 20 accessions were of Australian descent, while the remaining 60 accessions were derived from open pollination of the Australian-descended accessions. Young, healthy leaves from each accession were sampled, placed in plastic bag containing silica gel. Upon arrival at the laboratory, all samples were immediately stored at -80 °C until further analysis.

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). The dried DNA pellets were resuspended in 50 µL TE buffer (10 mM Tris, pH 8.0; 1 mM EDTA), and DNA quality was assessed by 1% agarose gel electrophoresis. The DNA concentration and purity were measured using an Implen NanoPhotometer N60 UV/Vis spectrophotometer (Implen GmbH, Munich, Germany). All DNA samples were normalized to a final concentration of 10 ng µL⁻¹ with sterile deionized water and stored at -20 °C for subsequent analyses.

SSR-based DNA profiling

Twenty simple sequence repeat (SSR) markers specific to macadamia were used to amplify DNA from the 80 collected accessions (Ranketse et al., 2022; Wang et al., 2022). The polymerase chain reaction (PCR) amplification was carried out in a 15 µL reaction volume containing 10 ng genomic DNA, 1x PCR master mix, and SSR primers at a final concentration of 0.2 µM. The thermal cycling conditions were as follows: Initial denaturation at 95 °C for 10 min; 35 cycles of denaturation at 94 °C for 20 s, annealing at 53-57 °C for 30 s, and extension at 72 °C for 30 s; followed by a final extension at 72 °C for 7 min and a hold at 12 °C for 5 min. The PCR products were separated on a 6% non-denaturing polyacrylamide gel using 1x TBE buffer. Gels were stained with ethidium bromide for 20 min, and DNA bands were visualized under UV light using a transilluminator and documented with a digital imaging system.

Genetic diversity and population structure analysis

The PCR visualization results, represented as DNA banding patterns, were converted into binary data: A score of '1' was assigned for the presence of a band at a specific allele, and '0' for its absence. The binary data were analyzed using PowerMarker version 3.25 (Liu and Muse, 2005) to calculate the number of alleles, major allele frequency, gene diversity, observed heterozygosity and polymorphic information content (PIC).

To assess genetic diversity and relationships among accessions, similarity coefficients were calculated using the sequential agglomerative hierarchical and nested (SAHN) clustering algorithm with the unweighted pair group method with arithmetic mean (UPGMA) implemented in NTSYS version 2.02 (Rohlf, 2000). The resulting data was used to construct a phylogenetic tree. In addition, population structure was analyzed using STRUCTURE software (Pritchard et al., 2000) to infer the underlying genetic diversity and subpopulation structure within the macadamia accessions. The optimal number of genetic clusters (K) was determined by performing five independent runs for each K value, using a burn-in period of 10 000 iterations followed by 10 000 Markov chain Monte Carlo (MCMC) repetitions. The analysis employed a model incorporating admixture and correlated allele frequencies (Falush et al., 2003). The most likely value of K was identified using the ΔK method (Evanno et al., 2005), as implemented in the web-based tool STRUCTURE Harvester (Earl and von Holdt, 2012).

RESULTS AND DISCUSSION

Genetic variation of macadamia accessions using SSR Markers

Twenty simple sequence repeat (SSR) markers were employed to assess the genetic diversity of 80 macadamia accessions. Of these, 18 markers displayed clear polymorphism, indicating their effectiveness in differentiating the accessions and therefore were selected for subsequent analyses. To confirm the robustness and applicability of these previously reported markers in macadamia, we further evaluated their informativeness by calculating relevant statistical parameters, including allelic diversity and polymorphic information content (Table 1).

A total of 117 alleles were detected across the SSR markers, with an average of 6.5 alleles per locus, ranging from 2 (Mac009, Mac013 and mg7SSR3) to 10 (Mac011). The degree of polymorphism at each locus was further assessed by examining alleles frequencies. The major allele frequency varied from 0.18 (Mac002) to 0.94 (Mac013), with an average of 0.51, indicating substantial variation among loci. Observed heterozygosity (H_o) ranged from 0.06 (Mac013) to 0.99 (Mac010, Mac019 and mgSSR12), while expected heterozygosity (H_e) ranged from 0.11 (Mac013) to 0.88 (Mac011). On average, the observed heterozygosity (0.67) slightly exceeded the expected heterozygosity (0.63), suggesting a moderate excess of heterozygotes in the population. The polymorphic information content (PIC) values ranged from 0.10 (Mac013) to 0.87 (Mac011), with a mean of 0.59, confirming that most markers used in this study were highly informative for assessing genetic diversity. These results demonstrate that the SSR markers employed provide sufficient resolution to capture genetic variation within macadamia, making them valuable tools for diversity assessment and future breeding applications.

In this study, a total of 117 alleles were detected, which is lower than the 169 alleles reported by Ranketse et al. (2022) for local South African macadamia accessions together with introduced accessions from Hawaii and Australia. Such differences likely reflect variation in the genetic backgrounds of the accessions analyzed and the number of SSR markers applied, rather than a direct reduction in genetic diversity. Notably, our analysis included five SSR markers that were not used in the study by Ranketse et al. (2022). Despite the lower allele count, the results provide meaningful insights into the genetic variability of the studied macadamia accessions and highlight their potential value for breeding programs. Furthermore, the observed heterozygosity (H_o) slightly exceeding

expected heterozygosity (H_e) suggests the presence of heterozygote excess in the population. This pattern could result from admixture of previously isolated genetic groups, non-random mating such as disassortative mating, or selection favoring heterozygotes. A negative inbreeding coefficient further supports the presence of this heterozygote surplus (Allendorf et al., 2013; De Meeûs, 2018). On the other hand, approximately 77.8% of the SSR markers used in present study were classified as highly polymorphic and informative ($PIC > 0.5$) according to the criteria of Botstein et al. (1980). Of the remaining markers with lower informativeness, four exhibited moderate ($0.25 < PIC \leq 0.5$) or low ($PIC \leq 0.25$) PIC values. Particularly, marker Mac013, which showed a low PIC value in this study, was also reported to have low informativeness in previous investigations (Langdon et al., 2019; Ranketse et al., 2022). Despite its limited individual discriminatory power, Mac013 remains useful when applied in combination with other markers for DNA fingerprinting and accession identification. Overall, the SSR markers employed here proved effective in differentiating Australian-descent macadamia accessions that have adapted to the Indonesian climate.

Table 1. Summary statistics of 18 polymorphic simple sequence repeat (SSR) markers used for genetic diversity analysis. PIC: Polymorphic information content.

SSR Marker	Allele number	Major allele frequency	Gene diversity	Observed heterozygosity	PIC
Mac001	7	0.46	0.67	0.94	0.62
Mac002	9	0.18	0.87	0.95	0.86
Mac005	9	0.36	0.75	0.62	0.72
Mac007	8	0.53	0.68	0.74	0.65
Mac008	9	0.53	0.67	0.50	0.64
Mac009	2	0.73	0.39	0.49	0.32
Mac010	8	0.31	0.83	0.99	0.81
Mac011	10	0.2	0.88	0.98	0.87
Mac013	2	0.94	0.11	0.06	0.10
Mac014	4	0.72	0.42	0.41	0.37
Mac017	7	0.53	0.67	0.80	0.64
Mac018	8	0.55	0.65	0.67	0.62
Mac019	6	0.49	0.66	0.99	0.61
mg2SSR26	7	0.57	0.60	0.52	0.55
mg4SSR26	9	0.21	0.85	0.96	0.84
mg7SSR3	2	0.85	0.26	0.025	0.22
mg8SSR43	3	0.56	0.58	0.47	0.52
mg9SSR12	7	0.38	0.76	0.99	0.73
Mean	6.5	0.51	0.63	0.67	0.59

Phylogenetic relationships among macadamia accessions

Genetic relationships among 80 macadamia accessions were assessed using the unweighted pair group method with arithmetic mean (UPGMA) clustering method, which generated a phylogenetic tree with similarity coefficients ranging from 0.43 to 0.94, indicating considerable genetic variation. At a similarity threshold of 0.53, the accessions were clustered into three main groups (Figure 1). Most accessions (77) were clustered in Group I, while the remaining three accessions formed Groups II and III. At a higher similarity coefficient of 0.62, Group I was further divided into three subgroups: Subgroup I (62 accessions), Subgroup II (5 accessions), and Subgroup III (10 accessions).

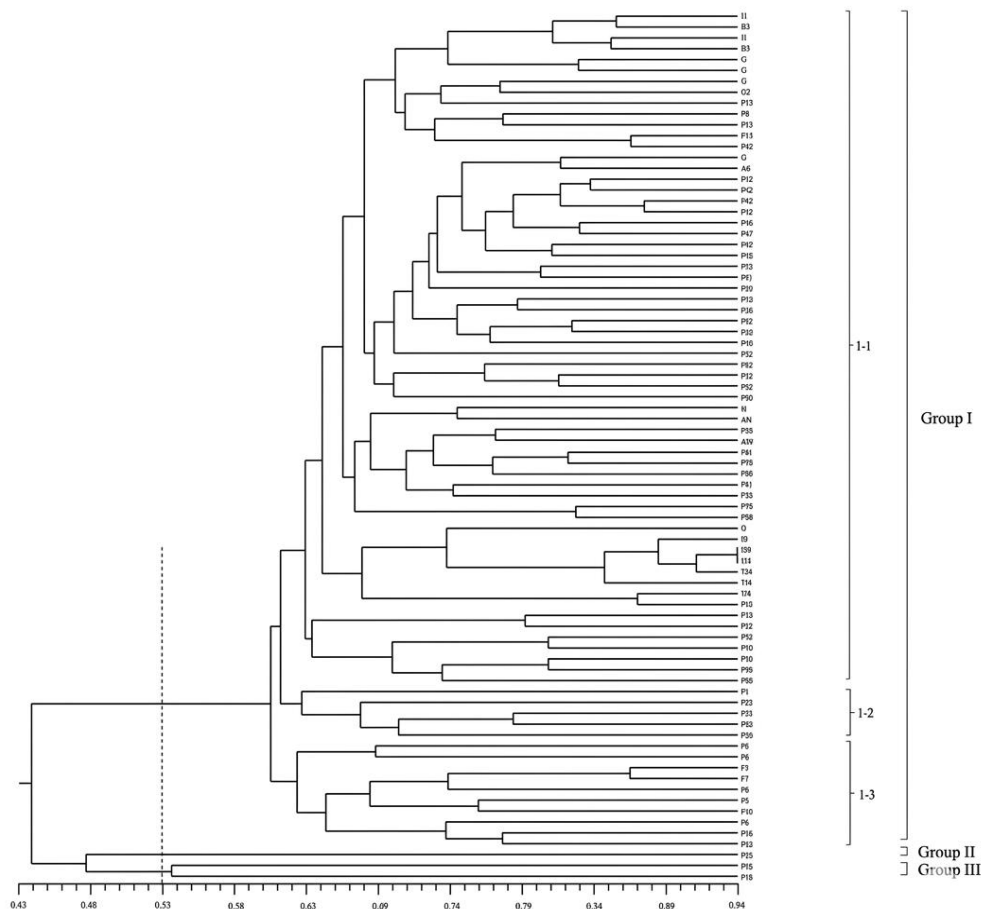


Figure 1. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram depicting the genetic relationships among 80 macadamia accessions based on simple sequence repeat (SSR) markers.

Interestingly, all 20 Australian-descent macadamia accessions were consistently grouped within Group I, specifically in Subgroup I, indicating a close genetic relationship among accessions from the same geographic origin. In contrast, Groups II and III were predominantly composed of open-pollinated accessions, which may reflect broader genetic variation within the local populations. These results align with the findings of Ranketse et al. (2022), who showed that South African selected macadamia germplasm tends to cluster according to region of origin, with strong differentiation between Australian, Hawaiian, and local South African lines. Additionally, studies of wild macadamia germplasm have similarly revealed phylogeographic structure: For example, a study of 302 wild accessions using thousands of single-nucleotide polymorphisms (SNPs) and silicoDArT markers identified distinct genetic groups corresponding to *M. integrifolia*, *M. tetraphylla*, *M. janseni*/*M. ternifolia*, and hybrids (Mai et al., 2020). Our study focused on accessions originating from Australia and Indonesia (open-pollinated accessions), whereas Ranketse et al. (2022) included materials from Australia, Hawaii, and South Africa, and Mai et al. (2020) examined multiple macadamia species distributed across eastern Australia. The consistent clustering patterns observed across these studies highlight the strong influence of geographic origin on the genetic structure of macadamia germplasm.

Notably, several open-pollinated accessions in our study clustered with Australian-descent macadamia accessions in Subgroup I, likely reflecting parental relatedness resulting from natural cross-pollination. High outcrossing rates and pollen-mediated gene flow in *Macadamia* have been confirmed in recent studies conducted by Richards et al. (2020), Trueman et al. (2024) and Zheng et al. (2026), supporting the possibility that open-pollinated genotypes may genetically group with introduced parental lines. This finding demonstrates that the development of modern cultivars has been strongly influenced by extensive hybridization among macadamia genotypes.

We also calculated pairwise genetic distances among the macadamia accessions to quantify genetic divergence between genotypes. Estimates of genetic distance provide breeders with an objective criterion for selecting parental combinations that maximize genetic complementarity, manage inbreeding, and potentially increase hybrid performance (Geng et al., 2021; Würschum et al., 2023). In this study, seven genotype pairs exhibited high genetic distance values (> 0.80), including P55-P19 (0.86), P19-I14 (0.85), P41-P19 (0.84), P42-P19 (0.81), P49-P19 (0.81), P50-P19 (0.81), and P52-P19 (0.81). The substantial divergence among these genotypes indicates a broad genetic base, which is advantageous for hybridization programs. Similar approaches have been applied in previous study, where parental selection based on molecular marker data has been used to broaden genetic diversity and identify superior breeding combinations (Nock et al., 2014; Langdon et al., 2020). Therefore, these genotype pairs can be recommended as promising parental combinations for future macadamia breeding programs to maximize genetic gain and maintain population diversity.

Population structure of 80 macadamia accessions

Population structure analysis of the 80 macadamia accessions revealed a clear ΔK peak at $K = 3$, indicating that the accessions can be classified into three distinct genetic populations (Figure 2). Among these, Population 2 contained the largest number of accessions (41; 51%), followed by Population 1 (33; 41%) and Population 3 (6; 8%) (Figure 3; Table 2). Population structure analysis infers genetic relationships by assigning individuals to clusters based on their degree of relatedness, which are visually represented by different colors corresponding to genetically differentiated groups (O'Connor et al., 2019). In this study, accession classification was determined using a membership threshold of 0.80 to distinguish pure genotypes from admixed individuals (membership coefficient < 0.80), following Niu et al. (2019). Based on this criterion, 14 accessions were categorized as admixed genotypes (Figure 3).

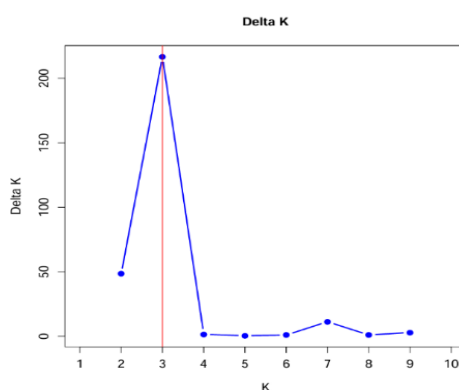


Figure 2. Estimation of optimal number of populations by ΔK at $K = 3$. K: Optimal number of genetic clusters.

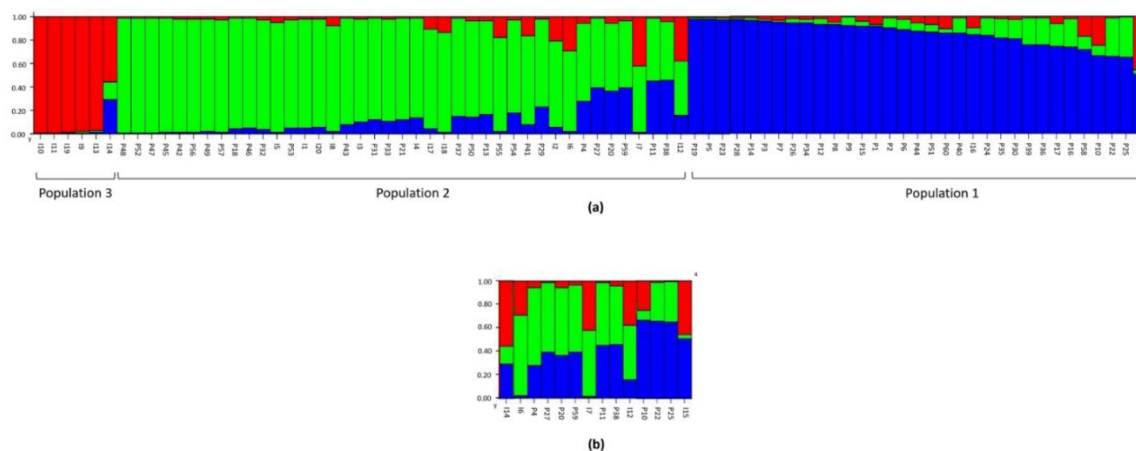


Figure 3. Structure clustering of 80 macadamia accessions at $K = 3$. Each vertical bar represents an accession, and colors indicate population clusters. K: Optimal number of genetic clusters.

Table 2. Proportion of membership and genetic variation within and among 80 macadamia accessions. PIC: Polymorphic information content.

Population	Number of accessions	Proportion of membership	Mean number of alleles	Mean expected heterozygosity	Mean observed heterozygosity	Mean PIC value
Population 1	33	0.41	6.00	0.64	0.64	0.59
Population 2	41	0.51	6.06	0.60	0.69	0.57
Population 3	6	0.08	2.89	0.46	0.65	0.40
Average			4.98	0.57	0.66	0.52

The clustering pattern obtained from the STRUCTURE analysis was largely consistent with that of the UPGMA dendrogram. However, several accessions assigned to Group I in the UPGMA dendrogram were further subdivided into three distinct groups in the STRUCTURE analysis, indicating the higher resolution of genetic differentiation achieved by the model-based approach. Australian-descent macadamia accessions were distributed across all three groups. In Groups I and II, these accessions clustered together with open-pollinated accessions, suggesting a close genetic relationship. In contrast, several introduced accessions formed a distinct cluster in Group III, indicating a degree of genetic differentiation. This distribution likely reflects differences in genetic background and breeding history. The clustering of Australian-descent and open-pollinated accessions in Groups I and II suggests shared ancestry or gene flow, possibly resulting from the use of related parental lines or natural hybridization following introduction. Conversely, the distinct grouping of some Australian-descent accessions in Group III may indicate unique genetic backgrounds, limited gene flow, or independent selection histories prior to their introduction.

Similar patterns have been reported in previous macadamia genetic diversity studies, where introduced and cultivated accessions were not clearly separated but instead exhibited substantial admixture, reflecting a narrow genetic base and shared ancestry among cultivars (O'Connor et al., 2019; Nock et al., 2019). Furthermore, the predominance of genetic variation within populations, rather than among populations, supports the relatively weak genetic differentiation observed among accessions. This pattern is typical of outcrossing, long-lived species, in which mating systems that promote gene flow and recombination facilitate the accumulation and maintenance of genetic diversity. Such genetic characteristics are important for enhancing adaptive potential and increasing resilience to environmental change (Yang et al., 2025). However, the presence of distinct clusters, as observed for Group III in this study, has also been reported and is often associated with accessions derived from different breeding programs or geographic origins, highlighting the existence of unique and potentially underutilized genetic diversity within macadamia germplasm (Zheng et al., 2026).

Calculations of genetic diversity across the three macadamia populations revealed that the mean H_o was slightly higher than the mean H_e in all populations (Table 2). This pattern indicates an excess of heterozygotes, suggesting prevalent outcrossing and possible admixture among accessions. The observed excess of heterozygosity ($H_o > H_e$) may reflect factors such as frequent hybridization, gene flow among populations, or selection favoring heterozygous individuals. In terms of marker informativeness, Population 1 exhibited a higher PIC value than Populations 2 and 3 (Table 2), indicating a greater level of allelic diversity and discriminatory power of the markers within this group. Additionally, the higher PIC value in Population 1 suggests that it may harbor more diverse or informative alleles, potentially making it a valuable genetic resource for breeding programs and conservation efforts.

CONCLUSIONS

This study demonstrates that simple sequence repeat (SSR) markers are effective tools for assessing genetic diversity and population structure in macadamia. The relatively high levels of allelic variation and polymorphic information content indicate that the selected markers provide sufficient resolution for differentiating accessions. The observed excess of heterozygosity suggests prevalent outcrossing, gene flow, and admixture among the evaluated germplasms. Both cluster and population structure analyses consistently revealed three genetic groups, with patterns largely influenced by geographic origin and breeding history. The close clustering of Australian-descent and open-pollinated accessions highlights shared ancestry and the important role of

hybridization in shaping modern macadamia cultivars. At the same time, the presence of distinct genetic clusters indicates the existence of unique and potentially underutilized genetic resources.

The predominance of genetic variation within populations and the identification of highly divergent genotype pairs provide valuable insights for breeding programs. These findings can support the selection of genetically diverse parental combinations to maximize heterosis, maintain genetic diversity, and enhance long-term crop improvement. Overall, this study contributes important genetic information for the conservation, management, and strategic utilization of macadamia germplasm.

Author contribution

Conceptualization: N.K.I., M.S.D.I. Methodology: N.K.I., N.K.F. Software: C.T. Validation: R.R., D.D. Formal analysis: N.K.I., C.T. Investigation: N.K.F., S.S., E.R., D.H. Data curation: M.S.D.I., N.K.F., S.S., E.H.P. Writing original draft: N.K.I. Writing-review & editing: M.S.D.I. Visualization: E.H.P., D.D. Supervision: R.R., D.H. Project administration: S.S., E.R. Funding acquisition: M.S.D.I. All co-authors reviewed the final version and approved the manuscript before submission.

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References

- Allendorf, F.W., Luikart, G., Aitken, S.N. 2013. Conservation and the genetics of populations. 2nd ed. Wiley-Blackwell, Hoboken, New Jersey, USA.
- Botstein, D., White, R.L., Skolnick, M., Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32(3):314-331.
- Collard, B.C.Y., Mackill D.J. 2008. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B Biological Science*. 363(1491):557-572.
- De Meeûs, T. 2018. Revisiting F_{IS} , F_{ST} , Wahlund effects, and null alleles. *Heredity* 120(6):617-627. doi:10.1093/jhered/esx106.
- Doyle J., Doyle J. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19:11-15.
- Earl, D.A., von Holdt B.M. 2012. Structure harvester: a website and program for visualizing structure output and implementing the Evanno method. *Conservation Genetics Resources* 4(2):359-361.
- Evanno, G., Regnaut, S., Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* 14(8):2611-2620.
- Falush, D., Stephens, M., Pritchard, J.K. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164:1567-1587.
- Geng, X., Qu, Y., Jia, Y., He, S., Pan, Z., Wang, L., et al. 2021. Assessment of heterosis based on parental genetic distance estimated with SSR and SNP markers in upland cotton (*Gossypium hirsutum* L.) *BMC Genomics* 22:123. doi:10.1186/s12864-021-07431-6.
- Govindaraj, M., Vetriventhan, M., Srinivasan, M. 2015. Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. *Genetics Research International* 2015:431487. doi:10.1155/2015/431487.
- Kho, P.E., Rejab, N.A., Khalid, N., Liang, S.S., Mercer, Z.J.A., Hwang, S.S., et al. 2026. Genetic fidelity assessment of micropropagated pepper (*Piper nigrum* L.) plantlets using ISSR markers. *Chilean Journal of Agricultural Research* 86:725-731. doi:10.4067/S0718-58392026000500725.
- Langdon, K.S., King, G.J., Baten, A., Mauleon, R., Bundock, P.C., Topp, B.L., et al. 2020. Maximising recombination across macadamia populations to generate linkage maps for genome anchoring. *Scientific Reports* 10:5048. doi:10.1038/s41598-020-61708-6.
- Langdon, K.S., King, G.J., Nock, C.J. 2019. DNA paternity testing indicates unexpectedly high levels of self-fertilisation in macadamia. *Tree Genetics & Genomes* 15:29.
- Li, Z., Wu, C., Ma, J., Geng, J., Tao, L., He, X., et al. 2024. Genetic diversity analysis of macadamia germplasm in China based on whole-genome resequencing. *Tree Genetics & Genomes* 20:14. doi:10.1007/s11295-024-01648-8.
- Liu, K., Muse, S.V. 2005. PowerMarker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21:2128-2129.
- Mai, T., Alam, M., Hardner C., Henry R., Topp, B. 2020. Genetic structure of wild germplasm of Macadamia: Species assignment, diversity and phylogeographic relationships. *Plants* 9:714. doi:10.3390/plants9060714.
- Niu, S., Song, Q., Koiwa, H., Qiao, D., Zhao, D., Chen, Z., et al. 2019. Genetic diversity, linkage disequilibrium, and population structure analysis of the tea plant (*Camellia sinensis*) from an origin center, Guizhou plateau, using genome wide SNPs developed by genotyping-by sequencing. *BMC Plant Biology* 19:328. doi:10.1186/s12870-019-1917-5.

- Nock, C.J., Elphinstone, M.S., Ablett, G., Kawamata, A., Hancock, W., Hardner, C.M., et al. 2014. Whole genome shotgun sequences for microsatellite discovery and application in cultivated and wild macadamia (Proteaceae). *Applications in Plant Sciences* 2(4):1300089. doi:10.3732/apps.1300089.
- Nock, C.J., Hardner, C.M., Montenegro, J.D., Termizi, A.A.A., Hayashi, S., Playford, J., et al. 2019. Wild origins of macadamia domestication identified through intraspecific chloroplast genome sequencing. *Frontiers in Plant Science* 10:334. doi:10.3389/fpls.2019.00334.
- O'Connor, K., Kilian, A., Hayes, B., Hardner, C., Nock, C., Baten, A., et al. 2019. Population structure, genetic diversity and linkage disequilibrium in a macadamia breeding population using SNP and silicoDART markers. *Tree Genetics & Genomes* 15:24. doi:10.1007/s11295-019-1331-z.
- Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- Ranketse, M., Hefer, C.A., Pierneef, R., Fourie, G., Myburg, A.A. 2022. Genetic diversity and population structure analysis reveals the unique genetic composition of South African selected macadamia accessions. *Tree Genetics & Genomes* 18:15. doi:10.1007/s11295-022-01543-0.
- Richards, T.E., Kämper, W., Trueman, S.J., Wallace, H.M., Ogbourne, S.M., Brooks, P.R., et al. 2020. Relationships between nut size, kernel quality, nutritional composition and levels of outcrossing in three macadamia cultivars. *Plants* 9(2):228. doi:10.3390/plants9020228.
- Rohlf, F.J. 2000. NTSYS-PC, numerical taxonomy system for the PC, ExeterSoftware, Ver. 2.1. Applied Biostatistics, Setauket, New York, USA.
- Swarup, S., Cargill, E.J., Crosby, K., Flagel, L., Kniskern, J., Glenn, K.C. 2021. Genetic diversity is indispensable for plant breeding to improve crops. *Crop Science* 61:839-852. doi:10.1002/csc2.20377.
- Trueman, S.J., Penter, M.G., Malagodi-Braga, K.S., Nichols, J., De Silva, A.L., Ramos, A.T.M., et al. 2024. High outcrossing levels among global macadamia cultivars: implications for nut quality, orchard designs and pollinator management. *Horticulturae* 10(3):203. doi:10.3390/horticulturae10030203.
- Wang, P., Mo, Y., Wang, Y., Fei, Y., Huang, J., Ni, J., et al. 2022. Macadamia germplasm and genomic database (MacadamiaGGD): A comprehensive platform for germplasm innovation and functional genomics in *Macadamia*. *Frontiers Plant Science* 13:1007266. doi:10.3389/fpls.2022.1007266.
- Widiastuti, A.V., Asyiah, I.N., Pujiastuti, P. 2021. Morphology and economic value of macadamia (*Macadamia integrifolia* Maiden and Betcher). *Berkala Saintek* 9(4):153-159.
- 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* 136:186. doi:10.1007/s00122-023-04436-5.
- Yang, G., Ma, T., Wang, Z. 2025. Genetic diversity and population structure of *Sphaeropteris brunoniana* based on SSR molecular markers. *Global Ecology and Conservation* 62:e03720. doi:10.1016/j.gecco.2025.e03720.
- Yin, J., Zhao, H., Wu, X., Ma, Y., Zhang, J., Li, Y., et al. 2023. SSR marker-based analysis for identification and of genetic diversity of non-heading Chinese cabbage varieties. *Frontiers in Plant Science* 14:1112748. doi:10.3389/fpls.2023.1112748.
- Zheng, C., Liu, Z., Liu, N., Li, C., Niu, Y., Mao, C., et al. 2026. Genetic diversity analysis and core collection construction of *Macadamia* spp. from Yunnan using fourfold degenerate SNP sites. *BMC Plant Biology* 26:840. doi:10.1186/s12870-026-08663-y.