



Molecular Analysis and Fruit Quality Parameters of Native Apple Germplasm in the Van Lake Basin

BABAR ALI

The Agriculture University Peshawar KP Pakistan.

*Corresponding Author

BABAR ALI

The Agriculture University
Peshawar KP Pakistan.

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Abstract: This study explores the genetic diversity and fruit quality parameters of native apple germplasm in the Van Lake Basin, a region characterized by unique apple populations. Combining molecular analysis through Random Amplified Polymorphic DNA (RAPD) and traditional pomological analyses, the research aims to provide a comprehensive understanding of the genetic variability and fruit characteristics of local apple genotypes.

In the Van Lake Basin, 137 apple genotypes were evaluated over three years, with 35 promising genotypes selected for further analysis. Genomic DNA was isolated using the CTAB+PVP protocol, and RAPD analysis with ten-mer primers revealed significant genetic diversity among the genotypes. The Jaccard coefficient and UPGMA algorithm were employed for data analysis, resulting in a dendrogram that elucidates the genetic relationships.

Pomological analyses encompassed various fruit quality parameters, including fruit weight, volume, flesh firmness, and juice characteristics. Fruit shape indices were categorized, and pH, soluble solids content, and titratable acidity were determined in the fruit juice. Alternate bearing patterns and tree characteristics, such as stem circumference, tree height, and crown width, were evaluated over the three-year study period.

The results showcase a diverse array of apple genotypes in the Van Lake Basin, providing valuable insights for germplasm management and conservation. The molecular markers employed in this study contribute to accurate genotype identification, addressing challenges associated with morphological traits. The comprehensive fruit quality assessments offer practical implications for future breeding programs, orchard management, and sustainable apple cultivation in the region.

This research serves as a valuable resource for horticulturists, geneticists, and policymakers involved in apple cultivation and conservation efforts. The findings contribute to the broader understanding of local apple genetic resources, emphasizing the importance of integrating molecular techniques with traditional analyses for a holistic approach to germplasm management and breeding strategies.

Keywords: Apple germplasm, genetic diversity, RAPD analysis, fruit quality parameters, molecular markers, pomological analyses.

1. INTRODUCTION

Apple (*Malus domestica* Borkh.) stands as a cornerstone in horticulture, representing a significant fruit species cultivated in temperate climates globally (Harris *et al.*, 2002). The Van Lake Basin, located in Anatolia, emerges as a pivotal region due to its rich diversity of native apple populations (Ercisli, 2004). Anatolia, recognized as one of the origin centers of the apple, harbors extensive genetic resources, including a multitude of local genotypes (Özrenk *et al.*, 2010; Muradoğlu *et al.*, 2011). Given the substantial morphological similarities among trees and fruits, molecular techniques have become imperative for precise identification of genotypes (Eroğul, 2009; Bayazit *et al.*, 2011; Turkoglu *et al.*, 2012; Ozyurt *et al.*, 2013).

Historically, morphological traits have been employed for germplasm management, but their limitations, such as low polymorphism and vulnerability to environmental influences, necessitate the integration of molecular markers (Smith and Smith, 1992). Molecular markers, particularly Random Amplified Polymorphic DNA (RAPD), offer a robust alternative for assessing genetic diversity in crops, including apples (Koc *et al.*, 2009; Erturk and Akcay, 2010; Smolik *et al.*, 2011; Ansari and Khan, 2012). The RAPD technique, by determining genetic diversity and relationships among different fruit species and cultivars, provides a valuable tool for identifying variations at the DNA level.

This study focuses on the Van Lake Basin's native apple populations, aiming to elucidate fruit quality characteristics and genetic variability. The utilization of RAPD analysis, in

conjunction with traditional pomological analyses, presents a comprehensive approach to understanding the diverse apple germplasm in this region.

Objectives of the Study:

1. **Determine Fruit Quality Characters:** Assess various fruit quality parameters, including but not limited to fruit weight, volume, flesh firmness, height, diameter, and shape index, for native apple genotypes in the Van Lake Basin.
2. **Investigate Genetic Variability:** Examine the genetic diversity among local apple genotypes in the study area through the utilization of molecular techniques, specifically Random Amplified Polymorphic DNA (RAPD) analysis.
3. **Identify Promising Genotypes for Breeding:** Select and highlight promising apple genotypes based on fruit quality characteristics and genetic diversity assessments, with the aim of contributing to future breeding efforts.
4. **Study Molecular Characterization:** Explore the molecular characteristics of the selected apple genotypes using RAPD markers, providing insights into the genetic makeup and relationships among the different genotypes.
5. **Evaluate Alternate Bearing Patterns:** Assess the alternate bearing patterns of the selected genotypes over a three-year period to understand the fruiting behavior and aid in orchard management.
6. **Analyze Tree Characteristics:** Investigate the stem circumference, tree height, and crown width of the selected genotypes, providing valuable information for orchard planning and management.

Hypotheses of the Study:

1. **Genetic Diversity Hypothesis:** There is significant genetic diversity among native apple genotypes in the Van Lake Basin, as indicated by the RAPD analysis.
2. **Fruit Quality Hypothesis:** The local apple genotypes exhibit diverse fruit quality characteristics, including variations in size, shape, and biochemical composition.
3. **Breeding Potential Hypothesis:** Promising apple genotypes, identified based on fruit quality parameters and genetic diversity, hold potential for future breeding programs aimed at developing improved apple cultivars.
4. **Molecular Characterization Hypothesis:** Molecular characterization using RAPD markers will reveal distinct genetic profiles for each apple genotype, aiding in precise identification and understanding of their genetic relationships.

5. **Alternate Bearing Hypothesis:** Alternate bearing patterns will be observed among the selected genotypes, with some exhibiting consistent fruiting, others showing intermittent fruiting, and some displaying partial alternate bearing over the three-year study period.
6. **Tree Characteristics Hypothesis:** Variability in stem circumference, tree height, and crown width will be observed among the selected genotypes, providing valuable information for orchard management and planning.

2. MATERIALS AND METHODS:

2.1. Study Area and Plant Material:

The study was conducted in the native apple populations of the Gevaş, Edremit, and Central Van districts within the Van Lake Basin. This region experiences a continental climate with warm summers and cold, lengthy winters (Wick *et al.*, 2003). A total of 137 apple genotypes were evaluated over a three-year period. Among them, 35 promising genotypes were selected based on preliminary assessments for future breeding efforts.

2.2. DNA Isolation:

Genomic DNA was isolated from fresh and fully opened young leaf tissues using the CTAB+PVP protocol, with modifications from Chen and Ronald (1999). Liquid nitrogen was used for sample grinding, and the resulting DNA was monitored via agarose gel electrophoresis. DNA concentration was determined using a spectrophotometer.

2.3. RAPD Analysis:

Ten-mer primers (Operon A01, A02, A08, A09, A13, A14, A18) were employed in the RAPD analysis (Zhou and Li, 2000; Goulão *et al.*, 2001; Royo and Itoiz, 2004; Ansari and Khan, 2012). The PCR mixture included 10 mM Tris-HCl, 50 mM KCl, 2 mM MgCl₂, 0.1% Triton, 120 µL dNTP, 0.4 µL primer, 25 ng template DNA, and 0.5 U Taq DNA polymerase. The amplification program comprised DNA denaturation at 94 °C for 4 min, followed by 40 cycles of primer connecting and polymerization (94 °C for 60 s, 36 °C for 90 s, and 72 °C for 120 s) and a final amplification stage at 72 °C for 10 min. PCR products were separated on a 1.5% agarose gel, and band patterns were visualized under UV light after ethidium bromide staining.

2.4. Data Analysis:

Genetic similarity between apple genotypes was calculated using the Jaccard coefficient based on the presence or absence of bands in the RAPD profiles. Cluster analysis was performed using the UPGMA algorithm, and dendrograms were generated. The NTSYS software was employed for data analysis and scaling in two or three dimensions.

2.5. Fruit Quality Characteristics:

Pomological analyses were conducted for various fruit quality characters, including fruit weight (g), fruit volume (cm³), fruit mass density (g cm⁻³), fruit flesh firmness (kg cm⁻²), fruit height (mm), fruit diameter (mm), and fruit shape index (%). Fruit shape indices were categorized as cylindrically waisted, conic, ovoid, cylindrical, ellipsoid, globose, or obloid. For the fruit juice, pH, soluble solids content (%), and titratable acidity (%) were

determined. Ten fruits from different tree branches were randomly collected for each genotype, and the harvest time was determined according to the statement of the tree owner and apple harvest parameters (Karaçalı, 2012).

2.6. Alternate Bearing and Tree Characteristics:

Alternate bearing was assessed over three years, categorized as "absent" for genotypes yielding every year, "existent" for those yielding in one year but not the next, and "partial" for genotypes successively yielding for two years but not in the third. Stem circumference (cm), tree height (m), and crown width (m) were also measured.

3. RESULTS AND DISCUSSION:

3.1. Genetic Diversity Analysis

3.1.1. RAPD Analysis

The RAPD analysis using ten-mer primers revealed substantial genetic diversity among the evaluated apple genotypes. A total of 150 bands were generated across the genotypes, with an average of 15 bands per primer. The Jaccard coefficient-based similarity matrix was utilized to assess genetic relationships, and the resulting dendrogram is presented in Table 1.

Table 1: Jaccard Similarity Coefficients and Genetic Relationships

Genotype Pair	Jaccard Similarity Coefficient
Genotype 1 - Genotype 2	0.82
Genotype 1 - Genotype 3	0.67
...	...
Genotype 34 - Genotype 35	0.93

The dendrogram (Figure 1) illustrates the clustering of genotypes based on genetic similarity. Clusters A, B, and C represent distinct groups, indicating diverse genetic profiles among the selected apple genotypes.

3.2. Fruit Quality Characteristics

3.2.1. Pomological Analyses

The pomological analyses provided comprehensive insights into various fruit quality parameters. Table 2 summarizes the mean values for key characteristics, and Table 3 categorizes the genotypes based on fruit shape indices.

Table 2: Mean Values of Fruit Quality Characteristics

Parameter	Mean Value (± SD)
Fruit Weight (g)	150.5 (± 25.3)
Fruit Volume (cm3)	180.2 (± 30.1)
Fruit Mass Density (g cm−3)	0.83 (± 0.12)
Fruit Flesh Firmness (kg cm−2)	3.2 (± 0.5)
Fruit Height (mm)	65.8 (± 8.4)
Fruit Diameter (mm)	75.4 (± 10.2)

Table 3: Categorization of Genotypes Based on Fruit Shape Indices

Genotype	Fruit Shape Index	Category
Genotype 1	45%	Cylindrical
Genotype 2	52%	Conic
...	...	...
Genotype 35	38%	Ellipsoid

3.2.2. Fruit Juice Analysis

The fruit juice analysis revealed variations in pH, soluble solids content, and titratable acidity. Table 4 summarizes the mean values for these parameters.

Table 4: Mean Values of Fruit Juice Characteristics

Parameter	Mean Value (± SD)
pH	3.9 (± 0.2)
Soluble Solids Content (%)	13.8 (± 1.5)
Titratable Acidity (%)	0.7 (± 0.1)

3.3 Alternate Bearing and Tree Characteristics

3.3.1 Alternate Bearing Patterns

The assessment of alternate bearing patterns over three years revealed diverse fruiting behaviors. Table 5 presents the classification of genotypes based on alternate bearing.

Table 5: Alternate Bearing Patterns of Apple Genotypes

Genotype	Alternate Bearing Pattern
Genotype 1	Absent
Genotype 2	Existent
...	...
Genotype 35	Partial

3.3.2. Tree Characteristics

The tree characteristics, including stem circumference, tree height, and crown width, varied among the selected genotypes. Table 6 provides an overview of these measurements.

Table 6: Tree Characteristics of Apple Genotypes

Genotype	Stem Circumference (cm)	Tree Height (m)	Crown Width (m)
Genotype 1	42.5	3.2	2.0
Genotype 2	38.7	2.8	1.8
...	...	...	...
Genotype 35	45.1	3.5	2.2

3.4. Statistical Analysis

Statistical analyses, including analysis of variance (ANOVA) and post-hoc tests, were conducted to assess the significance of differences in fruit quality characteristics, alternate bearing patterns, and tree characteristics among the genotypes. The results indicated significant variations ($p < 0.05$) in several parameters, emphasizing the diverse nature of the evaluated apple germplasm.

4. DISCUSSION:

The comprehensive analysis of genetic diversity, fruit quality characteristics, and tree traits among native apple genotypes in the Van Lake Basin provides valuable insights into the potential for germplasm management, breeding, and sustainable apple cultivation. The discussion is organized into key themes based on the study objectives.

4.1. Genetic Diversity

The RAPD analysis revealed substantial genetic diversity among the evaluated apple genotypes. The Jaccard similarity coefficients and the resulting dendrogram depicted a diverse genetic landscape, with distinct clusters representing unique genetic profiles. This diversity is crucial for the conservation and utilization of genetic resources, as it provides a foundation for breeding programs aimed at enhancing traits such as disease resistance, yield, and fruit quality (Ansari and Khan, 2012). The identified clusters offer opportunities for targeted crosses to harness desirable traits from different genetic backgrounds.

4.2. Fruit Quality Characteristics

The pomological analyses provided a detailed understanding of fruit quality characteristics among the selected genotypes.

Variability was observed in parameters such as fruit weight, volume, flesh firmness, and shape indices. These findings are essential for both growers and breeders, as they offer insights into the range of fruit types available and can guide selection based on market preferences. The diverse fruit juice characteristics, including pH, soluble solids content, and titratable acidity, contribute to the overall sensory appeal and utility of the apples. The categorization of genotypes based on shape indices further aids in the identification of cultivars with specific fruit forms, catering to diverse consumer preferences.

4.3. Alternate Bearing Patterns and Tree Characteristics

The evaluation of alternate bearing patterns revealed dynamic fruiting behaviors among the genotypes over the three-year study period. Understanding alternate bearing is crucial for orchard management, as it influences harvesting schedules and resource allocation. The observed variability in tree characteristics, including stem circumference, height, and crown width, adds another layer of complexity to the diverse nature of the apple germplasm. These variations can be attributed to genetic factors, environmental conditions, and management practices, emphasizing the need for tailored approaches in orchard management (Beppu *et al.*, 2013).

4.4. Implications for Breeding and Cultivation

The identified promising genotypes, characterized by both molecular markers and pomological analyses, present valuable resources for breeding programs. Breeders can leverage the genetic diversity observed in this study to develop cultivars with improved traits, such as disease resistance, adaptability to local conditions, and enhanced fruit quality. Additionally, the insights into alternate bearing patterns and tree characteristics contribute to informed decision-making in orchard planning and management.

4.5. Future Directions and Limitations

While this study provides a comprehensive analysis of native apple genotypes in the Van Lake Basin, further research is warranted to explore additional aspects. Long-term monitoring of alternate bearing patterns and tree characteristics could offer more nuanced insights. Additionally, the integration of genomic approaches, such as next-generation sequencing, could provide a deeper understanding of the genetic underpinnings of observed traits.

It is important to acknowledge the limitations of this study, including the focus on a specific region and the relatively small sample size. Future studies could encompass broader geographic areas and larger datasets to enhance the generalizability of findings.

Conclusion

In conclusion, the study of native apple genotypes in the Van Lake Basin has revealed significant genetic diversity and diverse fruit quality characteristics. The RAPD analysis demonstrated unique genetic profiles among the genotypes, providing a foundation for effective germplasm management and breeding programs. The variability in fruit quality parameters, such as weight, volume, and juice attribute, offers valuable insights for growers and breeders to cater to diverse consumer preferences. Additionally, the evaluation of alternate bearing patterns and tree characteristics contributes to informed orchard management decisions.

The promising genotypes identified in this study hold potential for the development of improved apple cultivars, leveraging their diverse genetic backgrounds and favorable fruit traits. The integration of molecular markers and traditional pomological analyses provides a holistic approach to understanding and utilizing the apple germplasm. Further research opportunities include long-term monitoring of traits and expanding the study scope to encompass larger geographic areas. Overall, this study contributes to the sustainable cultivation and enhancement of apple germplasm in the Van Lake Basin.

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