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Biochemical Evaluation of Diverse Wood Apple (*Feronia limonia* L.) Genotypes under Tamil Nadu Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Wood apple (*Feronia limonia* L.) is a hardy, underutilized indigenous fruit tree known for its exceptional adaptability to harsh agro-climatic conditions, including drought, salinity, and degraded soils. Despite its rich nutritional and medicinal profile comprising vitamins, minerals, phenolic compounds, and pectin wood apple has seen limited attention in terms of systematic breeding, biochemical evaluation, and commercial cultivation. In the context of growing demand for nutritious, climate-resilient fruit crops and the need for value addition in lesser-known species, a deeper understanding of its genetic variability in quality traits is critical. Thus, this study was undertaken to explore the genetic diversity in key biochemical traits among selected wood apple genotypes with the aim of identifying elite lines suitable for varietal development, industrial processing, and nutritional enhancement.

Methodology: A field evaluation was conducted at the Department of Fruit Science, Horticultural College and Research Institute, TNAU, Periyakulam, Tamil Nadu during 2019-2024. A total of nine genotypes (WFL-01 to WFL-09), shortlisted from prior germplasm collections based on preliminary quality traits, were subjected to detailed biochemical assessment under Randomized Complete Block Design (RCBD) with three replications

Results: The current shows significant variability among the genotypes for quality parameters such as total soluble solids (TSS) ranging from 13.4 - 15.0 °Brix, acidity (2.40 - 3.60 %), TSS/acidity ratio (3.72 - 7.00), ascorbic acid (13.8 - 16.5 mg 100 g^{-1}), calcium (130 - 245 mg 100 g^{-1}), potassium (670 - 740 mg 100 g^{-1}), pectin content (4.70 - 5.40 %), total sugars (1.83 - 2.38 %), reducing sugars (0.96 - 1.29 %), and non-reducing sugars (0.85 - 1.06 %).

Conclusion: The present five-year study (2019–2024) revealed significant genetic variability among nine wood apple (*Feronia limonia* L.) genotypes. Among the genotypes, WFL-03 consistently recorded superior performance across all key quality traits, with TSS (15.0 °Brix), the lowest acidity (2.4 %), the highest TSS/acidity ratio (7.0), highest ascorbic acid (16.5 mg 100 g^{-1}), and highest pectin content (5.40 %), making it highly suitable for varietal development and commercial processing applications such as value-added products, nutraceutical formulations and functional foods. Its favorable sugar profile also indicates its acceptability for fresh consumption and industrial use. The observed variability highlights the genetic potential for crop improvement programs aimed at enhancing nutritional quality and promoting wood apple as a climate-resilient, underutilized fruit crop with applications in health, food, and sustainable agriculture.

Keywords: Wood apple; genotypes; evaluation; biochemical traits.

1. INTRODUCTION

Wood apple (*Feronia limonia* L.) is a monotypic species belonging to the family Rutaceae with a chromosome number of 2n = 18 (Dowarah et al., 2021). It is an underutilized indigenous fruit crop known for its hard shell and aromatic pulp. The unripe fruit is acidic, but when ripe, it develops a pleasant flavor suitable for culinary and processing applications (Shukla et al., 2025). In India, it is known by various vernacular names such as *Vilam Pazham* (Tamil), *Kaitha* (Hindi), *Kapitha* (Sanskrit), *Bal* (Assamese), *Koth bel* (Bengali), *Kothu* (Gujarati), *Beladahannu* (Kannada), *Vilam kai* (Malayalam), *Kavath* (Marathi), *Kaitha* (Oriya), and *Vellagapandu* (Telugu) (Shukla et al., 2024). The crop is widely distributed in Southeast Asian countries including Thailand, Malaysia, and Cambodia, and within India, it is naturally found in the plains of southern Maharashtra, West Bengal, Uttar Pradesh, Chhattisgarh, and Madhya Pradesh (Thakur et al., 2020).

Wood apple (*Limonia acidissima* L.) is known for its exceptional tolerance to drought, salinity, and nutrient-deficient soils, making it ideal for cultivation in arid and semi-arid climatic zones (Lagad et al., 2025). Though rarely grown in organized orchards, it is commonly seen along field borders, roadways, rail lines, and homestead gardens (Kumar and Deen, 2017). It is a deciduous, medium-sized tree that can grow up to 10 meters in height with a trunk girth ranging from 0.6 to 1.6 meters. The fruit is valued for its versatility, being consumed raw or processed into products like juices, jams, jellies, chutneys, and a traditional beverage called *sarbat* (Lamani et al., 2022). Nutritionally, the pulp is rich in β-carotene (a precursor to vitamin A), B-complex vitamins (especially riboflavin and thiamine), and moderate levels of vitamin C (Dyuti et al., 2022). It also offers a good amount of protein (up to 10%) and phenolic compounds (38.67 mg GAE g^{-1} DW), which contribute to its antioxidant properties. With pectin contents ranging from 3–8%, wood apple holds significant value for the processing industry. Phytochemical studies have identified compounds including flavonoids, phytosterols, glycosides, tannins, coumarins, and triterpenoids. Its compositional profile reveals high carbohydrate (70.14%), protein (13.8%), moderate dietary fiber (1.7 %), and essential minerals like calcium, magnesium, iron, and zinc, with comparatively low fat (4.3%), making it beneficial for bone health and anemia prevention (Dyuti et al., 2022).

Despite its nutritional richness and adaptability, wood apple remains an underutilized crop with limited systematic breeding and evaluation programs. However, wide genetic variability exists among native accessions, offering significant potential for crop improvement and development of superior genotypes for commercial cultivation. Therefore, in view of its underexploited potential and nutritional benefits, an evaluation study was undertaken to assess the diversity in biochemical traits among selected wood apple genotypes, aiming to identify elite accessions for future varietal development and value addition.

2. MATERIALS AND METHODS

2.1 Experimental Details

The present investigation on the evaluation of biochemical traits in wood apple (*Feronia limonia* L.) genotypes was conducted at the Central Block (10°07′21.8″N, 77°35′28.3″E) of the Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Tamil Nadu, India, during the period 2019-2024. The experimental site is located at an elevation of approximately 300 m above mean sea level and falls under a tropical climate. The region receives an average annual rainfall of 850–950 mm, with a mean maximum temperature of 36 °C and minimum of 22 °C. The soil is red loamy, well-drained, with a pH of 6.5 to 7.0, and moderate fertility.

Nine promising wood apple genotypes (WFL-01 to WFL-09), selected from genetically diverse regions of Tamil Nadu, were established under *ex-situ* field gene bank conditions. The trial was laid out in a Randomized Complete Block Design (RCBD) with three replications, each replication consisting of three trees per genotype. The trees were 10 to 15 years old at the time of biochemical sampling. Uniform agronomic practices were followed throughout the study, including weekly drip irrigation and application of recommended fertilizer doses (FYM @ 10 kg/plant and N:P:K @ 100:50:50 g/tree/year).

For biochemical analysis, 15 mature fruits per genotype (five fruits from each of three trees per replication) were harvested at full maturity. The analyses were performed in triplicate (three analytical replications per pooled sample) to ensure accuracy. The parameters evaluated during the study are listed below with its procedure.

2.2 Observation Recorded

2.2.1 Total soluble solids (°Brix)

Total soluble solids are determined by placing a few drops of clear fruit juice onto the prism of a hand-held refractometer and reading the value in °Brix (Brito et al., 2021).

2.2.2 Titratable acidity (%)

Titratable acidity is measured by titrating a known quantity of juice with standardized 0.1N sodium hydroxide solution, using phenolphthalein as an indicator (AOAC, 2005).

2.2.3 TSS/acidity ratio

It is used to calculate by dividing the value of total soluble solids (°Brix) by titratable acidity (%):

$$\text{TSS/Acidity Ratio} = \frac{\text{TSS (°Brix)}}{\text{Titratable Acidity (\%)}}$$

2.2.4 Ascorbic acid (mg 100 g^{-1})

The ascorbic acid content is estimated by extracting the sample in an oxalic acid solution and titrating with 2,6-dichlorophenolindophenol dye until a persistent pink color is observed. The concentration is calculated from a standard curve or titration factor (Sadasivam and Manickam, 1992).

2.2.5 Calcium (mg 100 g^{-1})

Calcium is analyzed using atomic absorption spectrophotometry (AAS), in which the fruit extract is compared against calcium standards (Zade, 2017).

2.2.6 Potassium (mg 100 g^{-1})

Potassium content is measured using flame photometry on digested fruit samples. The concentration is determined by calibration with potassium standards (Mutalik et al., 2011).

2.2.7 Pectin (%)

Pectin is extracted from fruit, precipitated as calcium pectate, and then dried and weighed, or alternatively quantified by reaction with copper and spectrophotometric determination (Roman-Benn et al., 2023).

2.2.8 Total sugars, reducing sugars and non-reducing sugars

All the sugar fractions were analysed from the standard method outlined by Ranganna (1986).

2.3 Statistical Analysis

The pooled data recorded over multiple seasons were statistically analyzed using Analysis of Variance (ANOVA) in R software (version 2.4.1), employing the agricolae package for performing mean comparisons and experimental design analysis (De Mendiburu, 2020). Treatment means were compared at the 5 % level of significance using the Critical Difference (CD) method to identify statistically significant differences among wood apple genotypes.

3. RESULTS AND DISCUSSION

The data presented in Fig. 1. reflect considerable genetic variability among the wood apple genotypes for key biochemical parameters such as TSS, acidity, ascorbic acid, pectin, calcium, and potassium content. This variation indicates significant potential for identifying superior genotypes for nutritional enhancement, processing quality, and value-added product development.

3.1 Total Soluble Solids

The Total Soluble Solids (TSS) content among the evaluated wood apple genotypes exhibited significant variation ($p < 0.05$), ranging from 13.40 ± 0.15 °Brix (WFL-01) to 15.00 ± 0.12 °Brix (WFL-03). The genotype WFL-03 recorded the highest TSS, followed closely by WFL-08 (14.80 ± 0.13 °Brix) and WFL-04 (14.50 ± 0.14 °Brix). These genotypes were found to be statistically superior to others based on the Critical Difference (CD) at 5% level. In contrast, the lowest TSS values were observed in WFL-01 (13.40 ± 0.15 °Brix) and WFL-07 (13.90 ± 0.11 °Brix). Genotypes WFL-03, WFL-08, and WFL-04 recorded the highest TSS values (>14.5 °Brix), which are considered highly desirable for enhanced sweetness, juice recovery, and processing efficiency.

The superior performance of WFL-03 in terms of TSS may be attributed to its inherent genetic potential, as all genotypes were evaluated under uniform environmental and agronomic conditions, minimizing external influences. Moreover, a positive association was observed between TSS and total sugar content, particularly in WFL-03, which exhibited both the highest TSS (15.0 °Brix) and the highest total sugars (2.38 %). This suggests that higher TSS values may reflect a greater accumulation of soluble carbohydrates, enhancing the fruit's palatability and commercial value. Similar correlations between TSS and sugars in wood apple were previously reported by Sharma et al. (2024), supporting the potential of these traits as selection indices in breeding programs.

3.2 Titratable Acidity and TSS/Acidity Ratio

Titratable acidity among the evaluated wood apple genotypes varied significantly ($p < 0.05$), ranging from 2.40 ± 0.07 % in WFL-03 to 3.60 ± 0.06 % in WFL-01 and WFL-07. These differences are primarily attributed to genotypic variability in organic acid synthesis and degradation pathways, particularly malic and citric acids. Since the fruits were grown under uniform environmental conditions, the variation can be ascribed largely to inherent genetic potential and enzyme activity influencing acid metabolism. Genotypes with lower acidity, such as WFL-03 and WFL-08, are more suitable for fresh consumption and juice processing due to their milder flavor, while higher acidity in genotypes like WFL-01 and WFL-07 may favor traditional processed products like chutneys and pickles (Singh et al., 2016 and Shukla et al., 2025).

The TSS/Acidity ratio (TAR), a key indicator of taste balance and flavor quality, also showed significant differences across genotypes. WFL-03 exhibited the highest TAR (7.00 ± 0.10), followed by WFL-08 and WFL-04, due to a favorable combination of high TSS and low acidity. These genotypes are ideal for fresh market and beverage formulations. In contrast, lower TAR values in WFL-01 and WFL-07 suggest a stronger tartness, supporting their use in culinary applications. The variation in TAR is directly influenced by the relative accumulation of sugars and organic acids, both of which are under genetic control. These findings align with Kumar and Deen (2017), who emphasized the importance of sugar–acid balance in determining consumer preference and processing suitability.

3.3 Ascorbic Acid

Ascorbic acid content varied significantly ($p < 0.05$) among the wood apple genotypes, ranging from 13.80 ± 0.11 mg 100 g^{-1} (WFL-07) to 16.50 ± 0.10 mg 100 g^{-1} (WFL-03). Genotype WFL-03 recorded the highest ascorbic acid content, followed by WFL-08 (15.50 ± 0.09 mg 100 g^{-1}) and WFL-04 (15.00 ± 0.08 mg 100 g^{-1}), all of which are statistically superior to the rest.

These genotypes are particularly promising for nutritional enhancement and functional food development, as they contribute substantially to daily vitamin C intake and offer strong antioxidant potential. The observed variation is mainly attributed to genetic differences influencing the biosynthesis and stability of ascorbic acid in fruit tissues. Since environmental conditions and cultural practices were kept uniform, the differences highlight the role of genotype-specific metabolic activity. Similar ranges were reported in the nutritional profiling of *Limonia acidissima* by Dyuti et al. (2022), affirming the influence of genetic background on vitamin C content.

3.4 Calcium and Potassium

Calcium and potassium content exhibited significant genotypic variability ($p < 0.05$) among the evaluated wood apple genotypes. Calcium content ranged from 132.45 ± 3.10 mg 100 g^{-1} in WFL-01 to 245.23 ± 2.80 mg 100 g^{-1} in WFL-03, while potassium ranged from 674.66 0 ± 4.50 mg 100 g^{-1} (WFL-06) to 742.47 ± 3.90 mg 100 g^{-1} in WFL-03. Genotypes WFL-03 and WFL-08, with elevated calcium (245.23 and 235.73 mg 100 g^{-1}, respectively) and potassium levels (742.47 and 721.56 mg 100 g^{-1}, respectively), are particularly valuable for both nutritional enhancement and processing potential.

The higher calcium levels in these genotypes are beneficial for improving structural integrity of fruit tissues, thereby extending postharvest shelf life due to enhanced cell wall rigidity. Potassium, on the other hand, plays a critical role in regulating osmotic balance, improving fruit sweetness, and supporting cardiovascular health. The observed variability is primarily attributed to genotypic differences in mineral uptake, transport efficiency, and nutrient partitioning within the fruit tissues, as environmental conditions were uniform across treatments. Similar ranges of calcium and potassium in wood apple were previously reported by Rajangam and Sankar (2022), who emphasized their importance in enhancing the functional food value of underutilized fruits.

3.5 Pectin

Pectin content showed significant variation among the wood apple genotypes, ranging from 4.70 ± 0.05 % in WFL-07 to 5.40 ± 0.04 % in WFL-03. Genotypes WFL-03, WFL-04, and WFL-08 recorded pectin contents exceeding 5.00 %, making them highly suitable for pectin extraction and industrial processing into jams, jellies, and fruit preserves. Higher pectin content is a key trait for commercial processing, contributing to desirable gelling properties, improved texture, and longer shelf life of fruit-based products.

This variability is primarily attributed to genetic differences affecting galacturonic acid biosynthesis and cell wall composition, which influence the extent of pectin accumulation in the fruit pulp. Since the experiment was conducted under uniform environmental conditions, the observed differences are mainly genotypic in origin. Similar findings were reported by Dowarah et al. (2021), who highlighted those genotypes of *Limonia acidissima* with high pectin levels are more appropriate for value-added processing and food formulation. The superior performance of WFL-03 in this context underscores its dual utility as both a nutrient-rich and processing-friendly cultivar.

3.6 Total Sugar, Reducing Sugar and Non-Reducing Sugar

Sugar fractions displayed significant genotypic variation, highlighting the influence of genetic background on sweetness and processing potential. Total sugar content ranged from 1.55 ± 0.06 % (WFL-07) to 2.38 ± 0.04 % (WFL-03), followed closely by WFL-08 (2.36 ± 0.04 %) and WFL-09 (2.34 ± 0.05 %). These genotypes also recorded high non-reducing sugar levels (>1.05 %), contributing to sweetness perception and improving shelf stability due to their resistance to fermentation and microbial degradation.

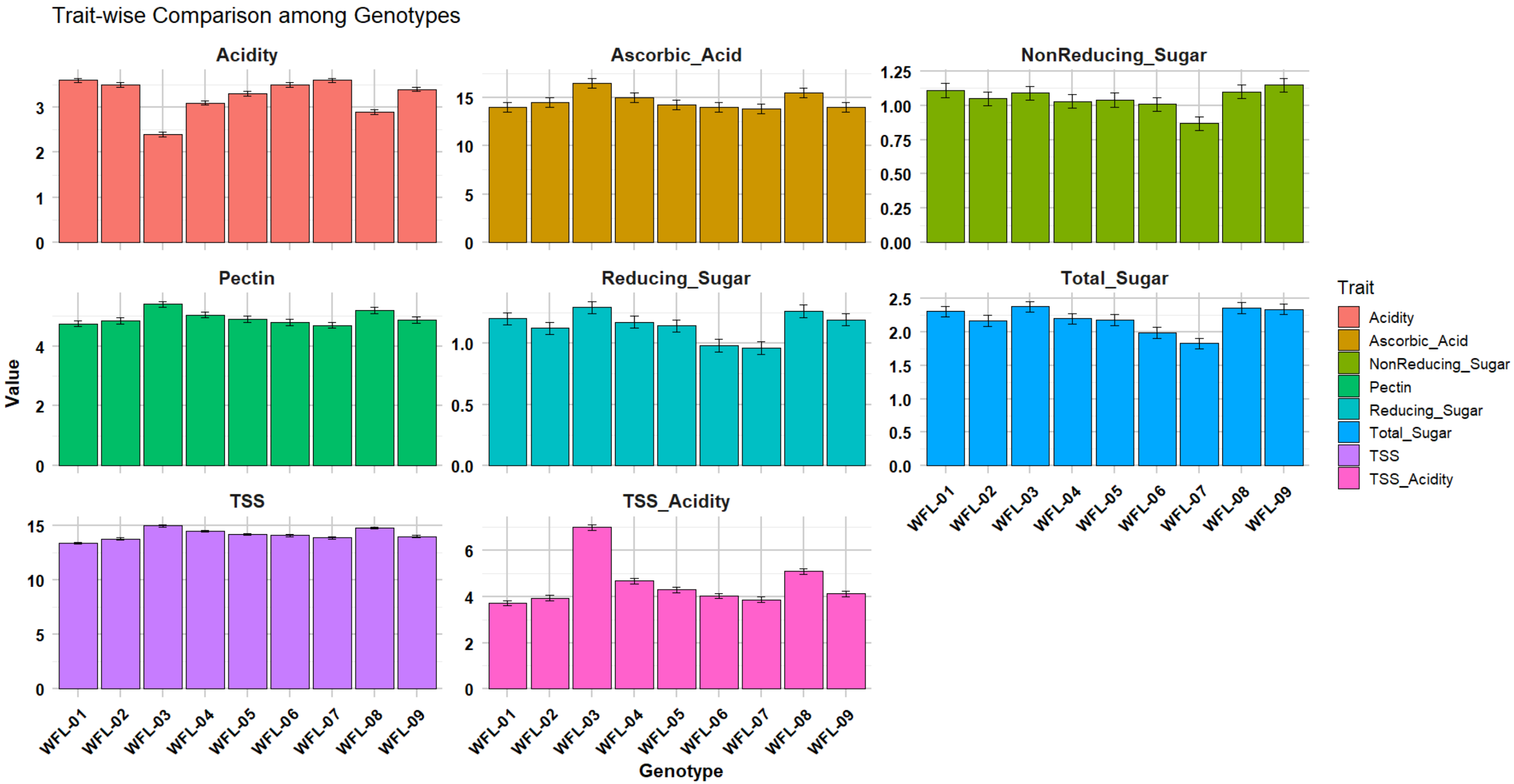

Fig. 1. Biochemical traits diversity among wood apple genotypes

Test the significant at P < 0.05 and Each bar represents the mean value ± standard error (SE) of pooled data across multiple seasons.

Reducing sugars, which play a crucial role in flavor development, browning reactions, and Maillard chemistry during thermal processing, ranged from 0.84 ± 0.03 % (WFL-07) to 1.29 ± 0.02 % (WFL-03). The high reducing sugar levels in WFL-03 and WFL-08 enhance their appeal for confectionery and pulp-based product development, particularly where flavor depth and caramelization are desired.

The variability in sugar fractions is primarily attributed to genotypic differences in carbohydrate metabolism, sugar transport, and enzymatic conversion rates between glucose, fructose, and sucrose. As environmental and management conditions were uniform, these differences reflect intrinsic genetic control. Similar trends were reported by Sharma et al. (2024), who emphasized that genotypes with a balanced profile of reducing and non-reducing sugars, combined with high TSS and favorable acidity, are ideal for juice extraction, fruit preserves, and sweetened concentrates. Thus, WFL-03 and WFL-08 emerge as promising candidates for commercial processing and value-added product development.

4. CONCLUSION

The evaluation of biochemical parameters among nine wood apple (Feronia limonia L.) genotypes revealed substantial variation, highlighting rich nutritional diversity and significant processing potential. Genotype WFL-03 emerged as the most promising, exhibiting the highest levels of TSS, ascorbic acid, calcium, potassium, pectin, and TSS/acidity ratio, making it highly suitable for both fresh consumption and value-added product development. Genotypes WFL-08 and WFL-04 also displayed favorable attributes such as balanced acidity and elevated sugar and pectin content, indicating their suitability for pulp-based processing and confectionery applications. The wide genotypic differences in sweetness, acidity, vitamin C, and mineral composition underscore the importance of selecting nutritionally rich accessions for varietal improvement. Based on these findings, it is recommended that superior genotypes like WFL-03 and WFL-08 be advanced for participatory breeding programs, multi-location trials to assess environmental stability and molecular characterization to identify trait-linked markers. Such efforts will aid in the development of elite cultivars tailored for both nutritional security and industrial use, reaffirming wood apple's potential as a health-promoting and commercially viable underutilized fruit crop.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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