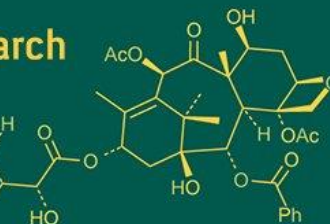
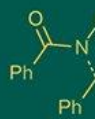


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Genotypic diversity and evaluation of wood apple: Morphological and biochemical variation, molecular evidence and priorities for genetic improvement

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Abstract

Wood apple (*Limonia acidissima* L.) is an underutilized fruit tree whose improvement depends on converting variation in seedling populations into reliable, end-use-specific planting material. This narrative review synthesizes evidence on tree architecture, fruit components, biochemical quality and molecular diversity, and critically examines the implications for genotype evaluation and conservation. Regional studies consistently identify variation in fruit size, pulp recovery, soluble solids, acidity and seed burden, providing opportunities for selection. However, differences among trees sampled in their original environments cannot be attributed exclusively to genotype, and published numerical ranges are affected by maturity, sampling procedures and analytical conventions. Molecular evidence based on simple sequence repeat markers confirm diversity that can complement phenotypic characterization, although marker distance alone does not establish agronomic superiority. An integrated evaluation framework is proposed that combines documented germplasm collection, standardized fruit sampling, replicated clonal trials, multi-season assessment, biochemical profiling and molecular identification. Selection should prioritize usable pulp yield, appropriate sugar-acid balance, manageable tree architecture and performance stability, with distinct objectives for fresh consumption and processing. Conservation should retain both promising selections and broader genetic representation. Progress will depend on linking diversity assessment to reproducible field performance rather than treating descriptive variation as sufficient evidence for cultivar recommendation.

Keywords: *Limonia acidissima*, germplasm characterization, fruit quality, phenotypic diversity, SSR markers, selection breeding, conservation

Introduction

Wood apple (*Limonia acidissima* L., Rutaceae) is a fruit tree associated with seasonally dry tropical environments. The accepted name and its synonym *Feronia limonia* (L.) Swingle refer to the same species; its native range extends across the Indian subcontinent to the Andaman Islands (Royal Botanic Gardens, Kew, 2026). Taxonomic precision is essential because literature on bael (*Aegle marmelos*) is sometimes discussed alongside wood apple. Findings from bael and other fruit crops may suggest evaluation methods, but they do not constitute direct evidence of variation in wood apple.

Seedling-origin trees offer material for selecting useful horticultural traits. Surveys in Gujarat, West Bengal and other Indian regions have examined fruit morphology and chemical composition, demonstrating the practical value of documenting existing variation (Singh *et al.*, 2016; Tamang *et al.*, 2020; Dowarah *et al.*, 2021)^[1, 8, 9]. For crop improvement, however, a promising tree is only the beginning of an evaluation process. Its apparent advantage must be reproduced in propagated material and maintained under the conditions in which farmers will cultivate it.

This review evaluates the evidence for wood apple diversity and develops a framework for translating characterization into selection, breeding and conservation. Particular attention is given to the distinction between phenotypic variation and genetic differentiation, the comparability of biochemical measurements, and the need to define selection objectives according to the intended use of the fruit.

2 Scope and approach of the review

A targeted narrative synthesis was undertaken using a literature compilation as the starting bibliography, followed by online searches for original wood apple studies and bibliographic

verification against publisher records. Searches combined wood apple, *Limonia acidissima* and *Feronia* with genotype evaluation, diversity, fruit quality, biochemical characterization and molecular markers. Searches and verification were conducted on 8 September 2026. This approach was intended to resolve source attribution and strengthen the thematic synthesis; it was not an exhaustive systematic search or a meta-analysis.

Priority was given to primary studies directly evaluating wood apple accessions, developmental changes relevant to sampling, and molecular diversity. Studies of other species were excluded from the numerical synthesis. Repeated descriptions of the same material were not treated as independent evidence. The South Indian morphological study was retained as a preprint and is identified accordingly. Molecular findings from Lata *et al.* (2024) [5] were limited to the accessible publisher abstract. No pooled estimates were calculated because sampling units, populations, maturity criteria and analytical procedures differed among studies. Recommendations below represent the synthesis and proposed evaluation framework, rather than results of a new experiment.

3 Germplasm resources and morphological diversity

Morphological characterization serves two related purposes: documenting germplasm identity and identifying traits with potential production value. Tree habit, branching, leaf form and fruit shape can assist accession description, while fruit components provide more direct information about usable yield. A descriptor is most useful when its measurement is reproducible and its relationship to the breeding objective is explicit. Large differences in tree size, for example, may reflect age or management and should not automatically be interpreted as evidence of superior genetic potential.

Tamang *et al.* (2020) [9] examined 11 wood apple plants from West Bengal during 2013-2015 and described variation in tree and fruit characteristics. The study illustrates the role of field exploration in identifying candidate material, but convenience and availability influenced sampling. Such collections should be regarded as an initial survey rather than a representative estimate of all diversity in a region.

In a South Indian preprint, Lamani and Hosakatte (2022) [4] characterized 32 populations or genotypes and reported tree heights of 7.68-16.69 m and pulp proportions of 53.91-72.93%. Principal component and cluster analyses produced four morphological groups. This evidence supports further evaluation of contrasting accessions, but the groups represent similarity in measured traits rather than independently confirmed genetic lineages. The inconsistent use of population and genotype also makes the sampling unit important when interpreting these results.

Future collections should assign a permanent identifier to each source tree and retain its coordinates, locality, estimated age, propagation history and management conditions. A source tree, a seed family and a clonally maintained accession should be recorded separately. Photographs and botanical vouchers would strengthen identification, while retaining the original local name would preserve links to farmer knowledge without making the local name the sole identifier.

4 Fruit components and yield evaluation

Fruit size is an accessible selection criterion, but its value depends on the proportion and quality of the usable pulp. Singh *et al.* (2016) [18] evaluated 25 Gujarat genotypes and

reported fruit weights of 156.45-432.93 g and pulp weights of 80.04-319.32 g. Their observations establish substantial variation within the sampled material. They do not establish that the largest-fruited accession is the most productive or economical under cultivation.

Dowarah *et al.* (2021) [1] reported fruit weights of 97.46-225.73 g and pulp weights of 50.73-117.54 g in 12 West Bengal genotypes. Their results also illustrate a component trade-off: WA-11 combined the highest fruit and pulp weights with the lowest reported pulp-to-seed ratio, 3.09. Thus, selection on fruit weight alone can overlook the seed burden. The useful comparison is the mass of recoverable pulp obtained per fruit and per tree under a clearly defined extraction procedure.

Raut *et al.* (2023) [6] described initial screening of 145 trees, followed by selection of 25 promising genotypes and detailed attention to nine elite types. The reported yield range of 66.47-170.80 kg per tree indicates an opportunity for selection among the assessed material. However, selection before detailed evaluation means that the final range is not an unbiased description of the original population. Some dimensions and component values are inconsistent between portions of that paper, so they should not be used to construct a pooled benchmark.

For future trials, usable pulp yield should be measured as the product of harvested fruit yield and the recoverable pulp fraction. The latter must specify whether seeds and fibre are included. Whole-fruit mass should be reconciled with shell, seed, pulp and documented processing losses. Yield efficiency per canopy area can supplement yield per tree when comparing contrasting growth habits, but it should be reported alongside absolute production and planting density. Repeated harvest records are needed to distinguish sustained productivity from a single favourable crop.

5 Biochemical variation and fruit quality

Soluble solids, titratable acidity, sugars and ascorbic acid provide complementary information about fruit quality. Total soluble solids should not be interpreted as a direct measure of total sugar because other dissolved constituents contribute to the refractometer reading. Likewise, pH and titratable acidity describe different aspects of acid chemistry. Studies should report both the numerical result and its analytical basis before comparisons are made.

In Gujarat material, Singh *et al.* (2016) [8] reported soluble solids of 11.07-19.36 °Brix and acidity of 3.23-6.86%. In the West Bengal study, soluble solids ranged from 11.16 to 22.08 °Brix, while ascorbic acid ranged from 7.45 to 24.98 mg/100 g (Dowarah *et al.*, 2021) [1]. These are study-specific ranges; their differences cannot be assigned entirely to regional genetics because maturity and analytical conditions may also contribute.

Shukla *et al.* (2025) [7] evaluated eight Bundelkhand genotypes. WABG-3 had the highest reported soluble solids, whereas WALD-2 had the highest total sugars and WABN-7 the highest reducing sugars. These different leaders demonstrate why a single quality measurement cannot define overall superiority. The study reported acidity of 1.85-3.60% and ascorbic acid of 14.43-17.20 mg/100 g. Such variation supports selecting complementary quality profiles rather than assuming that all desirable attributes occur together.

Fruit maturity is a major source of variation that must be controlled. Kumar and Deen (2017) [3] found that soluble

solids and sugars increased during growth and development, while total phenols declined; acidity and pectin increased until maturity and subsequently decreased. Consequently, comparing fruit harvested on the same calendar date may still compare different physiological stages. A genotype evaluation should record maturity indicators, time since harvest and storage conditions, and should distinguish measurements at harvest maturity from those at eating ripeness.

Phenolic and antioxidant assays can extend quality evaluation, but extraction studies are not genotype comparisons. Ilaiyaraja *et al.* (2015) [2] optimized recovery of bioactive compounds using response surface methodology, demonstrating the importance of analytical conditions. For selection, all accessions should be evaluated with the same extraction procedure and results expressed on a common fresh- or dry-mass basis. Antioxidant activity in a laboratory assay should remain an analytical trait; it does not independently establish a health benefit from consuming a selected genotype.

6 Molecular diversity and its relationship to phenotype

Molecular information provides a separate line of evidence that can assist accession identification and collection management. Lata *et al.* (2024) [5] assessed 96 samples from 16 Indian states using 24 polymorphic SSR markers developed through RNA sequencing. The publisher abstract reports 129 alleles, an average polymorphic information content of 0.62, and three groups identified through clustering and population-structure analyses. Molecular variance was greater within populations than among them.

The study supports sampling multiple individuals within localities as well as across regions. However, neutral or broadly distributed marker differences cannot, on their own, predict pulp yield, flavour or stress performance. Molecularly distant parents should therefore be selected together with evidence of complementary horticultural traits. A small marker panel may be useful for identity checking, but claims about adaptive variation require additional evidence.

An integrated collection should link the molecular profile and phenotypic records to the same physical accession. Analyses can then examine whether morphological groups correspond to molecular relationships or are largely explained by location and management. Disagreement between the two forms of evidence is informative: it may identify environmental effects, duplicate names, or different genetic routes to a similar phenotype. Dense-marker association studies would be most useful after reliable phenotyping and adequate sampling have been established.

7 Experimental and statistical priorities

The central experimental requirement is to separate the genotype from its original environment. Several fruits from one tree improve estimation of that tree's mean, but do not provide independent biological replication of its genotype. Similarly, repeated laboratory analyses estimate analytical precision rather than variation among trees. A strong evaluation should state the number of source trees, propagated trees, sampled fruits and laboratory replicates separately.

Candidate selections should be propagated and established in replicated blocks with comparable management. Rootstock identity, where relevant, should be documented.

Measurements across bearing seasons and sites would allow genotype, environment and their interaction to be examined. If resources initially permit only a single site, conclusions should remain specific to that environment until further testing is completed. Tree age, crop load and harvest maturity should be retained as explanatory variables rather than left as unrecorded differences.

Principal component analysis and clustering are useful for reducing many traits to interpretable patterns, provided that quantitative variables are appropriately scaled. Including several strongly correlated size measurements can disproportionately weight fruit size in a distance analysis. Qualitative descriptors require methods suitable for categorical data. The number of clusters should be treated as an analytical result sensitive to method and sampling, not as a fixed biological property of the species.

Heritability estimates require a design that separates genetic and environmental sources of variation. High phenotypic variability in a field survey does not demonstrate high heritability. Correlations also need biological interpretation: an association between fruit weight and pulp weight may partly follow from the fact that pulp is a component of the fruit. Selection indices should avoid repeatedly rewarding the same size dimension while overlooking flavour, seed burden or irregular bearing.

Reporting should include means, uncertainty, sample sizes and units, with raw observations retained where possible. Numerical precision should reflect measurement precision. Shell thickness should use a consistent unit, and canopy area must be distinguished from canopy volume. When source text and tables disagree, the discrepancy should be documented and the affected measurement excluded from synthesis unless a defensible resolution is available.

8 Selection objectives and conservation

Selection objectives should be defined before genotypes are ranked. For fresh pulp consumption, a candidate profile could combine acceptable flavour, favourable sugar-acid balance, low objectionable fibre and convenient pulp separation. For beverages, pulp recovery and sensory quality after dilution may be more relevant than fruit size. For pectin-oriented processing, extracted pectin yield and functional performance should be tested directly rather than inferred from fruit acidity alone. These are proposed end-use objectives, not validated selection thresholds.

A practical programme could begin with field collection and preliminary screening, retain complementary accessions, and advance a manageable subset to replicated clonal trials. The final stage should include processing or consumer assessment appropriate to the intended use, followed by evaluation of propagation success and planting-material identity. An accession that performs well at one stage should remain a candidate until its subsequent performance has been verified.

Conservation should extend beyond a few high-ranking trees. Restricting collections to large or sweet fruit could remove material useful for future traits or environments. A working collection should therefore include elite candidates, unusual trait combinations and representatives of broader diversity. In situ retention of source trees can complement field repositories, while duplicate holdings and stable accession records reduce the consequences of losing individual plants.

A core collection should be designed for representation rather than described simply as a list of the best genotypes. Combining molecular identity, geographic coverage and phenotypic variation would provide a more defensible basis for reducing collection size. Decisions about distributing planting material should also preserve the link between accession identity, source locality and observed performance.

9 Research gaps and future directions

The most immediate gap is the limited connection between regional surveys and comparable, replicated performance trials. Reassessing existing candidate material under shared protocols may yield greater practical value than repeatedly generating new accession lists with incompatible measurements. A common descriptor set and accession registry would make results easier to compare and reduce the risk of treating renamed material as independent germplasm.

A second priority is the integration of reproductive and seasonal traits with fruit quality. Flowering time, fruit set, harvest duration and consistency of bearing should be evaluated alongside composition. Controlled investigation of pollination and compatibility would strengthen parent selection, while repeated assessment of crop load would clarify whether quality differences persist across seasons. Claims of environmental resilience need genotype-level testing. The occurrence of a tree in a dry locality does not quantify its yield response to water shortage. Future experiments should measure performance under defined stress and comparison conditions, including recovery and fruit quality. Such evidence would allow selection for resilience to be distinguished from selection for survival alone.

Finally, analytical quality should become part of germplasm evaluation. Standardized maturity, validated biochemical methods, consistent mass bases and transparent sampling would strengthen the evidence more immediately than adding many poorly comparable assays. Molecular tools and predictive models can then build on reliable observations. Their value should be judged by whether they improve identification, conservation or selection decisions in independent material.

10 Conclusions

Wood apple research provides credible evidence of useful morphological, biochemical and molecular diversity. The available regional studies identify candidate material and contrasting quality profiles, but they do not justify a single universal ranking of genotypes. Differences in sampling, maturity and environment limit direct comparison, while molecular distance and morphological clustering address different aspects of diversity.

The next step is to connect characterization with replicated clonal performance, usable pulp yield, end-use quality and seasonal stability. Selection and conservation should proceed together so that improvement retains a sufficiently broad genetic base. A coordinated programme with consistent descriptors, accession identity and transparent analytical methods would provide a stronger foundation for dependable wood apple cultivars.

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