

Advances in Genetics in Bottle Gourd (*Lagenaria siceraria*): From Genetic Diversity to Genomics-Assisted Breeding

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For breeding purposes, a successful variety should perform well in the field while satisfying the needs of growers, traders, and consumers. For instance, fruit shape can affect market acceptance, whereas the time to maturity influences when harvesting can begin. Thus, variety assessment should consider not only yield but also quality, adaptation, and consistency across seasons (Mkhize *et al.*, 2021; Decker-Walters *et al.*, 2017). An important question in crop genetics is whether differences between plants arise from heredity, environmental conditions, or a combination of the two. Field experiments quantify observable differences, whereas molecular techniques investigate the genetic variation underlying them. Careful integration of both types of information helps breeders select promising plants and choose suitable parents for crossing (Mkhize *et al.*, 2021). Bottle gourd (*Lagenaria siceraria*) is grown extensively in tropical and subtropical areas and displays substantial diversity in fruit shape, size, colour, and other characteristics. The young fruits are widely consumed as a vegetable, whereas mature fruits and seeds serve other traditional and domestic purposes (Mkhize *et al.*, 2021; Decker-Walters *et al.*, 2017). The crop's diversity is useful to breeders because different markets and production systems require different fruit types, maturity periods, yields, and levels of stress tolerance. Conventional breeding usually relies on choosing plants with preferred traits, crossing selected parents, and assessing their progeny over successive generations. These approaches remain important; however, observable traits are also influenced by soil, temperature, water supply, and crop management, which can complicate the identification of genetic causes (Mkhize *et al.*, 2021). Molecular genetics provides DNA-based evidence that helps researchers investigate inheritance and detect plants carrying potentially beneficial variants. Integrating field observations and genomic techniques can improve the accuracy and efficiency of selection (Wang *et al.*, 2018; Zhao *et al.*, 2024).

Keywords: bottle gourd, genetic diversity, molecular markers, QTL mapping, GWAS, marker-assisted selection, genomics, crop improvement

Genetic Diversity in Bottle Gourd: Sources of Useful Traits for Breeding

Germplasm comprises plant material maintained for conservation, research, and breeding purposes. It may include traditional landraces, farmer-selected lines, breeding lines, and formally released varieties. A diverse germplasm collection is valuable because traits of limited current importance may become useful as production environments or market requirements change. Collections are most useful when their identity is documented and their major traits are evaluated consistently (Decker-Walters *et al.*, 2017; Mkhize *et al.*, 2021). Genetic variation should not be evaluated on the basis of only one characteristic. Two accessions may have similar fruit shapes but differ in maturity, seed traits, disease response, or genetic background. Conversely, accessions that look different may still share much of their genetic background.

Using field observations together with DNA markers gives a more complete picture of diversity and helps avoid selecting parents that are too similar (Wang *et al.*, 2018; Genome-wide SSR markers in bottle gourd, 2022). Genetic diversity describes heritable differences among individuals within the same crop species. It provides the foundation for crop improvement, since new varieties depend on the availability of useful variation within the gene pool (Decker-Walters *et al.*, 2017). Bottle gourd accessions may differ in vine growth, branching, flowering time, fruit number, fruit length and diameter, rind colour, seed traits, taste, and yield. Some accessions may also carry useful responses to diseases or environmental stresses (Mkhize *et al.*, 2021).

Phenotypic Characterization: Identifying Differences in Visible Plant Traits

Reliable comparisons require researchers to define traits clearly and apply uniform measurement procedures. Days to flowering should be recorded from a defined starting point, fruit dimensions should be measured at a consistent stage, and yield should be calculated using the same plot or plant basis for all accessions. Replicated observations help separate repeatable differences from random variation. Recording weather, soil, irrigation, and crop-management details also help explain why a genotype may perform differently between trials (Decker-Walters *et al.*, 2017; Mkhize *et al.*, 2021). Phenotypic data are especially important for validating molecular findings. A DNA marker may appear associated with a trait in one dataset, but the relationship is only useful for breeding if the trait itself has been measured accurately. Multi-location and multi-season testing can show whether a trait is stable or whether its expression depends strongly on the environment (Mkhize *et al.*, 2021). Phenotypic characterization involves systematically documenting plant traits that can be observed or quantified. For bottle gourd, this may include days to first flowering, male and female flower production, fruit weight, fruit shape, fruit number per plant, and marketable yield. Evaluating accessions in more than one environment is important because a plant may perform well at one site but less well under different weather or soil conditions (Decker-Walters *et al.*, 2017; Mkhize *et al.*, 2021).

Molecular Characterization: Using DNA Markers to Compare Germplasm

Different marker systems vary in cost, technical requirements, and the amount of genetic information they provide. SSR markers are based on short repeated DNA sequences, whereas SNP markers identify differences at individual DNA positions. Once validated, markers can help identify accessions, assess relatedness, and track selected regions of the genome. Marker data do not replace field evaluation; instead, they complement it by providing information that may not be visible in the plant (Wang *et al.*, 2018; Genome-wide SSR markers in bottle gourd, 2022). Population structure is another important consideration. If a group of accessions shares a common ancestry, they may also share particular DNA variants for reasons unrelated to the trait under study. Researchers therefore use appropriate statistical methods to account for relatedness when estimating genetic diversity or testing marker–trait associations (Zhao *et al.*, 2024). Molecular characterization assesses DNA variation instead of depending solely on observable characteristics. Markers such as RAPD, AFLP, ISSR, SSR, and SNPs can help estimate how closely related accessions are and identify genetically distinct parents for crossing. A 2022 study developed 45,066 genome-wide SSR markers and validated a subset for diversity and population-structure analysis involving 96 accessions from India's National Genebank, providing a useful resource for future research and breeding (Genome-wide SSR markers in bottle gourd, 2022). Put simply, these markers serve as DNA landmarks, allowing researchers to distinguish and compare accessions that may appear alike.

Molecular Genetics and Genomics: Understanding the Bottle Gourd Genome

An organism's genome contains its complete set of DNA instructions. A reference genome provides a shared framework for locating genes and DNA variants. The quality of the reference matters: gaps or assembly errors can make it harder to identify genes or compare samples accurately. Genome resources are therefore foundational tools, but their value increases when they are paired with well-characterized plant collections and reliable trait measurements (Xu *et al.*, 2021; Wang *et al.*, 2018). A pangenome or broad genome-variation resource extends the

idea of a single reference by examining differences across multiple accessions. This can reveal variants that are absent from, or not easily represented by, one reference individual. The 2024 analysis of 197 bottle gourd accessions provides a population-scale resource for investigating genetic diversification and the history of the crop (Zhao *et al.*, 2024). A reference genome is a carefully assembled representation of an organism's DNA. It provides a map that researchers can use to locate genes, compare varieties, and search for DNA differences linked with important traits. A long-read bottle gourd genome assembly supported research into the genetic basis of fruit-shape variation (Xu *et al.*, 2021). Later population-level genomic work examined variation across 197 accessions, creating a resource for studying the crop's history, diversity, and possible links between genetic variants and traits (Zhao *et al.*, 2024). Next-generation sequencing (NGS) can generate extensive DNA sequence data more rapidly than earlier sequencing approaches. Approaches such as genotyping-by-sequencing and whole-genome resequencing can identify many variants across a collection of plants. These variants can be used to estimate genetic relationships, develop markers, and support mapping studies (Wang *et al.*, 2018; Zhao *et al.*, 2024). Comparative genomics further advances analysis by examining bottle gourd alongside related cucurbits, including cucumber, melon, and watermelon. Similarities can point researchers towards candidate genes, but a gene's role in a related crop should not be assumed to be identical in bottle gourd without testing (Mkhize *et al.*, 2021).

Genetic Control of Important Horticultural Traits in Bottle Gourd

Many horticultural characteristics are quantitative, with values distributed across a range rather than divided into a few discrete groups. Fruit weight, fruit length, yield, and flowering time may be influenced by multiple genes, environmental conditions, and interactions between the two. This makes them more difficult to improve than traits controlled by a single gene with a large, consistent effect. For quantitative traits, large populations and repeated measurements usually provide stronger evidence than observations from only a few plants (Mkhize *et al.*, 2021; Xu *et al.*, 2021).

Fruit Shape and Size: Identifying Genetic Regions Linked to Market Preferences

Fruit-shape studies require standardized descriptions or measurements because labels such as 'long' or 'round' can be subjective. Geometric morphometrics uses measurable points or outlines to describe shape more precisely. Such data can be combined with DNA information to find regions associated with fruit form. The reported 11 fruit-shape QTLs provide useful leads, but breeders need to confirm their effects in additional populations before relying on them for routine selection (Xu *et al.*, 2021). Fruit size and shape may also be affected by plant vigour, water availability, nutrition, fruit load, and the developmental stage at measurement. A robust study should therefore record crop-management conditions and measure fruit at comparable maturity. This reduces the chance that environmental differences will be mistaken for genetic effects (Mkhize *et al.*, 2021). Fruit shape and size are important market traits because consumers and growers may prefer long, round, oval, cylindrical, or bottle-shaped fruits. These traits often involve several genes and may also respond to the growing environment. Research using genome resources and geometric measurements identified 11 QTLs associated with fruit-shape variation in bottle gourd; some candidate regions contained genes related to fruit shape in other cucurbit crops, including members of the SUN, OFP, and AP2 gene families (Xu *et al.*, 2021). A quantitative trait locus (QTL) is a DNA region statistically linked to variation in a measurable characteristic. The regions identified in a study are starting points for further testing, not automatic proof that a single gene controls the trait.

Flowering and Sex Expression: Their Roles in Pollination and Fruit Production

Flowering traits are important not only for yield but also for the practical organization of breeding programmes. The timing and abundance of male and female flowers can affect pollen availability, fruit set, and the scheduling of controlled crosses. Researchers should record flowering under consistent conditions and consider that temperature, day length, and plant vigour may influence the expression of these traits. Genetic explanations should be based on experiments in bottle gourd rather than transferred uncritically from related crops (Mkhize *et al.*, 2021). The timing of flowering and the number of male and female flowers influence

pollination, fruit setting, and the duration of the growing period. These characteristics are influenced by both inherited factors and environmental conditions. Research in related cucurbits provides useful hypotheses, but bottle-gourd-specific populations and experiments are needed to confirm how particular flowering traits are inherited in this crop (Mkhize *et al.*, 2021).

Fruit Colour, Taste, and Quality: Improving Consumer Acceptance

Improving quality involves balancing consumer preferences and safe use with good agronomic performance. Traits such as rind colour may be visually assessed, whereas taste, texture, and biochemical composition may need more specialized evaluation. For traits such as bitterness, researchers should use suitable, validated assessment procedures and avoid drawing conclusions from a small number of fruits. The identification of bitterness-associated QTLs shows the potential of genetic mapping, but practical selection tools require further validation (Mkhize *et al.*, 2021). Fruit rind colour, flesh texture, taste, and bitterness contribute to consumer acceptance and market value. Genetic mapping has identified QTLs associated with bitterness in bottle gourd, showing that DNA-based approaches can help investigate quality traits (Mkhize *et al.*, 2021). Because taste and other quality traits can change with fruit maturity and growing conditions, researchers should record developmental stage and evaluate plants consistently before drawing conclusions.

QTL Mapping, GWAS, and Marker-Assisted Selection: Finding and Using Useful DNA Variants

QTL mapping and genome-wide association studies (GWAS) address related, but distinct, research questions. QTL mapping often uses a population developed from a planned cross between selected parents, whereas GWAS usually compares naturally occurring variation across a diverse collection. Both methods can identify genomic regions associated with traits, but each can be affected by population size, genetic background, measurement quality, and statistical power. Results should be treated as evidence of association until further experiments clarify the biological cause (Xu *et al.*, 2021; Zhao *et al.*, 2024). Before a marker is used in a breeding programme, it should be tested in independent plant material and, where possible, across locations and seasons. Researchers should evaluate how accurately the marker predicts the trait and whether its use improves selection compared with field assessment alone. A marker with a strong association in the original research population may be less informative in a different genetic background (Mkhize *et al.*, 2021).

QTL mapping identifies chromosome regions associated with traits that vary continuously, such as fruit weight, yield, flowering time, and stress tolerance. Researchers typically measure a trait in a population, genotype the plants with molecular markers, and use statistical analysis to find DNA regions associated with differences in the trait. In bottle gourd, QTL studies have helped investigate fruit shape and taste-related characteristics, while many complex traits still need further study (Xu *et al.*, 2021; Mkhize *et al.*, 2021). Genome-wide association studies (GWAS) look for relationships between naturally occurring DNA variants and traits across a diverse set of accessions. A 2024 analysis of 197 bottle gourd accessions illustrates how population-scale genomic data can be used to examine diversification and investigate disease-resistance traits, including response to zucchini yellow mosaic virus (Zhao *et al.*, 2024). GWAS can identify promising regions, but reliable results depend on accurate trait measurements, sufficient genetic diversity, and appropriate statistical analysis.

Marker-assisted selection (MAS) supports breeding decisions by using DNA markers associated with desirable alleles. For example, once a marker for a useful fruit trait or a disease-resistance locus has been validated, breeders may be able to screen seedlings rather than waiting for the trait to become visible. This can save time and space, but markers must be tested in the target breeding material because a marker that works in one population may be less reliable in another (Mkhize *et al.*, 2021).

Genetic Improvement for Resistance to Diseases and Environmental Stresses

Resistance screening should use clear and repeatable criteria. For disease studies, this may include symptom scoring, pathogen confirmation, and repeated evaluation. A plant that shows few symptoms in one trial is not necessarily genetically resistant: it may have escaped infection or experienced less exposure. Molecular tests and appropriate controls can help distinguish true resistance from disease avoidance or experimental variation (Mkhize *et al.*, 2021; Zhao *et al.*, 2024). Stress tolerance is often a complex outcome. Under drought, for example, plants may differ in root growth, water-use efficiency, flowering, and their ability to maintain fruit development. Heat stress can affect pollen viability, fruit set, and plant growth. Because these responses interact with the environment, breeding decisions should be based on replicated trials under relevant stress conditions as well as suitable non-stress comparisons (Mkhize *et al.*, 2021). Biotic stress results from living agents such as viruses, fungi, bacteria, and insect pests. Abiotic stress factors include water deficit, high temperatures, salinity, and nutrient shortage. Both types of stress can reduce growth, fruit quality, and yield, so resistance and tolerance are important breeding objectives (Mkhize *et al.*, 2021). Conventional screening helps identify promising accessions, while QTL mapping, GWAS, and molecular markers can help investigate the inherited basis of their responses. Population-genomic research has explored bottle gourd variation relevant to zucchini yellow mosaic virus resistance, but resistance markers need careful validation before they are used routinely in breeding (Zhao *et al.*, 2024). Drought and heat tolerance are especially complex because plant responses may involve root development, water-use efficiency, flowering, and fruit development, each affected by several genes and the environment. Strong breeding programmes should therefore combine molecular data with replicated field testing across locations and seasons (Mkhize *et al.*, 2021).

Transcriptomics, Functional Genomics, and Metabolomics: Explaining How Genes Influence Fruit Development

Transcriptomic experiments measure RNA abundance and can show which genes are more or less active in a tissue at a particular time. Comparisons between developmental stages or between contrasting genotypes can identify patterns worth investigating. However, high gene expression does not by itself prove that a gene causes a trait; additional functional experiments are needed to test its role (Wang *et al.*, 2018; Combined genomic, transcriptomic, and metabolomic analyses, 2024).

Metabolomics measures small molecules, including sugars, amino acids, lipids, and other compounds. These measurements can help describe how fruit composition changes during development and can point to pathways that may influence quality. Combining metabolomic results with gene-expression and genomic information gives a broader view of biological processes, although results may still be influenced by fruit maturity, growing conditions, and sample handling (Combined genomic, transcriptomic, and metabolomic analyses, 2024). Genome sequencing identifies the DNA sequences present, while transcriptomics indicates which genes are active under specific conditions. Functional genomics uses experiments to test how genes contribute to plant processes and traits. These methods can help researchers move from identifying a DNA region to understanding what biological process may explain a trait (Wang *et al.*, 2018).

A 2024 study combined genomic, transcriptomic, and metabolomic data to examine bottle gourd fruit development. It reported developmental changes in amino acids, lipids, sugars, and terpenoids and identified gene-expression patterns associated with fruit expansion and ripening (Combined genomic, transcriptomic, and metabolomic analyses, 2024). Metabolomics measures small molecules in a sample, helping researchers compare biochemical composition among genotypes or developmental stages. When these data types are analysed together, they can suggest links between gene activity and fruit quality. However, a candidate gene suggested by these analyses still requires experimental confirmation before its function can be considered established.

Genome Editing and Emerging Biotechnology: Exploring Targeted Changes in Bottle Gourd DNA

Unlike conventional crossing, genome editing targets a chosen DNA location instead of recombining numerous regions from parental genomes. In research, an edit can help test whether a candidate gene is involved in a trait. For crop development, however, success requires more than producing an edited sequence: researchers must obtain regenerated plants, confirm the change, assess possible unintended effects, and test the plant's performance over time (Mkhize *et al.*, 2021). One major practical barrier for many crops is efficient tissue culture and plant regeneration. If edited cells cannot be regenerated into healthy plants reliably, the method will have limited breeding value. Any future use in bottle gourd should also consider local regulations, biosafety evaluation, public acceptance, and whether the proposed trait provides a clear advantage over available breeding methods. Genome editing, including CRISPR/Cas-based methods, allows researchers to make targeted changes to DNA and test gene function. In principle, this could help investigate genes related to fruit shape, bitterness, flowering, disease resistance, and stress responses in bottle gourd. Practical use depends on reliable methods for transforming plant cells, regenerating whole plants, checking the intended edit, and assessing unintended changes (Mkhize *et al.*, 2021). Genome editing should currently be viewed as a developing research option rather than a routine solution for every bottle-gourd breeding problem. Edited plants would still need to be assessed for trait stability, field performance, safety, and compliance with applicable regulations. Conventional breeding and molecular selection remain important because they can use existing variation without requiring targeted genome modification.

Applying Genomic Information to Breeding, Hybrid Development, and Nutritional Quality

A practical genomics-assisted breeding pipeline begins by defining the target trait and identifying suitable germplasm. Researchers then characterize the parents, make crosses if required, measure offspring performance, and use validated markers or genomic predictions to support selection. Selected lines must still be tested for yield, quality, disease response, and stability. This stepwise approach helps ensure that molecular findings translate into benefits under actual production conditions (Mkhize *et al.*, 2021; Zhao *et al.*, 2024). Hybrid performance depends on the combination of parental genes and cannot be predicted solely from how genetically different the parents appear. Molecular data can help describe diversity and choose promising parents, but field evaluation is essential to identify hybrids with useful yield, uniformity, maturity, and fruit quality. Nutritional and biochemical traits should likewise be measured with consistent methods and considered alongside consumer acceptability and agronomic performance (Mkhize *et al.*, 2021; Combined genomic, transcriptomic, and metabolomic analyses, 2024).

Genomics-assisted breeding combines DNA-based information with traditional crossing and field-based selection. Breeders can use markers to identify diverse parents, track validated alleles, and select offspring that combine useful characteristics. Hybrid development may improve yield, uniformity, earliness, or other traits when suitable parental lines are identified, but genomic differences alone do not guarantee a successful hybrid; field trials are needed to confirm performance (Mkhize *et al.*, 2021; Zhao *et al.*, 2024). Genomic selection is a related approach that uses information from many markers to predict the likely performance of plants for complex traits. It can be useful when large training populations, dependable phenotypic measurements, and robust prediction models are available. Bottle gourd improvement can also include nutritional and biochemical quality, such as taste, flesh properties, and mineral composition. Metabolomics can help measure biochemical differences, but quality should be evaluated alongside yield, consumer preference, and adaptation to local production conditions (Combined genomic, transcriptomic, and metabolomic analyses, 2024; Mkhize *et al.*, 2021).

Current Research Gaps and Future Priorities for Bottle Gourd Improvement

Future studies would benefit from shared descriptor lists, consistent measurement protocols, and better documentation of germplasm identity. Multi-location trials can estimate how stable a trait is across environments, while combining field records with DNA data can help separate genetic effects from environmental influences. Publicly accessible datasets and well-described experimental methods would also make it easier for other researchers to reproduce findings and build on earlier work (Decker-Walters *et al.*, 2017; Wang *et al.*, 2018). Another priority is to move from association to validation. Candidate genes and markers should be checked in independent material, and their effects should be measured for both target traits and possible trade-offs. For example, selection for one quality or resistance trait should not unintentionally reduce yield or adaptation. Integrating genetic analysis with plant pathology, physiology, horticulture, and food-quality assessment will make future breeding outcomes more useful to growers and consumers (Mkhize *et al.*, 2021; Zhao *et al.*, 2024).

Important challenges remain. Germplasm may not always be evaluated with the same methods or in the same environments, making comparisons difficult. Many important traits, particularly yield stability, stress tolerance, and resistance to multiple diseases, are complex and not yet explained by a single reliable marker. A marker discovered in one genetic background may not work equally well in another, so validation across diverse accessions and locations is essential (Mkhize *et al.*, 2021; Zhao *et al.*, 2024). Future work should prioritize systematic conservation and characterization of germplasm, multi-location field trials, and the validation of markers for traits that breeders and growers need most. Combining QTL mapping, GWAS, transcriptomics, metabolomics, and functional experiments may narrow the search for useful genes, while improved plant-regeneration methods could support future genome-editing research. Collaboration among geneticists, breeders, horticulturists, and food scientists will be needed to turn genomic discoveries into varieties that perform reliably in farmers' fields (Wang *et al.*, 2018; Mkhize *et al.*, 2021).

Practical Implications: How Researchers Can Apply These Tools in Breeding Programmes

A practical programme can begin with a clearly defined breeding objective, such as improved fruit shape, yield stability, or disease response. Researchers should first select a representative set of accessions, record their traits using standard descriptors, and retain accurate labels and seed records. DNA markers can then help estimate relatedness and identify promising parents, while replicated field trials establish whether the plants have the desired performance (Decker-Walters *et al.*, 2017; Genome-wide SSR markers in bottle gourd, 2022). For a trait with a validated marker, marker-assisted selection may help screen early-generation plants. For traits controlled by many genes, a combination of careful phenotyping, genomic information, and multi-environment testing is likely to be more informative. In either case, the final decision should be based on agronomic performance and quality, not molecular results alone (Mkhize *et al.*, 2021; Zhao *et al.*, 2024).

Conclusion: Combining Conventional Breeding with Modern Genomic Tools

The strongest strategy is to retain conventional breeding while applying genomic tools wherever they provide additional benefit. Field evaluation shows whether a plant performs well under production conditions; DNA-based approaches help explain inherited differences and may make selection more efficient. Genomic tools are most useful when the trait is important, the marker or prediction is reliable, and the result has been confirmed in the intended breeding material (Mkhize *et al.*, 2021; Xu *et al.*, 2021). Overall, the resources described in this review create a strong foundation for future bottle gourd improvement, but continued validation and collaboration are essential. The long-term goal is to develop varieties that combine market-preferred fruits, reliable yield, acceptable quality, and adaptation to local environments. Achieving this goal requires careful germplasm conservation, strong field experiments, transparent reporting, and responsible use of new biotechnology (Decker-Walters *et al.*, 2017; Zhao *et al.*, 2024).

Genetic diversity and modern genomic tools provide complementary ways to improve bottle gourd. Field characterization identifies useful plants, while molecular markers and genome-wide data help researchers understand their genetic relationships and locate regions associated with traits. Advances include genome resources, large SSR marker collections, fruit-shape QTLs, population-level genomic analyses, and multi-omics studies of fruit development (Xu *et al.*, 2021; Genome-wide SSR markers in bottle gourd, 2022; Zhao *et al.*, 2024; Combined genomic, transcriptomic, and metabolomic analyses, 2024). The next step is to validate these discoveries in breeding populations and real production environments. Combining conventional selection with well-tested molecular tools offers a practical path towards bottle gourd varieties with preferred fruit traits, better quality, and improved adaptation to changing environmental conditions (Mkhize *et al.*, 2021; Decker-Walters *et al.*, 2017).

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