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ADVANCED BREEDING TECHNOLOGIES FOR FRUIT CROPS: GENOMIC SELECTION, SPEED BREEDING AND GENOME EDITING

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ABSTRACT

Fruit crops are essential components of global agriculture, contributing substantially to nutritional security, farm income, employment and agricultural diversification. However, genetic improvement of perennial fruit crops is constrained by long juvenile phases, extended generation intervals, large plant size, high heterozygosity, polyploidy, reproductive barriers, complex quantitative trait architecture and strong genotype × environment interactions. These limitations make conventional crossing and phenotypic selection time-consuming, resource-intensive and often inefficient for traits that can only be evaluated after several years. Recent advances in genomics and biotechnology provide opportunities to overcome these constraints and accelerate genetic gain. Genomic selection (GS) enables genome-wide prediction of breeding values and early selection of superior individuals for complex traits, reducing dependence on prolonged phenotypic evaluation. Speed breeding complements genomic selection by shortening generation intervals through controlled environmental conditions, manipulation of flowering, grafting and other generation-acceleration strategies, thereby increasing the number of breeding cycles achievable within a given period. Precision genomics, particularly CRISPR/Cas-based genome editing, enables targeted modification of genes and regulatory regions associated with disease resistance, fruit quality, nutritional composition, plant architecture and reproductive traits. Recent developments in multiplex editing, promoter editing, base editing and prime editing are further expanding the precision and versatility of genome engineering. The integration of genomic selection, speed breeding and precision genomics with high-throughput phenotyping offers considerable potential to transform fruit breeding from a predominantly phenotype-driven process into a predictive, accelerated and precision-oriented system. This review summarizes the principles, applications, successes and limitations of these advanced breeding technologies and discusses their potential integration for developing high-yielding, high-quality, climate-resilient and market-preferred fruit cultivars.

KEYWORDS: Genomic selection, Speed breeding, Precision genomics, CRISPR/Cas genome editing, High-throughput, phenotyping, Fruit crop improvement, Climate-resilient cultivars

INTRODUCTION

Fruit crops occupy an important position in global agriculture because of their contributions to food and nutritional security, farm income, employment and agricultural diversification. Fruits provide essential vitamins, minerals, dietary fibre, antioxidants and diverse bioactive compounds associated with human health. They also have considerable economic value through fresh consumption, processing, export and value-added products. With increasing population, changing dietary preferences and growing demand for nutritious foods, fruit production must increase while maintaining desirable attributes such as yield, flavour, nutritional quality, firmness, storability, shelf life and market acceptability. Consequently, genetic improvement of fruit crops has become increasingly important for developing cultivars that combine productivity, quality and resilience (Zhou *et al.* 2020; Lee *et al.* 2024).

However, fruit production faces a growing combination of biological, environmental and socioeconomic challenges. Limited availability of productive land and water, climate change, emerging pests and diseases and increasing environmental variability threaten stable fruit production. Rising temperatures, altered precipitation and increasing frequencies of drought, heat and flooding can affect flowering, fruit set, development and quality, while changing climatic

conditions may also alter the distribution and severity of pests and pathogens. At the same time, consumers increasingly demand fruits with improved flavour, appearance, nutritional composition, uniformity and postharvest quality. Breeding programmes must therefore simultaneously address multiple, often quantitatively inherited traits while maintaining adaptation to changing production environments (Osorio-Marín *et al.* 2024; Yadav *et al.* 2023; Chaurasiya *et al.* 2026).
Conventional breeding based on hybridization, recombination and phenotypic selection has contributed substantially to the development of improved fruit cultivars. Nevertheless, its efficiency is considerably lower in perennial fruit crops than in many annual crops. The long juvenile phase and extended generation interval of fruit trees can delay flowering and fruit production for several years, while large plant size and substantial space requirements make the establishment and evaluation of large breeding populations expensive. Moreover, many important traits, including yield, fruit quality, flavour, maturity, storage characteristics and stress resistance, can only be reliably evaluated after plants reach reproductive or physiological maturity. Consequently, breeders may need to maintain thousands of seedlings for several years before superior individuals can be identified (van Nocker and Gardiner 2014; De Mori and Cipriani 2023).
The genetic and reproductive characteristics of fruit crops further complicate conventional improvement. Many species exhibit high heterozygosity, polyploidy, self-incompatibility, complex reproductive systems and substantial genotype × environment interactions. Important breeding objectives such as fruit size, sugar content, acidity, firmness, colour, flavour, maturity, yield and stress tolerance are generally controlled by multiple genes, often with relatively small individual effects. Phenotypic selection alone may therefore be inefficient, particularly for traits with moderate heritability or strong environmental influence. These limitations increase both the time and resources required to achieve meaningful genetic gain and emphasize the need for approaches that can improve selection accuracy while reducing the breeding cycle (Singh and Pradhan 2020; De Mori and Cipriani 2023; Lee *et al.* 2024).
Rapid developments in genomics, high-throughput genotyping, sequencing, bioinformatics and phenomics have created new opportunities to overcome these limitations. Molecular markers and genome-wide approaches allow breeders to characterize genetic variation and investigate the genetic architecture of complex traits. Marker-assisted selection, QTL mapping and genome-wide association studies (GWAS) have facilitated the identification of genomic regions and alleles associated with economically important traits. However, because many fruit traits are highly polygenic, identifying individual significant loci may not always provide sufficient predictive power for selection. Genomic selection (GS) addresses this limitation by using genome-wide marker information to estimate the breeding value of individuals, allowing superior seedlings to be selected before expensive and time-consuming phenotyping of mature plants. This approach has consequently attracted increasing attention in perennial fruit crops (Meuwissen *et al.* 2001; De Mori and Cipriani 2023; Lee *et al.* 2024).
Reducing the generation interval is equally important for increasing the rate of genetic gain. Speed-breeding approaches seek to accelerate plant development and reproduction through controlled environmental conditions, optimized photoperiod and temperature, manipulation of flowering, grafting, growth regulators and other generation-acceleration strategies. Although initially developed mainly for annual crops, these approaches are particularly relevant to fruit trees because their long juvenile phase represents one of the major barriers to rapid breeding. Early-flowering technologies provide an additional opportunity to shorten juvenile periods and generate successive breeding generations more rapidly, potentially allowing speed breeding to be integrated with genomic selection for early identification and rapid advancement of superior individuals (Ghosh *et al.* 2018; Watson *et al.* 2018; van Nocker and Gardiner 2014).
The development of precision genomics and genome editing has further expanded the possibilities for fruit crop improvement. CRISPR/Cas systems enable targeted modification of genes and regulatory regions, providing opportunities to generate desirable alleles directly in elite genetic backgrounds. Applications in fruit crops have demonstrated the potential of genome editing for improving disease resistance, fruit quality, nutritional composition, plant architecture and reproductive traits. Advances including multiplex editing, promoter editing, base editing and prime editing are further increasing the precision and range of possible genetic modifications. Such approaches are particularly valuable when favourable alleles are absent from available germplasm or when conventional introgression would require several generations of crossing and backcrossing (Zhou *et al.* 2020; Xia *et al.* 2021).
The future of fruit breeding will therefore depend increasingly on the integration of conventional breeding with genomic prediction, speed breeding, high-throughput phenotyping and precision genomics, rather than on replacing conventional approaches. Genomic selection can predict the breeding value of young individuals, speed breeding can accelerate generation turnover, and genome editing can precisely modify genes or regulatory regions identified through genetic and genomic analyses. Integration with pangenomics, multi-omics, environmental data and artificial intelligence may further improve the prediction and manipulation of complex traits. Together, these technologies have the potential to transform fruit breeding from a predominantly phenotype-dependent and time-intensive process into a predictive, accelerated and precision-oriented breeding system (Li *et al.* 2025; Lee *et al.* 2024).
Against this background, this review discusses the principles, applications, opportunities and limitations of genomic selection, speed breeding and precision genomics in fruit crops, with emphasis on their potential integration to increase genetic gain, shorten breeding cycles and facilitate the development of high-yielding, high-quality, stress-resilient and climate-adapted fruit cultivars.

Limitations and Bottlenecks in Fruit breeding

Fruit crop breeding is inherently more complex and time-consuming than breeding in many annual crops because of the perennial nature, reproductive biology, genetic architecture and environmental sensitivity of fruit-producing species. Although conventional breeding has generated numerous cultivars with improved yield, fruit quality and adaptation, its efficiency is constrained by long generation intervals, prolonged juvenile phases, high heterozygosity, polyploidy, narrow genetic bases, complex quantitative inheritance, reproductive barriers, high phenotyping costs and increasing climatic

variability. These limitations become particularly challenging when multiple traits, such as yield, fruit quality, pest and disease resistance and climate resilience, must be combined in a single cultivar (van Nocker and Gardiner 2014; Kole *et al.* 2015; De Mori and Cipriani 2023; Lee *et al.* 2024).

One of the most fundamental constraints is the long juvenile phase and extended generation interval characteristic of many perennial fruit crops. Unlike annual crops, many fruit trees require several years to reach reproductive maturity, delaying the evaluation of economically important traits such as flowering, yield, fruit size, maturity, quality and reproductive performance. Even after flowering begins, traits such as yield stability, fruit quality and alternate bearing may require evaluation across multiple seasons. Consequently, repeated cycles of crossing, progeny development and phenotypic selection can extend cultivar development over many years, reducing the rate of genetic gain per unit time (van Nocker and Gardiner 2014).

The genetic complexity of many fruit crops is further increased by polyploidy and complex genome organization. Multiple chromosome copies can result in polysomic, disomic or mixed inheritance, while allele dosage can influence phenotypic expression. In allopolyploids, the presence of related subgenomes also complicates the distinction between homologous and homeologous loci. These features can affect linkage mapping, QTL analysis, marker development, genome-wide association studies and genomic prediction (Ma *et al.* 2023). Polyploidy also presents challenges for genome editing because multiple copies of a target gene may need to be modified to produce a measurable phenotype (Ma *et al.* 2023).

High heterozygosity is another important characteristic of many fruit crops, particularly those with outcrossing systems and self-incompatibility. Highly heterozygous parents can produce progeny with extensive segregation, requiring large populations to identify favourable allele combinations. Developing homozygous lines through conventional inbreeding is often impractical because of self-incompatibility, inbreeding depression and long generation times. High heterozygosity can also complicate genome assembly, variant identification and haplotype reconstruction, making genotype phenotype relationships more difficult to predict (Ma *et al.* 2023; Germana 2006).

The availability and effective utilisation of genetic diversity also pose challenges. Although many fruit species possess considerable genetic diversity, breeding programmes often rely on a relatively narrow group of elite cultivars and parents. This can restrict access to alleles for emerging breeding objectives such as pathogen resistance, drought and heat tolerance, salinity tolerance and improved nutritional quality. Wild relatives, landraces and geographically diverse germplasm can provide valuable variation, but their incorporation into elite backgrounds may introduce undesirable characteristics (Sabety *et al.* 2024). Linkage drag may therefore require several generations of backcrossing and selection to recover the elite genetic background while retaining the desired allele (Sabety *et al.* 2024).

The improvement of fruit crops is further complicated by the quantitative inheritance of many economically important traits. Yield, fruit weight, firmness, soluble solids, acidity, flavour, nutritional composition, flowering time and tolerance to abiotic stresses are frequently controlled by multiple loci with relatively small effects. Their expression is also strongly influenced by environmental conditions and genotype × environment interactions (De Mori and Cipriani 2023; Lee *et al.* 2024). Phenotypic selection based on a single season or location may therefore provide an unreliable estimate of breeding value. Multi-year and multi-location evaluation is often necessary, substantially increasing the cost and duration of breeding programmes. This complexity also reduces the effectiveness of conventional marker-assisted selection for traits controlled by numerous small-effect loci (Meuwissen *et al.* 2001; Lee *et al.* 2024).

The reproductive biology of many fruit crops creates additional barriers to conventional hybridization. Self-incompatibility, pollen sterility, differences in flowering time, poor pollen viability, embryo abortion and interspecific or interploidy incompatibility can restrict the combinations of parents that can be successfully crossed (Anderson 2010; Germana 2006; van Nocker and Gardiner 2014). Even when valuable alleles are identified in wild or related species, their transfer into elite cultivars may require specialized approaches such as embryo rescue, tissue culture, chromosome manipulation and repeated backcrossing. These reproductive barriers consequently reduce the range of genetic combinations that can be explored (Anderson 2010).

Another major bottleneck is the large plant size, high resource requirement and difficulty of phenotyping associated with perennial fruit crops. Mature trees require substantial land, irrigation, nutrients and labour, limiting the size of breeding populations. This is particularly problematic because large populations are desirable for recovering rare favourable recombinations. While traits such as fruit size, colour and shape can be measured relatively easily, characteristics including flavour, aroma, texture, nutritional composition, metabolite profiles and storage behaviour may require specialized instruments, destructive sampling or biochemical analyses (van Nocker and Gardiner 2014; Lee *et al.* 2024). High phenotyping costs can therefore restrict population size and selection intensity.

The perennial nature of fruit crops also increases the difficulty of evaluating alternate bearing and genotype × environment interactions. In alternate-bearing species, high yield in one season may be followed by low yield in the next, complicating the assessment of long-term productivity. Similarly, genotype performance can vary considerably across environments because of differences in temperature, rainfall, soil conditions, altitude, management and pest or disease pressure (Smith and Samach 2013; Sharma *et al.* 2019). Reliable cultivar selection therefore often requires multi-environment and multi-year testing, further increasing breeding time and resource requirements.

Climate change adds another layer of complexity to fruit crop improvement. Rising temperatures, altered precipitation patterns, drought, heat waves, irregular chilling conditions and extreme weather events can affect flowering, pollination, fruit set, development and quality. Temperate fruit crops may be particularly sensitive to changes in winter chilling requirements, whereas heat during flowering can reduce pollen viability and fruit set. Drought and heat can impair photosynthesis and reproductive performance, while excessive rainfall and flooding can affect root function and increase disease pressure. Climate change may also alter pest and pathogen distributions (Yadav *et al.* 2023; Osorio-Marín *et al.* 2024). Developing climate-resilient cultivars is therefore challenging because tolerance and environmental adaptation are generally quantitative traits strongly influenced by genotype × environment interactions.

Collectively, these constraints create a fundamental mismatch between the biological timescale of fruit breeding and the increasing need for rapid genetic improvement. The major challenge is not simply generating genetic variation, but identifying, evaluating and deploying favourable variation efficiently within long-lived and genetically complex plants. These limitations provide a strong rationale for integrating advanced breeding technologies with conventional approaches (van Nocker and Gardiner 2014; Lee *et al.* 2024; Ma *et al.* 2023).

Genomic Selection

Genomic selection is a modern breeding method that uses DNA markers across the entire genome to predict the breeding value and performance of plants or animals before physical testing. It speeds up selection, lowers costs, and increases genetic gain compared to traditional methods (Meuwissen *et al.* 2001; Crossa *et al.* 2017).

Principles of genomic selection

Genomic selection (GS) represents a major shift from marker-assisted selection and QTL-based breeding because it uses genome-wide molecular marker information to predict the genetic merit of individuals rather than selecting individuals based on a limited number of significant markers. The concept was originally proposed by Meuwissen *et al.* (2001) and has subsequently become an important component of modern breeding programmes (Crossa et al. 2017; Meuwissen *et al.* 2001). In GS, a training population consisting of individuals with both genome-wide genotype information and reliable phenotypic records is used to estimate the relationship between marker variation and phenotypic performance. A prediction model is then developed to estimate the genomic estimated breeding value (GEBV) of individuals in a candidate population for which genotype data are available but phenotypic data may be absent or incomplete. The highest-ranking individuals can subsequently be selected as parents or advanced in the breeding programme. This is particularly advantageous in perennial fruit crops because seedlings can potentially be selected before reaching reproductive maturity, thereby reducing the need to maintain large numbers of trees until fruiting. A systematic review published in 2024 identified GS applications across 25 fruit and vegetable crops and emphasized its potential to increase selection intensity, prediction accuracy and genetic gain (Meuwissen *et al.* 2001; Minamikawa *et al.* 2017).

The basic GS pipeline therefore involves several interconnected stages: development of a representative training population, phenotyping, genome-wide genotyping, construction of a genomic relationship matrix, model training, prediction of GEBVs, validation, selection of superior individuals and incorporation of selected individuals into the breeding programme. The training population is particularly important because prediction accuracy depends strongly on its size, genetic diversity, relatedness to the candidate population, trait heritability and quality of phenotypic data (Jannink *et al.* 2010; Lee *et al.* 2024; Crossa *et al.* 2017). For perennial fruit crops, the training population can be constructed from breeding families, germplasm collections, historical breeding material or combinations of these sources. Once a prediction model is established, genotyping of young seedlings can allow breeders to predict their breeding values without waiting several years for fruiting phenotypes. This provides a direct mechanism for reducing the effective generation interval and increasing genetic gain per unit time (Meuwissen *et al.* 2001; Muranty *et al.* 2015).

A major conceptual advantage of GS over conventional marker-assisted selection is its ability to capture polygenic variation. Many economically important fruit traits including fruit weight, firmness, soluble solids, acidity, maturity, yield, disease resistance and environmental adaptation are controlled by numerous loci with relatively small effects. QTL mapping and MAS may fail to capture much of this variation because they focus on individual loci or genomic regions that reach statistical significance. GS instead attempts to capture the combined contribution of genome-wide markers. The markers do not necessarily have to be physically located within causal genes; they can act as proxies for causal variants through linkage disequilibrium. This makes GS particularly suitable for complex quantitative traits, provided that the marker density and training population are sufficient to capture the relevant genetic variation (Meuwissen *et al.* 2001; Crossa *et al.* 2017; De Los Campos *et al.* 2013).

Statistical models used in genomic selection

Several statistical and computational models have been developed to convert genome-wide marker information into genomic breeding values. The major approaches used in fruit crops include genomic best linear unbiased prediction (GBLUP), ridge regression, Bayesian models, kernel-based methods and machine-learning algorithms. These approaches differ primarily in their assumptions regarding marker effects, genetic architecture and relationships among individuals. No single model is universally superior because prediction performance depends on the trait, population structure, marker density, training population and genetic architecture (De Los Campos *et al.* 2013; Crossa *et al.* 2017; Lee *et al.* 2024).

Among these approaches, GBLUP is one of the most widely used genomic prediction models. GBLUP replaces the conventional pedigree relationship matrix with a genomic relationship matrix (G) calculated from genome-wide molecular markers. Individuals sharing a greater proportion of alleles are therefore assigned a higher genomic relationship, allowing the model to estimate their breeding values using both phenotypic information and genome-wide genetic relationships (VanRaden 2008; De Los Campos *et al.* 2013). The general mixed-model representation can be expressed as:

$$\mathbf{y = X\beta + Zu + e}$$

Where y represents phenotypic observations, β represents fixed effects, u represents genomic breeding values, X and Z are incidence matrices and e represent residual error (De Los Campos *et al.* 2013; VanRaden 2008).

GBLUP is particularly suitable for highly polygenic traits in which numerous loci contribute small effects. Its computational simplicity and relatively stable performance have made it one of the major approaches used in fruit crop genomic prediction. GBLUP can also be extended to incorporate dominance, genotype × environment interactions, multiple traits and multi-environment datasets (VanRaden 2008; Crossa *et al.* 2017; De Los Campos *et al.* 2013).

Bayesian genomic prediction models provide an alternative framework in which marker effects are assigned prior probability distributions. Major approaches include BayesA, BayesB, BayesC, Bayesian ridge regression and Bayes LASSO. These models differ in the extent to which marker effects are allowed to vary and the degree of shrinkage applied to individual marker effects. Bayesian approaches can be advantageous when the genetic architecture contains a mixture of many small-effect loci and a smaller number of loci with relatively larger effects. For example, BayesB assumes that a proportion of markers have negligible effects while allowing the remaining markers to have variable effects. In contrast, Bayesian ridge regression applies relatively uniform shrinkage and is conceptually similar to ridge-regression-based genomic prediction (De Los Campos *et al.* 2013; Crossa *et al.* 2017; Meuwissen *et al.* 2001). These approaches have been evaluated in fruit crops alongside GBLUP, particularly for traits such as fruit quality, yield and disease resistance.

Kernel-based approaches, including reproducing kernel Hilbert space (RKHS) models, provide a means of capturing nonlinear relationships between genotype and phenotype. While GBLUP primarily models additive relationships, kernel models can potentially capture nonlinear genetic effects, including certain forms of epistasis. This can be useful when the relationship between genomic similarity and phenotypic similarity is not adequately represented by a simple linear model. However, kernel methods require careful parameterization and may not consistently outperform simpler linear models, particularly when the trait is predominantly additive (Crossa *et al.* 2017; De Los Campos *et al.* 2013).

The increasing availability of high-density genomic datasets has also encouraged the application of machine-learning approaches such as random forest, support vector machines, elastic net, LASSO, artificial neural networks and deep-learning models. These approaches can potentially capture nonlinear relationships, marker interactions and complex genotype–phenotype patterns. However, greater computational complexity does not necessarily result in greater prediction accuracy. In relatively small fruit breeding populations, conventional linear models such as GBLUP or Bayesian ridge regression can remain highly competitive because machine-learning models may overfit when the number of markers greatly exceeds the number of training individuals (Crossa *et al.* 2017; Azodi *et al.* 2019). Thus, machine learning should currently be viewed as a complementary strategy within genomic prediction rather than an automatic replacement for established mixed-model approaches.

Comparative studies in fruit crops demonstrate that model performance is trait- and population-dependent. For example, Minamikawa *et al.* evaluated several prediction approaches in citrus using 1,841 SNP markers and 17 fruit-quality traits and found that linear GBLUP/ridge approaches were highly competitive, whereas incorporating dominance effects improved prediction for some traits such as acidity and juiciness. These results illustrate an important principle for fruit breeding: model selection should be empirically optimized for each breeding population and trait rather than assuming that a more sophisticated model will always produce greater prediction accuracy (Minamikawa *et al.* 2017).

Similar evidence has been obtained in other fruit crops. In apple, Muranty *et al.* (2015) used 977 individuals, 7,829 SNPs and 10 traits related to productivity and fruit appearance; prediction accuracy varied substantially among traits and was strongly influenced by heritability and phenotypic distribution. Their results demonstrated the potential of genomic prediction to accelerate breeding in outbred fruit trees while reducing the dependence on extensive phenotyping of mature plants. In peach, Biscarini *et al.* (2017) investigated genome-enabled prediction for fruit weight and quality traits using repeated records, providing further evidence for the application of genomic prediction to perennial fruit breeding.

Evidence and success stories of genomic selection in fruit

The practical potential of GS has been demonstrated most extensively in apple, one of the best-developed perennial fruit systems for genomic breeding. Muranty *et al.* (2015) evaluated 977 apple individuals belonging to 20 pedigreed full-sib families using 7,829 SNP markers and assessed ten productivity and fruit-appearance traits. Genomic prediction accuracy reached up to approximately 0.50 for individual traits, with accuracy strongly influenced by heritability and the phenotypic distribution of traits. Importantly, the study demonstrated that genomic predictions could be translated into selection responses, providing early evidence that GS could move beyond theoretical prediction and contribute directly to apple breeding. Subsequent apple research has extended genomic prediction to additional fruit-quality traits, demonstrating the possibility of using genomic information to support earlier selection in breeding populations (Muranty *et al.* 2015).

Citrus represents another particularly important application because conventional citrus breeding is constrained by long juvenile periods and expensive field evaluation. Minamikawa *et al.* (2017) evaluated 676 citrus breeding individuals from 35 full-sib families using 1,841 SNP markers and 17 fruit-quality traits. Several genomic prediction models were compared, and models incorporating additive and dominance effects improved prediction for some characteristics, particularly acidity and juiciness. GBLUP using a linear ridge kernel was also more robust than the nonlinear Gaussian-kernel approach for observations at the tails of the phenotypic distribution (Minamikawa *et al.* 2017).

In another study involving hybrids derived from Pera sweet orange × Murcott tangor, Gois et al. (2016) evaluated 180 individual trees for ten fruit-quality characteristics. The hybrids were genotyped using 5,287 DArTseq markers, and marker effects were predicted using the rr-BLUP method. Predictive abilities ranged from approximately 0.53 to 0.64, while more than 35% of the genetic variance was explained by the markers. Although prediction accuracy from genomic selection was lower than that obtained from phenotypic selection, genomic selection produced greater genetic gain per unit time because it allowed earlier prediction and selection (Gois *et al.* 2016).

In peach, genomic selection has been evaluated for traits directly related to commercial fruit quality. Biscarini *et al.* (2017) investigated genome-enabled prediction in 1,147 individuals from eleven European peach populations. The study evaluated fruit weight, sugar content and titratable acidity, using an IPSC 9K SNP array. Plants were grown in France, Italy and Spain and phenotyped over three to five years. The study demonstrated the potential of repeated phenotypic records and genome-wide markers for predicting important fruit-quality traits in peach (Biscarini *et al.* 2017).

Grapevine has also emerged as an important crop for genomic prediction because breeding objectives frequently involve complex traits related to yield, berry composition, phenology and vigour. In a study designed to approximate a realistic

breeding situation, genomic prediction was evaluated across a diversity panel and ten bi-parental grapevine crosses for 15 traits. Prediction of cross means produced an average per-trait predictive ability of approximately 0.60, whereas prediction of individual progeny within crosses was lower, with an average predictive ability of approximately 0.26. The study demonstrated that genetic distance between parents, heritability and the magnitude of cross effects influenced prediction performance, highlighting the importance of training-population design and genetic relatedness in grapevine genomic prediction (Brault *et al.* 2022).

More recently, grapevine genomic breeding has begun to incorporate genomic variation beyond conventional SNP markers. A graph-based grapevine pangenome constructed from 29 assemblies identified more than 236,000 structural variants across 466 grapevine cultivars. Incorporating structural variants into GWAS improved estimated heritability for several traits, and genomic prediction was performed for 29 complex agronomic traits. These findings demonstrate the potential of integrating pangenomic and structural-variation information into genomic breeding rather than relying exclusively on SNP variation from a single reference genome (Liu *et al.* 2024).

The application of GS to mango is more recent but particularly promising because mango is a perennial, highly heterozygous fruit crop in which phenotyping mature trees can be expensive and time-consuming. A recent study evaluated genomic prediction for fruit blush colour, average fruit weight, fruit firmness and trunk circumference in 225 mango gene-pool accessions using GBLUP-based models. Importantly, when population structure was accounted for using principal components, predictive ability decreased substantially for all traits, indicating that apparently high prediction accuracy can partly arise from genetic differences among subpopulations rather than genuine prediction of within-population breeding values. The study therefore emphasizes the importance of accounting for population structure when developing and validating genomic prediction models in diverse mango germplasm (2025).

Collectively, evidence from apple, citrus, peach, grapevine and mango demonstrates that genomic selection can be applied across diverse perennial fruit crops and breeding populations. However, prediction accuracy and practical utility vary substantially among crops and traits. The strongest evidence has generally emerged from crops with well-developed genomic resources, adequately characterized training populations and reliable phenotypic datasets. A recent systematic scoping review identified 63 genomic-selection studies covering 25 fruit and vegetable crops, illustrating the expanding application of GS while also highlighting differences in training-population design, marker density, prediction accuracy and validation strategies among crops (Lee *et al.* 2024).

Challenges and future prospects

Despite its potential, GS faces several challenges in fruit crops. The first is the development of large and representative training populations. Unlike annual crops, where large numbers of individuals can often be phenotyped relatively rapidly, perennial fruit trees may require several years before reliable phenotypic data become available. Consequently, establishing a sufficiently large training population can itself be expensive and time-consuming. The training population must also capture sufficient genetic diversity and maintain an appropriate relationship with the candidate population; otherwise, prediction accuracy may decline when models are applied to genetically divergent individuals (Lee *et al*. 2024; Crossa *et al.* 2017; Jannink *et al.* 2010).

The second major challenge is phenotyping. Genomic prediction is dependent on the quality and reliability of the phenotypic information used to train the model. Fruit quality can be influenced by developmental stage, environmental conditions, location, management and year. Consequently, multi-year and multi-environment phenotyping can be important for obtaining reliable estimates of genetic values. Genotype × environment interactions can also reduce the transferability of prediction models between environments, making multi-environment genomic prediction an important area for future development (Crossa *et al.* 2017; Lee *et al.* 2024). The peach study of Biscarini *et al.* (2017), for example, used repeated records collected over several years and environments, illustrating the importance of robust phenotypic data for genomic prediction in perennial fruit crops.

Population structure and genetic relatedness also require careful consideration. High prediction accuracy can sometimes result from close relationships between training and validation individuals rather than from genuine genome-wide prediction across unrelated genetic backgrounds. The grapevine study by Roth *et al.* (2022) demonstrated that genetic distance between parents was an important determinant of prediction performance. Similarly, the recent mango study showed that accounting for population structure substantially reduced apparent predictive ability, indicating that some initial prediction accuracy was associated with differences between subpopulations (Brault *et al.* 2022).

Other limitations include genotyping cost, limited population size, low-frequency alleles, marker density, computational requirements and model overfitting. The optimal prediction model can also differ among traits, populations and genetic architectures. For example, Minamikawa *et al.* (2017) demonstrated that incorporating dominance effects improved prediction for some citrus traits, whereas the performance of different kernel approaches varied according to the phenotypic distribution. Therefore, model comparison and validation under realistic breeding scenarios are essential before GS is implemented operationally (Crossa *et al.* 2017; Lee *et al.* 2024; Minamikawa *et al.* 2017).

Future fruit breeding programmes will increasingly benefit from integrating GS with high-throughput phenotyping, multi-environment trials, pedigree information, pangenomics, environmental covariates and speed-breeding approaches. Pangenomic resources can expand the range of genetic variation available for prediction, including structural variants that may not be captured adequately by conventional SNP datasets. In grapevine, for example, integration of pangenomic structural variation with GWAS and genomic prediction has already demonstrated the potential of this approach for multi-trait genomic breeding (Liu *et al.* 2024).

Such integration could allow breeders to genotype seedlings soon after germination, predict their breeding values for multiple traits, eliminate inferior individuals before orchard establishment and prioritize selected genotypes for detailed phenotyping. In this context, GS is not simply a replacement for phenotypic selection; rather, it is a mechanism for

reorganizing the breeding pipeline so that genomic information is used to make earlier and more informed decisions. The ultimate objective is therefore to increase genetic gain per unit time, rather than simply maximizing prediction accuracy in an experimental dataset (Lee *et al.* 2024; Meuwissen *et al.* 2001; Crossa *et al.* 2017).

Speed Breeding

Speed breeding (SB) refers to a collection of approaches that shorten the interval between successive generations by manipulating plant growth, flowering, seed development and environmental conditions. Although the concept was initially developed and extensively demonstrated in annual crops, its importance is potentially greater in perennial fruit crops because the long juvenile phase represents one of the major constraints on genetic gain. In conventional fruit breeding, breeders may have to wait several years before seedlings flower and produce fruit, with juvenile periods varying substantially among tree crops. The objective of speed breeding in fruit crops is therefore not simply to make plants grow faster, but to compress the seed-to-seed or crossing-to-crossing interval. This can involve controlled environments, optimized photoperiod and temperature, manipulation of flowering pathways, grafting, growth regulation, early seed recovery and, where appropriate, genetic approaches for precocious flowering (van Nocker and Gardiner 2014; Blinkov *et al.* 2025; Mir *et al.* 2026).

An important distinction should be made between speed breeding and early flowering. Speed breeding is the broader strategy for accelerating generation turnover, whereas early-flowering technologies represent one mechanism for overcoming juvenility and accelerating successive breeding cycles. This distinction is particularly relevant to fruit crops because simply increasing vegetative growth does not necessarily cause a juvenile tree to flower. Consequently, fruit-tree speed breeding may require integration of controlled environments with technologies that specifically manipulate flowering pathways (Mir *et al.* 2026; van Nocker and Gardiner 2014).

Technologies for speed breeding

Speed breeding combines several complementary technologies rather than relying on a single treatment. Major components include photoperiod manipulation, light-quality and light-intensity optimization, temperature control, CO_2 enrichment, optimized nutrition, plant-growth regulators, grafting, high-density cultivation, early seed harvesting and embryo culture, together with genetic manipulation of flowering pathways where appropriate. Modern speed-breeding systems therefore function as integrated approaches in which several stages of the plant life cycle are accelerated (Blinkov *et al.* 2025).

Photoperiod manipulation is one of the major environmental approaches used in speed breeding. Extending the daily photoperiod can accelerate flowering in photoperiod-responsive species, while appropriate light intensity and spectral composition can influence photosynthesis and developmental rate. Standardized speed-breeding systems for annual crops commonly combine extended photoperiods with controlled temperature and optimized light conditions. However, these systems cannot necessarily be transferred directly to woody fruit crops because their developmental and architectural requirements differ substantially (Blinkov *et al.* 2025).

Temperature regulation is another important component. Maintaining plants within an appropriate temperature range can accelerate vegetative development and influence flowering. In perennial fruit crops, environmental manipulation may additionally interact with dormancy and chilling requirements. Other approaches include optimized nutrition, CO_2 supplementation, manipulation of plant density, pruning and shoot management, grafting and plant-growth regulators. Thus, speed breeding should be regarded as an integrated environmental and developmental strategy rather than the manipulation of a single environmental parameter (Blinkov *et al.* 2025).

For perennial fruit crops, early flowering and rapid seed-to-seed cycling can be particularly valuable once flowering has been achieved. Early-flowering systems can substantially shorten the interval between crossing generations. Therefore, the breeding cycle can potentially be compressed at both ends: rapid induction of flowering at the reproductive stage and accelerated recovery of the next generation after seed production (Mir *et al.* 2026; van Nocker and Gardiner 2014).

Controlled-environment systems

Controlled-environment systems provide the physical infrastructure required for speed breeding. Growth chambers, controlled-environment rooms and greenhouse systems allow breeders to manipulate light duration, light intensity, temperature, humidity, CO_2 concentration and irrigation. Such systems can maintain plants under conditions favourable for rapid development throughout the year and can reduce seasonal restrictions on breeding cycles (Blinkov *et al.* 2025).

For fruit crops, however, controlled environments present greater challenges because mature woody plants require considerably more physical space than annual crops. Large canopy size and extensive root systems make conventional tree forms difficult to accommodate in small growth chambers. Consequently, compact growth forms, pruning, grafting, dwarfing rootstocks and high-density cultivation can potentially become important components of fruit-tree speed breeding. These considerations mean that speed-breeding protocols developed for annual crops cannot simply be transferred to perennial fruit crops without crop-specific modification (van Nocker and Gardiner 2014; Mir *et al.* 2026).

Controlled environments can also potentially be integrated with genomic selection and high-throughput phenotyping. Young plants can be genotyped and evaluated under standardized conditions, genomic predictions can be generated, and superior individuals can be prioritized for further breeding. Thus, controlled environments may contribute not only to faster generation turnover but also to more efficient selection.

Early-flowering technologies

Because juvenility is one of the most serious obstacles in perennial fruit breeding, induction of precocious flowering represents a particularly important component of rapid-cycle breeding. One extensively investigated strategy involves

manipulating genes involved in the flowering pathway, including FLOWERING LOCUS T (FT) and other flowering regulators. Such approaches can induce flowering substantially earlier than would occur under normal juvenile development (van Nocker and Gardiner 2014).

The BpMADS4-mediated rapid-cycle breeding system in apple

A particularly strong example is provided by apple. Flachowsky *et al.* (2007) demonstrated that overexpression of BpMADS4, a MADS-box gene from silver birch (Betula pendula), induced early flowering in transgenic apple. The approach subsequently became the basis of a rapid-cycle breeding system in which the early-flowering apple line T1190 was used as a breeding parent (Flachowsky *et al.* 2007).

Flachowsky *et al.* (2011) demonstrated that the BpMADS4-based system could reduce the generation interval dramatically. Transgenic progeny flowered within weeks to months rather than requiring the several years normally associated with juvenile apple plants. Using the early-flowering line, the researchers demonstrated that a breeding generation of approximately one year or less could be achieved, allowing rapid introgression of traits from wild apple material (Flachowsky *et al.* 2011).

The approach was subsequently applied to fire-blight resistance breeding. Using the BpMADS4-transgenic apple line T1190, researchers accelerated introgression of the Malus fusca-derived fire-blight resistance locus. Schlathölter et al. (2018) reported the development of advanced fire-blight-resistant apple selections representing the fifth generation within seven years, demonstrating the practical value of early flowering for accelerating perennial fruit breeding. Importantly, the final selections could be recovered as null segregants lacking the BpMADS4 transgene, meaning that the early-flowering transgene did not have to remain in the final breeding material (Schlathölter *et al.* 2018).

This example is particularly important because it demonstrates that early flowering is not merely a physiological phenomenon but can be incorporated into an actual breeding pipeline. The strategy combines early flowering, rapid crossing, molecular/phenotypic selection, segregation of the flowering transgene, thereby reducing the time required for successive generations.

Similar manipulation of flowering pathways has subsequently been investigated in other woody perennial crops, including citrus and plum, indicating that genetic acceleration of flowering has potential beyond apple. However, the strength and stability of the response are species- and genotype-dependent, so these approaches should not be considered universally transferable across fruit crops.

More recently, Mir *et al.* (2026) proposed a broader framework for speed breeding in perennial fruit crops that incorporates naturally early-bearing genotypes as intermediate parents. Their review highlights examples such as columnar apple and lateral-bearing walnut as potential sources of precocious phenotypes that could complement environmental and genetic approaches for shortening breeding cycles (Mir *et al.* 2026).

Applications in fruit crops

The application of speed breeding to fruit crops is currently less extensive than its application to annual crops, but several important examples demonstrate its potential. Apple is probably the strongest example. Conventional apple seedlings may require several years before flowering, whereas early-flowering technologies have reduced this interval to approximately ten months in experimental systems. Speed breeding combined with early flowering and marker-assisted selection has therefore been used to accelerate the introgression of traits such as disease resistance (Flachowsky *et al.* 2011; Schlathölter *et al.* 2018).

Kiwifruit (*Actinidia chinensis*) represents an important emerging target. Kiwifruit breeding is complicated by long generation time, large plant size, dioecy, different ploidy levels and chilling requirements for budbreak and flowering. Consequently, researchers have proposed integrating rapid breeding approaches with manipulation of flowering and dormancy-related processes to accelerate breeding. This is particularly relevant under climate change, where reduced winter chilling may itself disrupt flowering and reproductive synchrony (Herath *et al.* 2023).

Citrus is another major candidate because breeding cycles commonly extend over several years. Early-flowering approaches involving FT and LFY have demonstrated the possibility of reducing citrus juvenility and accelerating breeding cycles. This has particular significance for disease-resistance breeding, where rapid introgression of resistance from related germplasm is needed (van Nocker and Gardiner 2014).

The application of speed breeding is also being explored conceptually and experimentally in mango, walnut and other perennial fruit and nut crops. Mango is particularly attractive because of its long juvenile phase and large breeding populations, although the physical size of trees and the lack of robust transformation/regeneration systems remain important barriers (Mir *et al.* 2026).

Genome editing

The CRISPR/Cas9 system enables targeted modification of a plant genome through an RNA-guided nuclease mechanism. A single-guide RNA (sgRNA) directs the Cas9 nuclease to a complementary genomic sequence located adjacent to an appropriate protospacer-adjacent motif (PAM), where Cas9 generates a double-strand break (DSB). The resulting DSB is predominantly repaired through the error-prone non-homologous end joining (NHEJ) pathway, generating small insertions or deletions (indels) that can disrupt the reading frame and produce loss-of-function alleles. Alternatively, when a suitable repair template is available, homology-directed repair (HDR) can be used to introduce defined sequence changes, although HDR is generally less efficient in plants than NHEJ. Thus, by selecting appropriate genomic targets, CRISPR/Cas9 can be used to knock out genes, modify promoters or regulatory elements, and, through multiplexing, simultaneously modify several genes. This targeted mechanism is particularly valuable for fruit crops because it allows specific traits to be

modified directly in elite cultivars without necessarily requiring multiple generations of conventional crossing and backcrossing (Zhou *et al.* 2020).

Advances in CRISPR for plant genome editing

Since the first demonstrations of CRISPR/Cas genome editing in plants in 2013, the technology has progressed from simple Cas9-mediated gene knockout to increasingly sophisticated forms of genome engineering. Improvements in sgRNA design, Cas9 variants, multiplex editing, transformation systems, DNA-free ribonucleoprotein (RNP) delivery and transgene removal have increased the flexibility of the technology. In parallel, base editors enable targeted nucleotide substitutions without creating a conventional DSB, while prime editing provides a broader capacity for introducing precise sequence changes. Catalytically inactive Cas proteins (dCas9) have also been fused with transcriptional regulators or epigenetic modifiers to alter gene expression without changing the underlying DNA sequence. These developments are particularly important for fruit crops because many desirable traits are controlled by regulatory variation rather than complete loss of gene function. Nevertheless, regeneration and transformation remain major bottlenecks in woody and perennial fruit crops, meaning that technological advances must be accompanied by cultivar-specific regeneration systems (Xia *et al.* 2021; Zhou *et al.* 2020).

CRISPR for Fruit improvement

The application of Cas9 to fruit crops has progressed from proof-of-concept editing, frequently using PHYTOENE DESATURASE (PDS) genes to generate easily recognizable albino phenotypes, towards direct modification of genes controlling disease resistance, fruit quality, nutritional composition, plant architecture, flowering and reproductive biology. This progression is important because successful editing of PDS demonstrates that the editing machinery functions in a crop, whereas modification of a trait-associated gene provides stronger evidence for the practical application of genome editing in breeding. Early fruit-crop studies established CRISPR/Cas9 systems in apple, banana, citrus, grapevine, kiwifruit, pear, watermelon and other horticultural species (Erpen-Dalla Corte *et al.* 2019; Zhou *et al.* 2020).

Disease resistance

One of the strongest applications of CRISPR/Cas9 in fruit breeding is the modification of host susceptibility genes. A landmark example is citrus canker resistance. Jia *et al.* targeted the CsLOB1 susceptibility gene in Duncan grapefruit. Editing the coding region generated lines with markedly reduced canker development following *Xanthomonas citri* inoculation; several edited lines showed no canker symptoms at four days post-inoculation and substantially reduced disease development subsequently (Jia *et al.* 2017). A complementary strategy was subsequently demonstrated in Wanjincheng sweet orange, where rather than knocking out CsLOB1, CRISPR/Cas9 was used to edit the effector-binding element (EBE) in the CsLOB1 promoter. This region is recognized by the bacterial TAL effector PthA4 to induce CsLOB1 expression. Editing the EBE prevented pathogen-mediated activation of the susceptibility gene, and several edited lines displayed enhanced resistance; two lines showed no visible canker symptoms in the reported assay. This is a particularly important example for precision breeding because it demonstrates that CRISPR can modify a regulatory element rather than simply destroying a coding sequence (Peng *et al.* 2017). Apple provides another important example of susceptibility-gene editing. MdDIPM4 interacts with the *Erwinia amylovora* effector DspA/E and contributes to fire-blight susceptibility. CRISPR/Cas9-mediated knockout of MdDIPM4 in susceptible apple cultivars produced edited lines with substantially reduced susceptibility. In the reported study, an editing efficiency of approximately 75% was obtained, and several loss-of-function lines displayed significantly reduced disease susceptibility. The researchers additionally incorporated an FLP/FRT system to facilitate removal of the CRISPR/Cas9 T-DNA (Pompili *et al.* 2020). Grapevine has similarly been targeted for fungal disease resistance. Wang *et al.* used CRISPR/Cas9 to mutate VvMLO3 and VvMLO4, members of the mildew-resistance-locus-O family that can function as susceptibility factors for powdery mildew. Among the edited Thompson Seedless lines, several VvMLO3-edited plants displayed enhanced resistance to *Erysiphe necator*. The study also illustrates an important challenge of editing perennial fruit crops: different edited genotypes showed substantially different phenotypes, with some lines displaying senescence-like chlorosis and necrosis (Wan *et al.* 2020).

Fruit quality and nutritional improvements

Tomato has become the most extensively exploited fruit system for CRISPR/Cas9-mediated improvement of fruit quality. Although botanically a fruit, tomato is generally treated as a vegetable crop in production statistics, and it has an exceptionally well-developed transformation and regeneration system. CRISPR/Cas9 has been used to modify genes involved in fruit ripening, sugar accumulation, carotenoid metabolism, fruit colour, firmness, shelf life, fruit size and nutritional quality (Tiwari *et al.* 2023).

One notable study used multiplex CRISPR/Cas9 editing of five genes in the carotenoid pathway to increase lycopene accumulation in tomato. The resulting edited fruits accumulated approximately 5.1-fold more lycopene than the control, demonstrating how simultaneous modification of several pathway components can be used for metabolic engineering of fruit nutritional quality (Li *et al.* 2018). Sugar content has also been directly improved. Knockout of SlINVINH1 and SlVPE5, genes that negatively influence soluble sugar accumulation, increased glucose, fructose and total soluble solids in tomato fruits. Combining mutations in both genes produced an even stronger improvement than either single knockout, illustrating the potential of multiplex or stacked genome editing for quantitative fruit-quality traits (Wang *et al.* 2021). A related study targeting the cell-wall invertase inhibitor SlINVINH1 produced lines with increased soluble-solids content while maintaining fruit weight close to that of the original cultivar, demonstrating that genome editing can potentially improve quality without necessarily imposing a yield penalty (Kawaguchi *et al*. 2021).

Fruit colour has also been engineered using multiplex CRISPR/Cas9. By simultaneously editing PSY1, MYB12 and SGR1, researchers converted the red-fruited tomato cultivar 'Ailsa Craig' into lines exhibiting a range of fruit colours, including yellow, brown, pink, light-yellow, pink-brown and light-green phenotypes. Importantly, the strategy generated transgene-free plants with altered fruit colour within less than one year, demonstrating the potential of multiplex editing for rapidly modifying multigenic horticultural traits (Yang *et al.* 2023).
More recently, fruit firmness has been targeted through simultaneous knockout of FIS1 and PL. Single and double mutants displayed enhanced fruit firmness, with the double mutant showing the strongest improvement while retaining broadly comparable fruit-quality characteristics. This illustrates the potential of CRISPR/Cas9 to improve postharvest characteristics without conventional introgression of firmness-associated alleles (Yang *et al.* 2024).

Plant architecture, flowering and reproductive traits
CRISPR/Cas9 has also been used to modify traits indirectly affecting fruit production. Kiwifruit provides an important example because its dioecious reproductive system creates challenges for breeding and orchard management. Varkonyi-Gasic and colleagues targeted the SyGI gene, a suppressor of female development, in tetraploid *Actinidia chinensis*. CRISPR/Cas9-generated mutations produced premature stop codons in SyGI, demonstrating the feasibility of editing a sex-determining gene in a polyploid perennial fruit crop. Although phenotypic assessment was constrained by the long juvenile period of regenerated kiwifruit, the study demonstrated the potential of genome editing for manipulating reproductive biology (De Mori *et al.* 2020).

CONCLUSION
Fruit breeding is increasingly moving from conventional phenotype-driven selection towards an integrated, data-driven and precision-oriented approach. The long juvenile phase, polyploidy, high heterozygosity, narrow genetic diversity, complex reproductive biology and increasing effects of climate change make rapid development of improved fruit cultivars particularly challenging. The evolution from conventional breeding to marker-assisted selection, QTL mapping and GWAS has improved the identification and utilization of useful genetic variation, while genomic selection (GS) has further expanded this capability by enabling genome-wide prediction of breeding values for complex traits. By combining dense molecular markers with phenotypic information, GS can facilitate early selection of superior seedlings and reduce the need for extensive phenotyping of inferior individuals. Speed breeding complements this approach by shortening generation intervals through controlled environments, optimized photoperiod and temperature, early flowering, grafting and other approaches, with early-flowering technologies providing particular opportunities for perennial fruit crops. Together, genomic prediction and accelerated generation advancement can substantially increase genetic gain per unit time.
The emergence of precision genome editing, particularly CRISPR/Cas9, represents a further shift from selecting existing variation towards deliberately creating or modifying desirable alleles. Applications in citrus, apple, grapevine, tomato, cacao, kiwifruit and other fruit crops have demonstrated the potential of genome editing for disease resistance, fruit quality, nutritional enhancement, plant architecture and reproductive traits, while advances such as multiplex editing, promoter editing, base editing and prime editing are expanding the precision and scope of this technology. Looking forward, the greatest impact is likely to arise from integrating rather than independently applying these technologies: GWAS and genomic analyses can identify candidate loci, genomic selection can predict superior individuals, speed breeding can rapidly advance generations, and genome editing can precisely introduce desired changes where conventional variation is insufficient. Integration with high-throughput phenotyping, pangenomics, multi-omics, artificial intelligence and environmental data could ultimately establish a prediction-guided and precision-based breeding framework for fruit crops, accelerating the development of productive, nutritious, climate-resilient and consumer-preferred cultivars while overcoming many of the limitations inherent in conventional perennial fruit breeding.

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