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## ROOTSTOCK BREEDING AND GRAFTING INNOVATIONS IN PERENNIAL FRUIT TREES: A REVIEW

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### ABSTRACT

Rootstock breeding and grafting technology are central to the establishment and maintenance of perennial fruit tree farming. Rootstocks are key in determining tree vigour, environmental stress tolerance, disease resistance, and compatibility with various scions. The choice and utilization of rootstocks were formerly based on conventional wisdom and observational methods. But recent breeding strategies have implemented cutting-edge tools like marker-assisted selection (MAS), genomic selection, and CRISPR-mediated gene editing to increase the accuracy and efficiency of rootstock breeding. New grafting methods have elevated the success of scion-rootstock unions and facilitated propagation of fruit trees in various agro-climatic environments. Molecular tools have immensely speeded up the breeding process by facilitating early selection and minimizing reliance on extended field tests. These developments are especially pertinent in the context of climate change and mounting pressure on horticultural systems to be more resource-efficient and resilient. This review documents the history, aims, and advanced methodologies in rootstock breeding and grafting of perennial fruit crops. It highlights the necessity for merging traditional practice with biotechnology in order to meet current and future challenges in fruit production. By emphasizing genetic enhancement, stress resistance, and sustainability, new rootstock science innovations are opening the way for more productive and climate-tolerant orchard systems.

**Keywords :** Rootstock breeding, Marker-assisted selection, CRISPR-Cas, Genomic selection, Abiotic stress tolerance, Wild germplasm, Dwarfing rootstocks, Sustainable horticulture, Biotechnological tools.

### Introduction

In perennial fruit crops, grafting and rootstock selection are crucial factors that influence the productivity, disease resistance, and adaptability of the fruit trees. Grafting is a process of combining two different parts of a plant the rootstock and the scion where the rootstock supplies the root system and the scion develops the fruiting portion (Jain *et al.*, 2023).

Rootstocks play a key role in what will become the overall performance of the tree, including growth rate, fruit weight, resistance to diseases, and abiotic stress tolerance, for example, drought or soil salinity (González *et al.*, 2020). Grafting is crucial in horticulture, as it allows the propagation of high-performance cultivars, especially when sexual reproduction of some fruit trees becomes problematic

due to long juvenile phases (Lloyd & Jackson, 2018). Moreover, grafting provides the opportunity to combine positive characteristics of two various plant varieties, thus enhancing yield and resistance. Selection of rootstock is central to fruit orchards' long-term sustainability since it influences tree size, vigor, and nutrient uptake, thus impacting the quantity and quality of fruit yield (Bai *et al.*, 2019). For instance, the application of dwarfing rootstocks in apple trees has transformed orchards to enable dense planting and straightforward management, including harvesting (Robinson *et al.*, 2017). The capacity to breed rootstock genetics to suit varied climatic conditions and combat diseases such as phytophthora root rot (*Phytophthora spp.*) or root-knot nematodes (*Meloidogyne spp.*) further positions them as critical in the production of perennial fruit trees.

### Scope and Limitations

While the review will cover a wide range of perennial fruit crops, including apple, pear, citrus, and stone fruits, it will place particular emphasis on the advancements in rootstock breeding and grafting for temperate and subtropical fruit trees. It will focus on innovations within the last two decades, as they represent the most significant breakthroughs in the field. Yet, the limitations are brought about by the natural variability of tree performance from one region or climate to another that can affect the generalizability of some innovations. Moreover, the review will not thoroughly investigate the biotechnological approaches to rootstock development but highlight more on traditional and hybrid methods.

### Historical Perspective

The historical development of rootstock and grafting practices reflects a continuum of innovation, evolving from empirical practices to science-driven breeding and propagation strategies. This foundation continues to support modern horticultural systems, providing tools for sustainable intensification and adaptation to emerging global challenges.

### Evolution of rootstock use in horticulture

The application of rootstocks and grafting procedures in horticulture has a venerable past that dates back to ancient cultures. Historical accounts from China, Egypt, and the Mediterranean indicate that crude grafting methods were utilized as far back as 2000 BCE for the propagation of valuable fruit tree types (Janick, 2005). These early methods were based on observation lore handed down through generations, focusing on the compatibility of plant material in order to achieve successful join and yield.

### Traditional grafting methods

Rootstocks in the past were selected mainly based on availability and their ability to suit local conditions. With time, the role of rootstocks in regulating tree vigor, disease resistance, and environmental adaptability was realized. The Romans, especially, played a great role in disseminating grafting technology throughout Europe as they appreciated the potential to enhance fruit quality as well as guarantee consistency in orchard performance (Mudge *et al.*, 2009).

### Landmark innovations in rootstock breeding

Key developments in rootstock breeding in the late 20th and early 21st centuries involved combining genetic and molecular approaches to streamline selection procedures. Marker-assisted selection (MAS), quantitative trait loci (QTL) mapping, and more recently genome editing with CRISPR-Cas tools have added to the rootstock development arsenal (Kumar *et al.*, 2021; Osakabe *et al.*, 2016). They allow breeders to select and incorporate complex traits with greater accuracy and efficacy.

### Rootstock Breeding

Breeding for rootstocks has become a key element of sustainable fruit tree production, particularly in the context of mounting climatic variability, soil erosion, and disease attacks. While scions impart quality to fruit, rootstocks control a broad range of physiological and adaptability characteristics such as tree size, stress tolerance, and soil compatibility. The new breeding of rootstocks aims at integrating conventional breeding methods with molecular technologies to speed up the production of elite rootstocks that can address the changing challenges of international horticulture.

### Breeding Objectives

#### Vigour control

Tree vigour control is one of the main objectives in rootstock breeding. By selecting rootstocks that limit vegetative growth, breeders can produce dwarf or semi-dwarf trees, which are required in high-density plant systems and decreased orchard maintenance (Robinson *et al.*, 2017). Vigour control results in earlier fruiting, increased yield efficiency, and reduced pruning and harvesting costs. In apple, for instance, the Malling rootstock series (e.g., M.9, M.26) has revolutionized the world fruit industry by providing uniform tree size and early productivity (Lordanet *et al.*, 2019).

### **Stress tolerance (drought, salinity, cold)**

Abiotic stress resistance is another fundamental goal in rootstock breeding. Rootstocks should be capable of sustaining scion growth under adverse conditions like drought, saline soil, or high/low temperatures. Drought-resistant rootstocks like 'Krymsk' in stone fruits or trifoliolate hybrids in citrus enable fruit production in arid and semi-arid areas (González *et al.*, 2020). In citrus, salinity tolerance is imperative, especially in coastal or irrigated areas, and rootstocks like *Citrus macrophylla* and 'Swingle' citrumelo have been found to be highly adaptable (Castle *et al.*, 2011). Winter hardiness is essential for fruit cultivation in temperate areas; rootstocks from *Poncirus trifoliata* are widely used in citrus for their cold tolerance (Grosser & Gmitter, 2021).

### **Disease and pest resistance**

Resistance to insect pests and soil-borne pathogens is a major concern in rootstock breeding programs. Nematodes, fungi (e.g., *Phytophthora* spp.), and viruses (e.g., citrus tristeza virus) inflict widespread damage to fruit crops globally. In grapevines, resistant rootstocks '110R' and '3309C' are widely used to manage phylloxera (*Daktulosphaira vitifoliae*) and nematode infestations (Smith *et al.*, 2015). Apple rootstocks from the Geneva Research Station (e.g., G.11, G.41) have resistance to fire blight (*Erwinia amylovora*) and woolly apple aphid (*Eriosoma lanigerum*) (Fazio *et al.*, 2013). These disease-resistant rootstocks reduce dependence on chemical applications, resulting in more sustainable and environmentally friendly production systems.

### **Genetic Resources and Germplasm**

#### **Wild relatives and landraces**

Wild species and old landraces are important genetic reservoirs for rootstock enhancement. They tend to harbor traits lost in cultivated varieties through successive selection. *Poncirus trifoliata*, a wild citrus relative, is a keystone in citrus rootstock breeding for tristeza virus resistance, phytophthora, and cold (Grosser & Gmitter, 2021). Wild apple species like *Malus sieversii* offer pest and disease resistances and are currently being used in new breeding schemes (Volk *et al.*, 2015). These genetic resources are essential in the incorporation of new alleles for rootstock adaptability.

#### **Hybridization approaches**

Hybridization continues to be a core technique in rootstock breeding. It entails crossing compatible genotypes or species to merge desirable characteristics like vigour control and disease resistance. In stone

fruits, interspecific hybrids of peach (*Prunus persica*), almond (*Prunus dulcis*), and wild relatives have produced rootstocks with enhanced tolerance to calcareous soils and nematodes (Jiménez *et al.*, 2020). In citrus, hybrids like 'Carrizo' citrange and 'Swingle' citrumelo blend characteristics from sweet orange and trifoliolate orange, balancing vigour, compatibility, and disease resistance (Castle *et al.*, 2011). Hybridization is usually followed by clonal propagation to maintain the desirable genotype.

### **Biotechnology and molecular tools**

Biotechnology accelerates rootstock improvement by providing breeders with tools to identify and manipulate traits with precision. Techniques such as tissue culture, somatic hybridization, and genetic transformation have complemented the arsenal of tools in the hands of breeders. Somatic hybridization, especially in oranges, enables the combination of the genomes of two species to produce rootstocks with unique combinations of traits (Grosser *et al.*, 2020). Molecular markers are used for following traits and genotype determination, reducing the time of the breeding cycle and increasing the efficiency of selection. These advances allow the rapid screening of large germplasm collections for specific traits such as resistance to Verticillium wilt or high boron tolerance.

### **Modern Breeding Techniques**

#### **Marker-assisted selection**

Marker-assisted selection is a state-of-the-art breeding tool that uses DNA markers associated with traits to inform selection decisions. MAS shortens the breeding cycle by enabling early and non-destructive detection of favorable traits in seedlings. In apple, MAS was used to introduce resistance to fire blight and scab into rootstocks (Fazio *et al.*, 2013). In grapes, phylloxera and drought resistance markers increasingly enter breeding pipelines (Smith *et al.*, 2015). MAS is especially useful for rootstocks, where phenotype assessment can be time- and resource-consuming because of the long juvenile phase in perennial fruit trees.

#### **Genomic selection**

Genomic selection (GS) is a more recent approach that predicts breeding candidates' performance based on genome-wide marker data. In contrast to MAS, which is targeted at specific loci, GS evaluates the collective effect of all genes throughout the genome, and thus is very efficient for complex, quantitative traits such as vigour or drought tolerance (Crossa *et al.*, 2017). In rootstock breeding, GS can be used to predict trait values with precision prior to field testing, greatly

accelerating the selection process. This is especially promising for fruit trees, in which long life cycles and delayed expression of traits are great challenges to conventional breeding.

### **CRISPR and gene editing**

The application of gene-editing tools such as CRISPR-Cas9 is beginning to transform rootstock breeding. CRISPR allows for precise modifications to specific genes responsible for key traits, offering unprecedented control over the breeding process. In apple and citrus, gene editing has been explored to confer resistance to pathogens by targeting susceptibility genes or enhancing immune responses (Malnoyet *et al.*, 2016). Unlike traditional transgenic methods, CRISPR can create non-GMO plants by inducing mutations without introducing foreign DNA, which improves regulatory acceptance and consumer perception. As regulatory frameworks evolve, CRISPR has the potential to become a mainstream tool in rootstock development.

### **Advances in Grafting Techniques**

Grafting is a vital horticultural method that allows the propagation of better fruit tree cultivars by uniting a wanted scion to a compatible rootstock. Grafting offers the potential for incorporating desirable qualities like disease resistance, stress tolerance, and better fruit quality. Since then, grafting procedures have developed considerably over time and have progressed from crude manual techniques to highly advanced, precision-based techniques with support from improvements in physiological, molecular, and robotic systems. Knowledge of these breakthroughs is critical to improve propagation efficiency, compatibility, and orchard productivity over the long term.

### **Traditional Grafting Methods**

Conventional grafting methods are the backbone of vegetative plant propagation in horticulture. Conventional methods such as whip and tongue grafting, cleft grafting, and bark grafting are each used depending on plant species, size, and season.

### **Whip and tongue, cleft, bark grafting**

Whip and tongue grafting is preferred for young, active growth plants, often in nurseries. It offers maximum cambial contact area between scion and rootstock to encourage strong unions. Cleft grafting for thicker rootstocks or older plants consists of cutting a V-shaped cleft in the rootstock and placing one or more pieces of scion. Bark grafting is done when there is active growth and the bark can be removed from the wood. The scion is placed under a bark flap, which facilitates cambial contact (Hartmann *et al.*, 2010).

Although these techniques are quite simple, their success relies largely upon the practitioner's skill, the environment, and the physiological status of the plant material. They can be influenced with varying success by factors such as variations in temperature, availability of moisture, and the presence of pathogen at the grafting junction (Singh *et al.*, 2024).

### **Modern Innovations**

The use of biotechnology and mechanization in horticulture has led to the development of new grafting technologies. These are micrografting, in vitro grafting, and automated and robotic grafting systems, which focus on enhancing precision, success, and scalability.

### **Micrografting**

Micrografting includes grafting very small scions, like shoot tips or meristems, onto seedling rootstocks under sterile conditions. It is especially important for virus eradication and rejuvenation of elite but aging clones. Practiced extensively in citrus and apple breeding programs, it has been shown to be effective in the production of pathogen-free planting material (Navarro *et al.*, 1975).

### **In vitro grafting**

In vitro grafting provides even more regulated conditions, enabling scientists to investigate graft union initiation, reconnection of vascular tissues, and pathogen-host interactions. It is also a great platform for high-speed screening of compatibility between various combinations of rootstocks and scions (Melnyk and Meyerowitz, 2015). This technique supports molecular research that targets the initial physiological and biochemical processes after grafting.

### **Robotic and automated grafting systems**

Robotized and automated grafting machines have transformed nursery operations by standardizing cutting techniques, pressure, and angle of graft, hence lowering labor and human error expenses. Japan and South Korea have led the way in developing such technologies for fruit trees as well as vegetable crops. These machines are capable of doing hundreds of grafts an hour, thus facilitating bulk propagation on a commercial nursery scale (Kubota *et al.*, 2008).

### **Physiological and Molecular Aspects of Grafting**

Graft union formation is guided by several complicated physiological and molecular interactions. These involve tissue recognition, callus formation, vascular differentiation, and signal exchange between the rootstock and scion.

### Graft compatibility and incompatibility

Graft compatibility and incompatibility are caused by anatomical, biochemical, and genetic interactions. Successful vascular integration is achieved from compatible grafts, whereas incompatibility can cause inadequate growth or failure of union. Signs of incompatibility are necrotic layers, inadequate callus formation, and altered vascular tissues (Goldschmidt, 2014).

### Hormonal regulation and signal transduction

Hormonal regulation plays a vital role in the coordination of healing and union processes. Auxins induce cell elongation and vascular differentiation, while cytokinins stimulate cell division and tissue proliferation. Gibberellins and ethylene are inhibitors of developmental responses required for effective grafting. Imbalance in hormones can lead to incompatibility and delayed formation of a union.

### Role of RNA and proteins in graft union formation

Signal transduction pathways, including macromolecule transport of RNAs and proteins, facilitate long-distance communication between scion and rootstock. Mobile RNAs like siRNAs and mRNAs have the ability to regulate gene expression in tissues that are far away, controlling traits such as stress, growth regulation, and flowering (Kehr and Buhtz, 2008).

### Role of Rootstocks in Fruit Tree Performance

Rootstocks play a pivotal role in determining the overall performance, health, and productivity of perennial fruit trees. Their influence extends across several key physiological and morphological traits, including tree architecture, vigour, fruit yield, fruit quality, and resistance to various biotic and abiotic stresses. Selecting appropriate rootstocks tailored to specific environmental conditions and scion cultivars is a strategic approach to optimizing orchard sustainability and profitability.

### Impact on tree architecture and vigour

One of the primary functions of a rootstock is to regulate the vigour of the scion. Vigour control allows growers to manage tree size, improve orchard efficiency, and optimize light interception. Dwarfing and semi-dwarfing rootstocks are especially valued in high-density orchard systems because they facilitate easier canopy management, reduce pruning requirements, and enable mechanical harvesting (Webster, 2004). The ability of rootstocks to influence tree architecture is largely due to their effects on hormone production and nutrient uptake. Dwarfing

rootstocks generally have reduced auxin transport capacity and lower cytokinin levels, which limit excessive shoot elongation and promote compact growth habits (Tworkoski and Fazio, 2011)

### Impact on fruit yield and quality

Rootstocks also significantly affect the fruit yield and quality of grafted trees. Yield potential is influenced by rootstock-induced vigour, precocity, nutrient uptake efficiency, and water use efficiency. High-performing rootstocks not only improve yield consistency but also enhance fruit size, sugar content, and color development. In grapevine (*Vitis vinifera*), rootstocks such as 1103P and 101-14 Mgt have been reported to improve drought tolerance with high-quality fruit under water-limited conditions (Ollat *et al.*, 2016). In citrus, rootstocks such as Carrizo citrange are used widely due to the fact that they have been reported to improve total soluble solids, fruit weight, and juice content (Al-Jaleel *et al.*, 2005). Fruit quality traits such as texture, firmness, and shelf life are indirectly controlled by the rootstock via their influence on nutrient assimilation, particularly calcium and potassium. Rootstocks may also affect the level of secondary metabolites, flavour and aroma composition, important in wine and juice production (Keller, 2020).

### Influence on abiotic and biotic stress tolerance

One of the most important functions of rootstocks is to impart tolerance to environmental stresses and pest/pathogen pressures. Abiotic stresses like drought, salinity, and cold can have adverse effects on perennial fruit trees. Rootstocks impart a genetic buffer that enables scions to function better under stressful conditions. Drought-tolerant rootstocks are especially vital in arid and semi-arid regions. For instance, in apple, Geneva rootstocks such as G.41 and G.935 exhibit superior drought tolerance compared to older types due to their robust root systems and water use efficiency (Fazio *et al.*, 2013). In mango (*Mangifera indica*), rootstocks like '13-1' have demonstrated improved drought and salinity tolerance (Singh *et al.*, 2005). Biotic stress resistance is equally critical. Rootstocks can provide protection against soil-borne pathogens, nematodes, and insect pests. In grapes, rootstocks like Freedom and Harmony confer resistance to root-knot nematodes and phylloxera (Granett *et al.*, 2001). Similarly, in stone fruits, Myrobalan rootstocks are commonly used for their resistance to root rot and crown gall diseases (Layne and Bassi, 2008).

### Species-Specific Case Studies

Rootstock choice and grafting technology have varied consequences in perennial fruit species. Compatibility of rootstock, tolerance of stress, and yield performance have been extensively examined in various species, and yield useful information to breeding and orchard management.

#### Apple (*Malus domestica*)

Rootstocks in apples have been responsible for transforming commercial orchards into high-density plantations, with an amazing rise in productivity and profitability. Most widely used rootstocks are the Malling series (e.g., M.9, M.26) and the Geneva series (e.g., G.41, G.935). M.9 dwarfing rootstock has made high-density planting of orchards and early fruiting possible (Webster, 2004). Geneva rootstocks have been developed for their improved resistance to fire blight (*Erwinia amylovora*), tolerance to replant disease, and compatibility with modern training systems (Fazio *et al.*, 2013). Geneva rootstocks also show improved water and nutrient uptake, resulting in improved fruit quality. Research has also proven that the rootstocks reduce labor inputs significantly while either sustaining or improving fruit yields (Robinson *et al.*, 2011).

#### Citrus

Studies on citrus rootstocks have targeted disease resistance, salt tolerance, and fruit quality enhancement. Trifoliate orange (*Poncirus trifoliata*) and rootstock hybrids like Carrizo citrange and Swingle citrumelo have been used extensively due to their compatibility with the majority of scions and resistance to *Phytophthora* spp., citrus tristeza virus (CTV), and other root pathogens (Castle, 2010). Rootstocks such as Volkamer lemon are selected because of their yield potential, but at the expense of compromised fruit quality. Cleopatra mandarin, however, has acceptable fruit quality but is fairly slow-growing and calcareous soil-sensitive (Al-Jaleel *et al.*, 2005). Rootstock-scion interactions in citrus have a major impact on tree size, productivity, and juice quality.

#### Grapevine (*Vitis vinifera*)

The use of rootstocks in viticulture is primarily to combat phylloxera (*Daktulosphaira vitifoliae*) and abiotic stress management. American rootstocks such as *Vitis riparia*, *Vitis rupestris*, and *Vitis berlandieri* and their hybrids are commonly utilized. For instance, 1103 Paulsen is preferred in dry conditions since it possesses deep roots and is water-frugal, while 101-14 Mgt performs optimally in fertile, moist soils since it possesses moderate vigour and date of ripening (Ollat

*et al.*, 2016). Rootstocks have an effect on wine quality by regulating vine vigour and berry composition, phenolics and aroma volatiles (Keller, 2020). Viticultural regimes in conjunction with appropriate rootstocks have a considerable effect on the metabolic grape composition.

#### Stone fruits (peach, cherry, plum)

Stone fruit tree crops have traditionally relied on seedling and clonal rootstocks for propagation. For peaches (*Prunus persica*), Lovell and Guardian rootstocks are commonly used because they are tolerant to peach tree short life (PTSL) and root-kNOT nematodes (Rieger, 2006). For cherries (*Prunus savium*), Gisela rootstocks are valued for dwarfing properties and enhanced precocity, enabling high-density plantings and machine harvesting (Lang, 2005). Plum rootstocks such as Myrobalan and Mariana hybrids give disease resistance and tolerance to a wide range of soil conditions. Intergeneric hybrids such as Citation (peach x plum) are also employed in order to control vigour and disease pressures (Layne and Bassi, 2008).

#### Tropical fruits (mango, guava, sapota)

The tropical fruits, though less studied, also have promising rootstock-scion relationships. In mango (*Mangifera indica*), rootstocks '13-1', 'Olour', and 'Bappakai' showed differential influence on tree vigour, salinity tolerance, and yield of fruit (Singh *et al.*, 2005). Rootstocks 'Kurukkan' and 'Vellaikolumban' cause drought resistance and improve retention of the fruit retention. Guava (*Psidium guajava*) fruit production is improved with rootstocks like *P. friedrichsthalianum* and *P. cattleianum* that impart wilt resistance and improve the quality of the fruit. Micropropagation and in vitro grafting techniques are increasingly used to speed up rootstock production and screen for compatibility (Rai *et al.*, 2010). Rootstock breeding in sapota (*Manilkara zapota*) has been limited, although preliminary studies indicate that some species like *Manilkara hexandra* can offer disease resistance along with tolerance to saline soil (Siddiqui *et al.*, 2011).

### Challenges and Limitations

Perennial fruit crop improvement is still constrained by a number of issues, even with notable advancements in grafting and rootstock breeding techniques.

### Graft incompatibility

Graft incompatibility is among the most urgent issues. Early tree decline, delayed incompatibility symptoms, or poor graft union formation are all signs of rootstock and scion incompatibility (Goldschmidt, 2014). This physiological problem is caused by vascular discontinuity, mismatched biochemical pathways, or different growth rates. Graft incompatibility is still a significant obstacle to using otherwise promising rootstocks in stone fruits like cherries and apricots (Errea *et al.*, 2001). Interspecific or intergeneric grafting, which is frequently sought to transfer desirable traits like disease resistance, may also be limited by incompatibility. Comprehensive screening systems are still required, despite the fact that molecular diagnostics and *in vitro* grafting techniques are helping with early incompatibility detection (Pina and Errea, 2005).

### Breeding complexity in perennials

Long juvenile phases, heterozygosity, and polygenic trait inheritance make breeding perennial fruit trees more difficult. Perennials have longer generation times than annual crops, which slows down the breeding cycle and reduces the number of selections a researcher can make in their career (Brown and Maloney, 2009). Furthermore, several genes control many desirable traits, including disease resistance, abiotic stress tolerance, and dwarfing caused by the rootstock. This makes phenotypic evaluations time- and resource-intensive and complicates conventional breeding efforts. In many species, trait integration is still difficult and unpredictable, even with the development of genomic tools and marker-assisted selection (Gessler and Pertot, 2012, Singh *et al.*, 2023).

### Limited genomic resources for some species

The accessibility of genomic resources significantly differs among various fruit species. Although species like apples, grapes, and citrus have profited from extensive genome sequencing initiatives, other fruits such as sapota, guava, and numerous tropical varieties are devoid of detailed molecular maps and databases (Varshney *et al.*, 2014). This inconsistency hinders molecular breeding, gene identification, and trait mapping, especially concerning rootstock-specific characteristics. The ongoing development of high-throughput phenotyping technologies and transcriptomic tools is underway, yet their implementation in less-studied species continues to be limited (Torkamaneh *et al.*, 2018). Additionally, wild relatives and landraces which often represent

significant resources for disease and stress resistance remain underexploited due to insufficient characterization and lack of accessible germplasm collections (Warschefsky *et al.*, 2014).

### Future Prospects

The outlook for innovations in rootstock breeding and grafting for perennial fruit trees is bright, fueled by advancements in biotechnology, climate-resilient farming practices, and precise horticultural techniques. Rootstocks will be crucial in improving the adaptability, sustainability, and yield of fruit crops as they confront global issues like climate change, soil depletion, and escalating biological pressures.

### Climate-resilient rootstocks

Developing rootstocks that can endure extreme weather conditions is increasingly becoming a key focus of research. The anticipated effects of climate change, such as more frequent and intense droughts, heatwaves, and floods, present a significant danger to the survival and productivity of fruit trees (Gupta *et al.*, 2020). It is essential for climate-resilient rootstocks to sustain tree vigor and facilitate optimal fruit yields under these challenging circumstances. Scientists are prioritizing the identification and utilization of germplasm from arid and semi-arid regions, particularly wild relatives and local varieties known for their resilience to drought, salinity, and heat (Warschefsky *et al.*, 2016). In citrus, rootstocks such as 'Carrizo' and 'Swingle' have demonstrated notable drought resistance, but further improvements through crossbreeding and molecular approaches are necessary to tackle more extreme environmental challenges (Castle *et al.*, 2011).

### Integration of omics tools

Contemporary rootstock breeding increasingly utilizes multi-omics strategies, encompassing genomics, transcriptomics, proteomics, and metabolomics, to decipher the intricate traits related to graft compatibility, resistance to diseases, and tolerance to abiotic stresses (Kumar *et al.*, 2021). Genomic selection (GS) and genome-wide association studies (GWAS) enable breeders to forecast phenotypes based on genotypes, thus expediting the identification of superior rootstock lines. Transcriptomic studies provide insights into gene expression patterns during the formation of graft unions and responses to stress, highlighting crucial genes associated with compatibility and signaling processes (Melnyk & Meyerowitz, 2015). Metabolomic research further assists in pinpointing the biochemical pathways modulated by rootstocks,



particularly those associated with nutrient absorption and hormone production, which affect scion development and fruit quality. (Singh *et al.*, 2024).

CRISPR-Cas9 technology has created new possibilities for accurate genome modification in fruit crops. While there are still regulatory challenges in certain areas, efforts are being made to refine rootstock genomes for traits such as root structure, disease resistance, and water-use efficiency (Dalla Costa *et al.*, 2019). As methodologies become tailored to specific species and transformation success rates increase, the use of these technologies is expected to become commonplace in rootstock development initiatives.

### Sustainable and precision horticulture approaches

The efficient performance of rootstocks under various agro-ecological situations and the prudent use of inputs are intimately related to sustainability in fruit production. Orchards can drastically lessen their environmental impact by using rootstocks that require less irrigation, fertilizer, and pesticide use (Massai *et al.*, 2015). Additionally, efforts are being made to create rootstocks that support organic or low-input farming methods and improve nutrient-use efficiency. In order to track rootstock performance and soil conditions, precision horticulture incorporates technology such as machine learning, GPS mapping, and remote sensing. When combined with real-time data, smart rootstocks enable producers to make well-informed decisions on the management of their orchards (Singh *et al.*, 2025). Additionally, automated nursery operations and robotic grafting systems provide consistency and efficiency in the propagation and deployment of rootstock. . Additionally, the idea of designer rootstocks which are made to fit certain scion requirements and site circumstances is becoming more and more popular. These rootstocks can be chosen for their mechanical system compatibility as well as their agronomic advantages, which will help modernize harvest operations and orchard designs.

### Conclusion

Modern horticulture has undergone tremendous change as a result of the development of rootstock breeding and grafting techniques in perennial fruit trees. Because they affect tree vigor, stress tolerance, disease resistance, and fruit quality, rootstocks provide the basis of resilient, productive, and sustainable orchard systems. The historical evolution of rootstock use, breeding developments, grafting techniques, and species-specific applications across a variety of fruit crops, such as apple, citrus, grapevine, stone fruits, tropical fruits, and emergent crops like pomegranate, have all been covered in detail in this review.

One important takeaway from this review is how important breeding goals are for rootstock development, including vigor control, disease and insect resistance, and abiotic stress tolerance. Modern techniques like genomic selection, marker-assisted selection, and gene editing tools like CRISPR have been added to traditional procedures. These developments make it possible to create specialized rootstocks for a range of settings and production objectives. Likewise, advancements in grafting methods, including as automated systems, micrografting, and whip and cleft grafting, have simplified the development and growth of orchards.

The ramifications for the fruit sector are significant. Rootstocks are essential for enabling high-density planting techniques, lowering input needs, and adjusting fruit crops to climatic change. Their impact goes beyond agronomic characteristics to post-harvest quality and marketability, emphasizing their strategic and financial worth. New avenues to optimize rootstock performance and improve sustainability are made possible by the combination of precision horticulture technologies and omics techniques.

However, there are significant research gaps. Long-term productivity is limited by graft incompatibility, particularly in novel scion-rootstock pairings. Breeding efficiency is hindered by perennials' complicated genetic makeup and underused species' sparse genomic resources. Future studies should concentrate on identifying the molecular underpinnings of graft compatibility, growing libraries of rootstock germplasm, and utilizing digital agriculture technologies to track real-time rootstock-scion interactions.

Innovations in rootstock breeding and grafting constitute a vibrant new frontier in the production of perennial fruit trees. Researchers, breeders, and industry stakeholders must work together to fully utilize rootstocks in the development of high-performing, climate-resilient, and sustainable orchard systems in the future.

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