

Review

Macadamia Breeding for Reduced Plant Vigor: Progress and Prospects for Profitable and Sustainable Orchard Systems

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Abstract: Vigor control in tree crops plays an important role in increasing orchard efficiency and sustainability. It has enabled high-density plantations to maximize yield efficiency while reducing production costs. Although traditional methods such as frequent hedging and pruning are still used, dwarfing rootstocks and low-vigor cultivars are the most effective and sustainable means of vigor control, as these methods reduce labor and management costs while maintaining yield efficiency. Considerable variation among cultivars and rootstocks for vigor has been identified; however, mechanisms by which rootstocks affect scion vigor in slow-maturing tree crops remain unclear. With the lack of adequate information required for early and rapid selection, breeding programs in tree crops such as macadamia still utilize manual phenotyping, which is laborious, time-consuming, and expensive. Providing insights on emerging technologies that enhance breeding programs via rapid selection, this review summarizes the current state of vigor management and underlying mechanisms of vigor control in tree crops. It provides further understanding of the prospects of applying those techniques in rootstock and scion breeding for low-vigor and yield-efficient cultivars in tree crops, with specific reference to macadamia.

Keywords: macadamia; breeding; selection; vigor; rootstock; scion; high-density orchards; genomics-assisted breeding



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1. Introduction

The genus *Macadamia* comprises four species: *Macadamia integrifolia*, *M. tetraphylla*, *M. janseni*, and *M. ternifolia* [1]. All four species are indigenous to the mid-eastern coastline of Australia, and commercial cultivation of this high-value crop has spread around the world, including China, Kenya, South Africa, the USA, Guatemala, Malawi, and Brazil. With 50,300 tonnes (MT) of nut-in-shell production reported in 2020, each year, an increase in planting by around 5000 hectares is being recorded in Australia. Both *M. tetraphylla*, *M. integrifolia* and their hybrids are cultivated for edible nuts, while the kernels of *M. ternifolia* and *M. janseni* contain cyanogenic glycosides, making them inedible [2]. *M. ternifolia* and *M. janseni* are wild species that are comparatively smaller in size, while commercially cultivated trees can grow up to 15 m high and 10 m wide upon maturity [3].

High plant vigor at maturity is one of the key obstacles to profitable and efficient orchard systems in macadamia. Yield in tree crops largely depends on the maximization of photosynthesis via efficient light capture and effective partitioning of the resulting photosynthates between vegetative and reproductive growth [4]; excessive tree vigor therefore competes with fruit production by limiting resources for reproductive growth. In addition, vigorous growth leads to canopy shading and reduces production by interfering with light interception [5]. Another problem associated with high vigor is a shaded orchard

floor, which may lead to increased soil erosion along with loss of crop and nutrients [4]. Crowding due to excessive growth also restricts machinery access for harvesting and reduces the efficiency of pesticide application [4,5]. To prevent orchard shading and crowding at maturity, growers are compelled to restrict their orchards to low-density planting. Trees with a larger stature also require the use of expensive machinery and labor, increasing the cost of production [6]. Controlling excessive vigor has thus become highly important to maintain the economic viability of tree crop production.

Vigor control has been utilized in several crops to maximize yield efficiency and introduce high-density orchards. Following vigor control, planting density in apple orchards increased from 70–100 trees/ha in the early 1900's to up to 10,000 trees/ha in the 2000's, substantially boosting yield efficiency [7]. In the early 1980s, macadamia growers, increased the planting density from around 210 trees/ha to 357 trees/ha to increase economic returns; however, upon maturity, the orchards were heavily crowded following reduced yield [8]. This suggests the need for effective vigor control in macadamia to manage tree size without affecting the yield efficiency.

In traditional systems, tree size has been managed by training, pruning, and hedging. In crops like apples and pears, limb bending to control branch angle has been used as an effective way to control growth [9]. Similarly, Plant Growth Regulators (PGRs) are effectively used to control shoot growth in apples [10] and to induce a dwarfing effect in avocados [11]. In macadamia, the most common form of vigor management is through mechanical hedging or pruning [4]. However, severe disadvantages like intensive labor use, yield reduction, and wastage of assimilates are associated with the traditional methods of tree size control [12]. A reduction in yield following severe hedging was reported by Warner and Gitlin [13]. Another study by McFayden et al. [4] reported a 21% yield loss due to hedging in macadamia, highlighting the requirement for a different form of vigor control without affecting productivity.

Tree vigor in many fruit crops is currently controlled using dwarfing rootstocks, which restrict tree size and increase precocity (defined as a shorter time to first cropping) without impacting yield [14]. The vigor of crops such as apple, pear, plum, sweet cherry, peach, apricot, and nectarine is effectively controlled via unique rootstocks [6,15]. These rootstocks have transformed the European and American industries through vigor control to reduce labor costs and increase precocity [16]. Similarly, low-vigor scion cultivars are another choice for effective vigor control. Low-vigor cultivars with high precocity would greatly assist in high-density planting systems.

Currently, there are no vigor- and size-managing rootstocks and low-vigor scion cultivars commercially available in macadamia. Although extensive studies of vigor management have been conducted in several crop species, relatively few investigations have been made in macadamia, restricting its development. This review discusses the current state of vigor management and its underlying mechanisms in tree crops and explores the prospect of using emerging techniques in macadamia breeding for low-vigor, yield-efficient cultivars.

2. Manipulation of Tree Vigor for Orchard Profitability and Sustainability

Tree vigor strongly affects the efficiency of commercial production and is of major importance in orchard management, design, intensification, and productivity enhancement [17]. Vigor traits, including tree height, width, and volume, are known to influence light interception, yield, fruit quality, and production efficiency [5]. Light distribution is one of the major factors that affects crop productivity, while vigor parameters such as tree height, branching angle, and tree canopy determine the amount of light penetration. In macadamia, for instance, yield is known to increase up to 84–95% with increased light interception [8]. Vegetative and reproductive growth share the available photosynthates via resource partitioning; the share utilized by vegetative growth or vigor is important as it determines the resources available for reproductive growth [18]. Vigor also has a major impact on orchard management as it affects the decision of agronomic practices

such as training and pruning, choice of rootstock and scion, fertilization and irrigation. Management practices undertaken to optimize vigor, such as pruning, hedging, and branch bending, all influence the cost of production in orchards [4]. Tree vigor is thus an important parameter that determines crop productivity and orchard profitability.

While excessive vigor is detrimental to productivity and profitability, economic studies of fruit crop production have revealed clear advantages of early vigor (EV) in tree crops. Early vegetative growth or vigor at the juvenile stage is an important factor for orchard establishment and uniformity. Strong growth of young orchards is important; it determines the profitability of young orchards as productivity is largely a function of tree size [19]. Early vigor also has significant positive implications for plant adaptation to climate change, such as in response to drought stress [20]. Early root growth and establishment are dependent on the transport of photosynthates from the shoot, and a more expansive root system boosts crop resilience against stresses [21]. High early shoot and root growth are found to be associated with drought and heat-stress tolerance mechanisms in crops like cotton, in *Arabidopsis*, and some forest trees [20,22,23]. Furthermore, the stem diameter of young crops is known to be related to several plant attributes related to water absorption and transportation which also play a role in plant survival during water-stress [24].

Further highlighting the significance of EV in tree crops, a positive relationship between EV and precocity has also been established for many years [25]. A study evaluating 4- and 5-year-old macadamia trees observed a strong positive influence of early canopy volume on early yield, suggesting increased light interception as one of the contributing factors [26]. As early growth increases the amount of new shoot biomass, the crop thus occupies a larger area and captures more radiation [27], which is beneficial for early crop yield. However, vigor at maturity is an undesirable agronomic trait, and the existence of this relationship between precocity and vigor at maturity may have negative genetic implications. Thus, the goal should be to obtain trees with high initial vigor but low vigor at maturity. Establishing a correlation between EV and vigor at maturity may be beneficial. For instance, a lack of correlation between early seedling vigor and mature vegetative vigor in olives allowed the selection of high EV and precocious cultivars with low vigor at an adult stage, which could be adapted to high-density orchards [28]. This can also be achieved via investigation of the vigor trend of each genotype. Investigating similar relationships of early and mature age vigor along with precocity in macadamia is therefore important for the selection of cultivars for maximum profitability.

3. Genetic and Environmental Control of Plant Vigor

Vigor traits such as tree height, canopy size, trunk circumference, and branching patterns vary substantially in tree species. This variation persists either due to genetic or environmental control. Many crop species consist of multiple varieties/cultivars, which naturally tend to grow more vigorously or are naturally dwarfing. For example, the early cashew (*Anacardium occidentale*) is known to be naturally smaller in size than common cashew; the 'Amrapali' variety of mango is known to reduce the size of the mango tree [29]. In macadamia, *M. ternifolia* and *M. jansonii* are wild species that are smaller in size compared to the cultivated species [3]. This clearly suggests genetic control of vigor in these species. Similarly, high heritability estimates obtained for vigor traits in crops such as almond (0.7–0.8) and olive (0.47–0.58) [30,31] also show strong genetic control of these traits.

The utilization of rootstocks in tree species to control plant vigor also implicates genetic control of scion vigor. In several tree crops, rootstocks are classified into low, medium, and high vigor based on their effect on scion growth. Genetic mapping and quantitative trait loci (QTL) analysis have been used to detect several genomic regions associated with vigor traits. Two prime QTLs (*Dw1* and *Dw2*) were identified in a dwarfing apple rootstock 'M9' [32]. A study of genetic control of rootstock-induced dwarfing in pear identified an allele associated with apple *Dw1* [33], suggesting that vigor in tree crops is under genetic control.

When traits display diversity in distinct environmental backgrounds, even within a genotype, a clear environmental control of that trait can be observed. Environmental factors such as water and nutrient availability, light interception, soil properties, and temperature determine the extent of growth and vigor in crops; therefore, the same genotype may exhibit altered growth under different environmental settings. This has also been shown by the low heritability estimates of vigor traits in some studies. In mango, for instance, the heritability of trunk cross-sectional area (TCA), which is primarily used as an indicator of vigor, was found to be low (0.23) [34]. Similarly, low heritability estimates were identified in *Populus* for tree height and diameter (~ 0.2) [35].

Studies in macadamia have revealed low to high heritability estimates of growth traits. Toft et al. [36] identified a low heritability in young cultivars for canopy volume (0.07), while other traits, such as tree height (0.43) and TCA (0.32), showed moderate heritability. In another study by Hardner et al. [37], broad-sense heritability estimates of tree girth and canopy width were 0.21 and 0.28, respectively, considered to be low. Similarly, Mai et al. [38], in another study on macadamia, identified moderate to high heritability estimates (0.51–0.60) for mature age vigor traits such as height, canopy width, canopy length, and trunk circumference. These results suggest that both genetic and environmental factors are equally important for trait variability, while a slightly higher genetic control dictates a better opportunity for improvement via breeding.

4. Genotype X Environment Interaction on Plant Vigor

The performance of related genotypes can vary according to the testing environments, i.e., a genotype superior in one environment may not always be superior elsewhere; this phenomenon is defined as the genotype by environment interaction (GxE) [39]. The extent to which a trait is affected by GxE is an important factor that governs the degree of testing over years and the selection of locations where tests should be conducted to measure crop performance [40]. GxE has been widely studied in tree breeding as a means of producing superior cultivars with desirable traits.

Vigor traits are complex, highly plastic, and greatly affected by environment factors such as temperature, water availability, and soil characteristics; therefore, the observed phenotype is not only a genetic or environmental effect but a combination of both. A significant effect of GxE on growth traits was observed in forest trees [41]. These interactions are known to complicate the trial and selection in breeding programs and, therefore, reduce the overall genetic gain. While understanding existing patterns of GxE explainable by any repeatable factor is advantageous as elite genotypes may be selected for the specific environment, if no repeatable factor is identified that predicts patterns, GxE is treated as a complexity factor. Assessing these interactions requires devising special designs, such as multi-location designs, which are costly and time-consuming, especially in perennial species [42].

GxE effects have been incorporated into quantitative genetic models via genetic correlations within and between individual genotypes [43]. The use of predictive models based on linear and non-linear responses has also been suggested by several authors [44]. Linear mixed models, which include genetic effects as random factors, are the current method to evaluate crop genetics, involving the prediction of the best linear unbiased (BLUP) genetic effect [45]. Recent approaches to investigate GxE involve statistical analysis based on these linear mixed models, such as factor analytic (FA) models, which determine the genetic covariance structure of a multi-environmental trial [46].

Several studies have investigated GxE in forest trees such as poplar and pine [41,47] and some fruit trees including apple and olive [42,48]. Most of the studies included traits related to yield and quality of fruit production, with growth and vigor traits less represented. Sadok et al. [48] studied GxE for the early growth traits of olive; however, no GxE was found despite a strong environmental effect on most of the traits. Relatively few studies have investigated the GxE interaction in nut crops. In macadamia, a small number of

studies identified evidence of the effect of GxE on yield, [37,49,50]; however, none have examined GxE effects on economically important plant vigor traits.

5. Progress of Macadamia Breeding for Reduced Vigor

5.1. Scion Breeding

The 150-year domestication and breeding history of macadamia is relatively short in comparison to many other tree crops, and modern cultivars are only two to five generations out of the wild [51]. Most of the commercial cultivars currently being used are based on *M. integrifolia* and *M. tetraphylla*, which are indigenous to Queensland and northern New South Wales, respectively [52]. In the 20th century, the initial development of macadamia via selection and agronomic research occurred in Hawaii at the Hawaii Agriculture Experiment Station (HAES); research expanded into Australia with the first commercial orchards established in the 1920s, followed by the selection of new cultivars in the mid-1930s [1,53]. Over 80% of existing cultivars are derived from HAES selection program [52], and the three most important macadamia cultivars are ‘HAES344’, ‘HAES741’, and ‘HAES246’, which were released in 1971, 1977, and 1948, respectively [54].

Over the years, a number of Australian macadamia breeding programs have developed. A private breeding program, Hidden Valley Plantation (HVP), was established at Beerwah, Queensland, in 1972, which released the A series of macadamia cultivars [55]. Selection objectives of this program include early bearing, high kernel yield, high kernel recovery, and kernel quality. Although the program has not yet focused on reduced vigor, a small precocious tree with high yield efficiency—‘A16’—and a medium-low vigor tree—‘A4’—were released in 1981 and 1987, respectively. Recently, ‘A447’ and ‘A538’ were released commercially as small and highly precocious cultivars [56].

In 1996, the industry-supported National Macadamia Breeding Program was established with a plantation of 2000 seedlings in 1997–1998 at Bundaberg, Queensland, and Alstonville, New South Wales [1]. The major selection objectives of this program were yield-related traits, precocity, and tree size. This program has produced two generations of seedling progenies to date [54]. The first-generation breeding crosses (B 1.1. and B 1.2.) were planted from 1997–2003 from 14 and 47 parents, respectively; 20 and 23 elite progenies were selected from B 1.1. and B 1.2. and planted in regional variety trials for second-stage evaluation. Similarly, second-generation progenies (B 2.1, B 2.2, and B 2.3) were planted from 2011 to 2018 from 34, 39, and 17 parents, respectively; 10 elite progenies have been selected from B 2.1 so far [57]. From the elite selections of B 1.1 populations, four cultivars were released in 2017. One of these cultivars, “MIV1-P”, is a small to medium precocious tree that is potentially suitable for high-density planting [58].

Scion breeding in most macadamia breeding programs is more focused on selection for yield and quality traits. In addition to cultivars ‘A16’, ‘MIV1-P’, ‘A446’, and ‘A538’, ‘NG8’ (an early Australian selection) and ‘HAES814’ (a HAES selection) are two older cultivars considered as small trees and commercially grown in Australia [3]. However, highly efficient low-vigor cultivars particularly suited for high-density orchards are yet to be developed.

5.2. Breeding Rootstocks to Improve Orchard Productivity and Efficiency

Rootstocks have been used to graft fruit trees for more than two millennia, mainly for clonal propagation. Using rootstocks for the multiplication of varieties for commercial plantation is a common practice in many fruit crops such as apples, pears, peaches, grapes and apricots. In tree crops like macadamia, rootstocks play a critical role in crop performance. Nutrient transportation between the rootstock-scion graft union affects many important traits, including tree size, longevity, fruit quality and yield [59]. As part of a dual plant system, rootstocks regulate water and nutrient transportation to the scion and influence plant growth and development. Rootstocks with strong tap root systems offer plants resilience to a variety of biotic and abiotic stimuli, as well as increased flexibility to harsh environmental conditions [60].

Rootstocks' potential to control tree vigor has been described as a significant benefit for fruit-crop growers as it reduces management costs, facilitates high-density plantations, increases yield per unit area, and increases orchard efficiency and productivity [60]. In comparison to a vigorous rootstock, a dwarfing rootstock produces less vegetative growth and directs a higher proportion of dry weight into reproductive growth. It offers a flexible and often less costly method of tree vigor control [61]. Thus, dwarfing rootstocks are employed widely in high-density orchards to restrict tree volume and promote precocity. Rootstock breeding for orchard efficiency has successfully been conducted in many crops, including citrus [62], apple [63], almond [64], and mango [65].

A rootstock breeding program for macadamia is not yet prioritized due to the current major emphasis on yield and quality. The second-generation National Breeding Program took the initiative to identify elite rootstocks for scion yield and vigor control as a part of the "transforming subtropical tree crop productivity" program; 30 genotypes propagated as seedlings and cuttings were planted in 2017 and phenotyped for growth and flowering characteristics and final selections are to be made in 2024 [57]. Another program in South Africa, the Agricultural Research Council–Tropical and Subtropical Crops campus, has established an orchard with 18 cultivars to evaluate yield quality with a long-term goal of rootstock breeding for small trees [3]. Major efforts to develop novel rootstocks are therefore required to enhance orchard efficiency in macadamia.

5.3. Challenges and Opportunities of Low Vigor Rootstock and Scion Breeding

In the past, traditional breeding strategies have been used to generate new macadamia progeny. This consists of 8 years of seedling screening and 8–10 years of second-stage selection in regional variety trials (Figure 1) [54]. Selective breeding can improve profitability, but it comes with significant hindrances affecting genetic improvement, including large tree size, lengthy juvenility, a long time to peak production, and a poor correlation of young and mature tree traits [3]. Trees naturally exhibit long juvenile periods (3–10 years) and long generation times. Apple seedlings, for example, have a juvenile period of 7–9 years [66]; this is 4–5 years in olive [67] and more than 6 years in macadamia. Juvenility in these species is thus a significant obstacle in breeding programs for fast and comprehensive seedling assessment. Thus, a varietal selection program requires 20–30 years in selection for quantitative traits that are governed by multiple genes. The selection cycle can be even longer if the desired trait comes from wild species that have not yet been integrated into breeding germplasm. In addition, mature macadamia trees can attain a height of 15 m and a width of 10 m, which reduces the cropping efficiency and decreases the genetic gain [3]. Rootstock breeding is much slower than scion breeding of the same species because the testing cycle is more time consuming. Traditional breeding approaches are overall costly and slow in tree crops, and reduce the rate of genetic gain in rootstock and scion breeding.

Incorporating newly emerging technologies, including high-throughput phenotyping and genotyping, offers better opportunities for increasing genetic gain in rootstock and scion breeding programs. In addition, pertinent questions as to how rootstock $\times$ scion interactions affect vigor need to be addressed; answers may provide new avenues to breed rootstocks and scions with desirable traits. Genomic-based approaches, such as marker-assisted selection (MAS), genome-wide association studies (GWAS), and genomic selection (GS), offer promising opportunities for rootstock and scion breeding [3]. Genomics-assisted selection, aided by large numbers of markers and statistical models to estimate genomic breeding values, has the potential to replace field trials and move quickly into regional variety trials, thus cutting cultivar development time in half. These methods have started to be deployed in macadamia improvement programs [54].

Another great opportunity for rootstock and scion breeding exists in exploring the largely untouched *Macadamia* gene pool. Most current cultivars comprise a limited genetic base of *M. integrifolia*. The wild species *M. janseni* and *M. ternifolia*, which are smaller in size than the cultivated species, have not been used in directed breeding due to the presence of cyanogenic glycosides in the mature nut [3]. These valuable genetic resources have

the potential to act as parents with desirable vigor traits that could effectively be utilized in rootstock breeding. Comprehensive germplasm utilization, coupled with genomic approaches, has the potential to add significant advances in rootstock and scion breeding in macadamia.

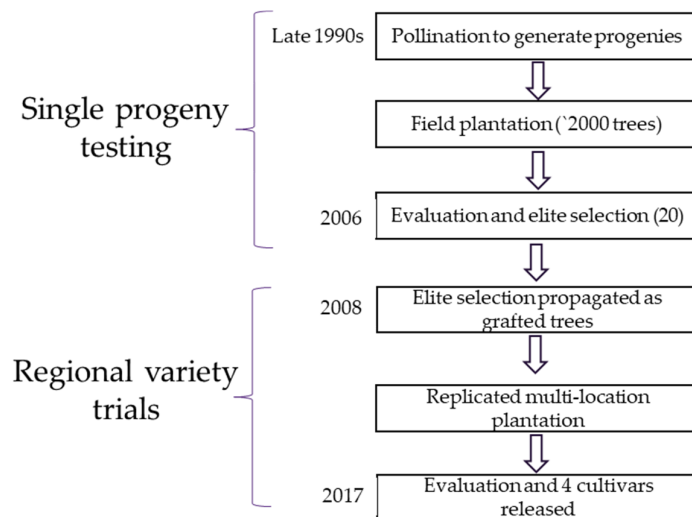


Figure 1. Traditional breeding timeline of the National Macadamia Breeding Program, Australia.

6. Genomics-Assisted Breeding: A Solution to Lengthy Breeding Cycle

6.1. Markers for Genomic Studies

Breeding programs of several crop species have utilized molecular markers for the identification of QTLs and candidate genes associated with important desirable traits. Molecular markers are useful in many fields of plant breeding and genetics, including studies of genetic diversity, population genetics, pedigree analysis, phylogenetics, and hybrid identification [40]. The development of genome-wide markers for high-throughput genotyping and construction of high-density genomic maps is enabled by the availability of fruit tree genome sequences [68]. Several molecular markers, such as Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs), and Next-generation sequencing (NGS), are utilized in fruit trees [69].

SSR markers, one of the most commonly used, are beneficial tools designed for QTL mapping, MAS, and diversity analysis. Bioinformatics applications are used to create them based on transcript and genomic sequences [42]. These markers are codominant, multi-allelic, reproducible, and amplifiable, which makes them advantageous as a genetic marker [70]. However, SNP markers are more cost-effective and allow higher throughput screening and higher density mapping in comparison to SSR markers [69]. ASNP is a single base-pair difference in the DNA sequence between individual members of a species. The genotyping by sequence (GBS) method is commonly used to produce SNPs by using next-generation sequencing (NGS) technologies [71].

In macadamia, several molecular markers have been developed to characterize genetic diversity within the genus. Aradhya et al. [72] studied the genetic variation of macadamia utilizing isozymes as molecular markers. A Randomly Amplified DNA fingerprinting (RAF)-based study was conducted to examine the genetic relationships among macadamia varieties [73]. Similarly, Machado et al. [74] conducted a Random Amplified Polymorphic DNA (RAPD)-based study on genetic variability in macadamia. With the discovery of increasingly complex genetic networks for important economic traits, applications for these marker systems are limited due to a low density of markers, poor genome coverage, and lower cost efficiency.

Diversity Arrays Technology (DArT), a cheap profiling technique for the whole genome, was developed to provide a practical and cost-effective whole-genome profiling capability [75]. This technique develops markers via a microarray hybridization method

and is capable of producing thousands of polymorphic loci in a single assay [76]. Using NGS technology, DArT platforms developed two types of markers: silicoDART and SNP markers. DArT markers have been successfully applied to investigate genetic diversity in cultivated and wild germplasm of macadamia [76]. Similarly, O'Connor et al. [77] studied the genetic diversity, population structure, and linkage disequilibrium (LD) of a macadamia population using DArTseq markers. Recently, Mai et al. [78] used DArTseq platforms for the genetic characterization of 302 wild accessions of macadamia. These studies suggest that DArTseq markers can be applied for genomic studies and selection in macadamia populations for rapid genetic gain.

6.2. Marker Discovery via QTL Mapping

Many economically important agronomic traits are quantitative and polygenic; that is, they are regulated by multiple genes on the same or separate chromosomes. The chromosomal regions containing these genes are called Quantitative Trait Loci (QTL). QTL mapping is a method of identifying gene locations that affect the trait of interest by utilizing molecular markers. It is based on the principle of assessing an association between the phenotype and genotype of a marker [40].

The basic steps of QTL mapping are described in Figure 2. It is vital that the parents chosen for QTL mapping are sufficiently polymorphic [79]. QTL detection can be performed using several methods, with the most prominent being Single-marker analysis (SMA), Simple-interval analysis (SIA), and Composite interval analysis (CIA) [80]. These procedures are based on the same principle: the mapping population's progenies are separated into two or three groups based on marker genotyping data for each plant; based on the phenotyping data, mean and variance for the target traits are estimated for each group, and the data sets are compared. Finally, if a significant difference is found between the genotype groups for the given marker, a conclusion is made that the investigated marker is associated with the targeted trait [40].

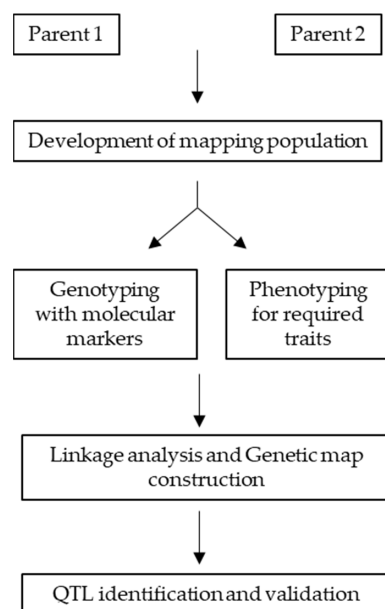


Figure 2. Basic steps of QTL mapping.

The genetic/linkage map provides the position and relative marker distance [81]. The first linkage map in macadamia was constructed using 328 randomly amplified DNA fingerprinting (RAF) and random amplified polymorphic DNA (RAPD) markers, and one sequence-tagged microsatellite site (STMS) marker; the map was based on 56 F1 progeny of 'HAES 246' × 'A16' [82]. However, these linkage maps could not be used for QTL detection due to low marker density and the absence of sequence-based markers [83]. Langdon

et al. [83] recently published the first sequence-based haploid-correlated linkage maps with high marker density; these maps are based on unselected progeny from self-pollinated ‘HAES741’, as well as its’ biparental cross with hybrid cultivars ‘A268’ and ‘A4’.

The ratio of linkage versus no linkage, known as the odd ratio, is used to calculate the linkage between markers, the Logarithm of Odds (LOD) [84]. LOD values of less than three are considered ideal for building linkage maps [80]. Molecular markers linked with traits of interest are identified using statistical programs such as R [85], QTLNetwork [86], PLABQTL [87], QGENE [88], and MapChart [89].

QTL mapping has been successfully applied in several tree and fruit crops for QTL identification, with most of the traits concerning disease and pest resistance, yield, and fruit quality (Table 1). Very few studies have conducted QTL mapping for vigor traits. Foster et al. [32] performed QTL analysis utilizing SSR markers and identified two major QTLs, ‘Dw1’ and ‘Dw2’, associated with rootstock-induced dwarfing in apples. Similarly, Knabel et al. [33] identified an allele associated with apple Dw1 segregating with dwarfing and precocity traits in pear using QTL analysis. However, QTL analysis has not been widely used in fruit tree breeding due to time and cost limitations. The preparation of an experimental population is slow and costly, and an expected marker effect may not be obtained in a population with different genetic backgrounds.

Table 1. QTL analysis and markers identified for vigor traits in tree crops.

Species	Markers	Traits	QTL	References
Apple (<i>Malus domestica</i>)	520 RAPD markers	Dwarfing trait of apple rootstock ‘M9’	<i>Dw1</i> associated with rootstock-induced dwarfing	[90]
Apple (<i>Malus domestica</i>)	SSR markers	Dwarfing trait of apple rootstock ‘M9’	<i>Dw1</i> and <i>Dw2</i> associated with rootstock-induced dwarfing	[32]
Pear (<i>Pyrus communis</i>)	710 SNP-based markers	Dwarfing trait of pear rootstock	<i>LG5</i> synthetic to <i>Dw1</i> in apple	[33]
Rubber (<i>Hevea brasiliensis</i>)	225 SSRs and 186 SNPs	Stem diameter, tree height, and no. of whorls	53 significant QTLs	[91]
Sweet cherry (<i>Prunus avium</i>)	842 SNPs	Fruit development time, maturity date, and 5 fruit-quality traits	18 significant stable QTLs	[92]
Sweet Orange (<i>Citrus sinensis</i>)	~30,000 DArTseq markers	12 fruit quality and quantity traits	19 significant QTLs	[93]

SNP, single nucleotide polymorphism; SSR, simple sequence repeat; RAPD, random amplified polymorphic DNA; DArT, Diversity Arrays Technology.

6.3. Marker Discovery through Genome-Wide Association Studies (GWAS)

Recent advancements in the field of high-throughput genotyping technologies have enabled more cost-effective high throughput genomic-based strategies such as GWAS [79]. GWAS is a method of candidate gene/QTL identification based on the association between genome-wide markers and phenotypes caused by Linkage disequilibrium (LD) between markers and casual genes or QTLs [69]. Linkage disequilibrium is the non-random association of alleles at different loci, which describes the unequal frequency of haplotypes in a population [79]. Although initially developed for the detection of genes associated with human diseases, GWAS has now evolved as a powerful tool in plant breeding [40].

Each marker in GWAS is tested individually to identify the association with the trait [94]. The basic method of conducting GWAS includes the selection of individuals from a natural population with a wide range of genetic diversity, followed by multiple instances of phenotyping for the traits of interest in different locations and environments for several years; the structure of the population and their relatedness are determined after genotyping with favorable markers [79]. For the quantification of LD, statistical procedures like D , D' , or r^2 are performed. Association between the phenotyping and genotyping data are

identified by using some statistical software programs [79]. Millions of SNPs are produced through GWAS, and SNPs are now commonly used markers for these studies [95].

As QTL mapping requires bi-parental populations, GWAS is more suitable for QTL detection in fruit trees than QTL Mapping. In GWAS, the allelic state of markers located on the segments of unrelated individuals is utilized for marker detection, which favors the impractical, laborious, and expensive family-based methods used in QTL mapping [96]. This method examines the broader germplasm pool, which includes a group of individuals sampled from wild populations, germplasm, and breeding cultivars, in addition to the multi-generation families formed through crossing parental-cultivars, as utilized in QTL analysis [69]. Multiple genes can be closely connected to the target gene due to the vast size of QTLs, which can be thousands of kilobases in length; this may result in linkage drag due to the presence of undesirable traits located closer to the desired ones [94]. As such, GWAS can overcome the detrimental effects of linkage drag as the marker intervals are shorter and can also identify smaller individual markers in LD with target traits in comparison to QTL mapping [94]. However, an increased rate of false positives in GWAS may be caused by factors such as population stratification and cryptic relatedness among individuals [97]. To control this rate of false positiveness, the statistical models used for GWAS should include correct terms for population structure and kinship relationships. Methods utilizing linear mixed models, which take multiple levels of relatedness into account, have therefore become a more standard procedure [69].

To date, many studies have been conducted on fruit trees to determine QTLs using GWAS, and most of the studies are conducted for fruit yield and quality traits (Table 2). In macadamia, GWAS was conducted for sticktight traits (diseased fruit pericarps retained in the canopy) in a breeding progeny of over 30 families [98]. More recently, GWAS was used in macadamia to identify the associations between 4352 SNP markers and 3 yield component traits [99]. O'Connor et al. [100] identified the potential of using genomics in macadamia breeding for varietal improvement. Although GWAS on vigor traits has not yet been conducted in macadamia, trunk circumference was studied as a yield component trait in a GWAS, and 44 significant SNPs were identified for this important vigor trait [101]. Further investigation on a different population may provide comprehensive information on the genetic structure of vigor traits in macadamia.

Table 2. GWAS in tree crops, population, marker size, and traits studied.

Species	Population Size	Marker Size	Traits	References
Apple (<i>Malus domestica</i>)	172 accessions	55,000 SNPs	11 fruit quality traits and 1 disease resistance	[102]
Apple (<i>Malus domestica</i>)	1200 seedlings	53 SSRs	Six fruit quality traits	[97]
Citrus (<i>Citrus spp.</i>)	111 varieties and 676 individuals	1841 SNPs	17 fruit quality traits	[103]
Citrus (<i>Citrus spp.</i>)	110 accessions	2309 SNPs	8 fruit-quality traits	[104]
Japanese chestnut (<i>Castanea crenata</i>)	99 cultivars and selections	162 SSRs and 741 SNPs	5 nut traits	[105]
Japanese pear (<i>Pyrus pyrifolia</i>)	76 cultivars	155 SSRs	4 fruit quality traits, harvest time, resistance to black spot, spur number and tree vigor	[106]
Macadamia (<i>Macadamia spp.</i>)	281 progenies	7126 SNPs	3 yield component traits	[99]
Macadamia (<i>Macadamia spp.</i>)	295 progenies	4113 SNPs	7 yield component traits, including trunk circumference	[101]
Peach (<i>Prunus persica</i>)	620 individuals	4005 SNPs	3 phenological and 11 fruit quality-related traits	[107]

SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

6.4. Marker-Assisted Selection (MAS)

MAS is a method in which a phenotype is selected based on the marker genotype [80]. Once markers significantly associated with genes or QTLs of interest are identified via QTL mapping or GWAS, breeders may use candidate marker alleles to identify plants carrying the genes or QTLs. In earlier times, marker-QTL association obtained from mapping studies were directly used in MAS, which has now been devised by adding QTL confirmation, QTL validation, and high-resolution mapping to eliminate inaccurate assessment of QTL position and effect due to factors such as sampling bias [108]. These additional procedures may convert markers into a form that is easier to detect.

MAS complements regular conventional breeding programs by increasing genetic gain per unit of time and reducing the length of the selection cycle. Once breeders identify tightly linked markers that accurately predict a trait phenotype, they may use these alleles to identify plants carrying the genes or QTLs, substituting large field trials by eliminating any unwanted genotypes and retaining only those plants possessing desirable genotypes. Some studies have mentioned that this process may eliminate almost 75% of plants after one cycle of MAS [80]. Besides substituting complex field trials, MAS eliminates unreliable phenotypic evaluation, making the selection process simpler by saving time, resources, and effort [69]. Selection can be carried out at the seedling stage; therefore, early selection can be made for traits that are expressed only at later developmental stages. While conventional methods don't allow individual plant selection due to environmental factors, MAS allows individual plant selection based on their genotype [80]. All these advantages can explicitly be utilized to accelerate breeding programs.

6.5. Genomic Selection (GS)

Traditional MAS is inefficient when many genes with small effects are segregating, and reliable markers have not been identified [69]. To overcome these limitations, a genome-wide approach called Genomic Selection (GS) was developed [109]. GS is a novel technique that predicts the genetic values of selected candidates depending upon the genome-estimated breeding values (GEBV), predicted by utilizing the high-density markers and eliminating the need to search for significant QTL-marker association [79]. GEBV is a prediction model that increases the prediction accuracy by combining phenotypic data with marker and pedigree data [79]. In comparison to MAS, GEBV depends on all markers with major and minor effects and utilizes those markers that cover the whole genome in such a way that all QTLs are in LD with at least a single marker [110]. GS, along with high throughput phenotyping, has revolutionized tree breeding by increasing the level of accuracy in selecting complex traits.

The important steps of GS, as outlined in Figure 3, involve two basic steps: to begin, a training population is created, consisting of individuals that have both genome-wide marker genotypes and target phenotypes. The effect of all markers on the phenotype is estimated, and a prediction equation is created. The equation is then used to forecast GEBVs, followed by an accuracy evaluation. For GS accuracy assessment, the predicted GEBV is cross-checked against known and accurate phenotypes in a testing population [109].

Accuracy among GS models depends on the model performance, sample size and relatedness, marker density, gene effects, heritability and genetic architecture, and the extent of LD between the marker and QTL [111]. In GS, accuracy is the key parameter as it compares various GS models and is also related to gains in selection. Several prediction models such as Best Linear Unbiased Prediction (BLUP), Genomic BLUP (GBLUP), ridge regression BLUP (RRBLUP), LASSO, Elastic Net, Random Forest, reproducing kernels Hilbert spaces regression (RHKS), and Bayes A,B,C, π have been developed for GS [112]. In addition to these statistical learning models, several machine learning methods have been developed recently, such as deep learning, random forest support vector machines, and gradient boost machines, among which Neural networks have been intensively studied and applied in genomic prediction [113]. These types of statistical machine learning methods

are implemented in GS because there is no best prediction model that can universally be used under all circumstances.

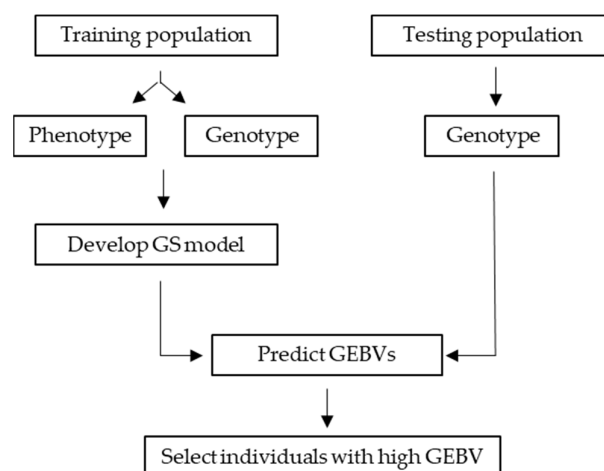


Figure 3. Basic steps of Genomic Selection.

For complex traits, some studies imply that GS is preferable to MAS and traditional phenotypic selection [114]. Since GS avoids the step of QTL identification, the issues of uncertainty in that step are also eliminated, which seems to be problematic in MAS [106]. Furthermore, in GS, markers are able to determine effects that might be too small to be declared as statistically significant [106]. In addition, GS avoids marker effect biases by utilizing all the available genetic markers with no significance threshold and thus produces more highly correlated measured and predicted BEVs [109]. These characteristics of GS make it efficient even for a polygenic trait with low heritability [115]. GS is thus capable of accelerating breeding cycles by enhancing the gain per unit time and cost.

With advancements in high throughput phenotyping and genotyping, studies on GS in tree crops are increasing (Table 3). Macadamia, with a selection cycle of more than 22 years, is a strong candidate for GS and would benefit highly from this technology as extensive phenotypic data are available with documented parentage [116]. Using GBLUP GS models, O'Connor et al. [117] recently assessed the accuracy of genomic prediction in a macadamia breeding population and concluded that including GS for yield could speed up rate of genetic gain. Although research conducting GS on vigor traits has not been prioritized yet, O'Connor et al. [101] identified 16 QTLs linked with trunk circumference in macadamia and suggested GS to be more appropriate than GWAS and MAS due to the large number of markers associated with this trait. Once successful, implementing GS for vigor traits in macadamia would require growing the progeny until the first leaf appears for DNA extraction, followed by genotyping, thus reducing the labor, plantation, and evaluation costs.

Table 3. GS studies in tree crops, including targeted traits, population and marker size, and prediction accuracy.

Species	Population Size	Marker Size	Phenotypic Traits	Prediction Accuracy	Reference
Apple (<i>Malus domestica</i>)	537 individuals	8294 SNPs	12 traits related to fruit texture	0.64 to 0.81	[118]
Apple (<i>Malus domestica</i>)	172 accessions	55,000 SNPs	Harvest date, 8 fruit-quality traits, and scab resistance	0.08 to 0.72	[102]
Cacao (<i>Theobroma cacao</i>)	287 individuals	5000 SNPs	4 fruit-quality and pathogen-resistance traits	0.42 to 0.59	[119]

Table 3. Cont.

Species	Population Size	Marker Size	Phenotypic Traits	Prediction Accuracy	Reference
Citrus (<i>Citrus</i> sp.)	111 varieties and 676 individuals	1841 SNPs	17 fruit quality traits	0.30 to 0.70	[103]
Eucalyptus (<i>Eucalyptus robusta</i>)	415 individuals	2919 SNPs	Volume at 49 months, Total lignin content, holo-cellulose content	0.05 to 0.79	[120]
European peach (<i>Prunus persica</i>)	1147 individuals	6076 SNPs	3 fruit-quality traits	0.60 to 0.72 (average)	[121]
Grapevine (<i>Vitis vinifera</i>)	3000 individuals	90,000 SNPs	4 traits	up to 0.90	[122]
Japanese Chestnut (<i>Castanea crenata</i>)	99 cultivars	162 SSRs and 741 SNPs	Nut harvest date, nut weight, pericarp splitting, insect infestation, and specific gravity	0.60 to 0.84	[105]
Japanese pear (<i>Pyrus pyrifolia</i> Nakai)	86 varieties and 765 trees from 16 full-sib families	1506 SNPs	18 traits	0.50 to 0.70 (single trait GP)	[114]
Macadamia (<i>Macadamia</i> sp.)	295 full-sib progenies	4113 SNPs	Nut yield and yield stability	0.14 to 0.79	[117]
Maritime pine (<i>Pinus pinaster</i>)	818 individuals	4436 SNPs	3 growth traits	0.70 to 0.85	[123]
Norway spruce (<i>Picea abies</i> L.)	1370 control-pollinated individuals	111,765 SNPs	3 vigor traits	0.49 to 0.97	[124]
Oil Palm (<i>Elaeis guineensis</i> Jacq.)	112 individuals	221 SSRs and 46,933 SNP s	6 fruit-quality traits	0.18 to 0.28 (SSR-based models), 0.23 to 0.43 (SNP-based models)	[125]
Rubber (<i>Hevea brasiliensis</i>)	435 individuals	30,546 SNPs	Diameter and height	0.59 to 0.75	[126]
Table grape (<i>Vitis vinifera</i>)	203 individuals	243 SSR markers	8 fruit and flower traits	0.57 to 0.77	[127]

SNP, single nucleotide polymorphism; SSR, simple sequence repeat; GP, Genomic Prediction; RAPD-STs, random amplified polymorphic DNA—sequence-tagged site.

7. Rapid Phenotyping for Vigor Traits

Phenotyping for growth traits is an important aspect of research in breeding tree crops. Information obtained from phenotyping is largely used by farmers in their decision-making process. In addition, accurate analysis of phenotypic traits is important for marker-assisted and genomic selections since selection is largely based on the data obtained from phenotyping. However, conventional methods of phenotyping are manual, laborious, and expensive with less accuracy. A lack of accurate phenotypic data has led to poor outcomes in gene identification, limiting the significance of genomics-assisted breeding [128]. Therefore, generating high-throughput, thorough, and effective data is needed for accurate crop evaluation and successful breeding.

Advanced technologies have allowed researchers to develop rapid and more accurate methods for the study of plant phenotypes. A variety of high-throughput platforms developed using the principles of spectroscopy, digital photography, spectral imaging, and LiDAR scanning are being used in fruit tree phenotyping [129]. In the case of tree growth measurements, traditional remote sensing methods based on manual interpretation of aerial imagery using photogrammetry are largely used [130]. Multispectral and hyperspectral imaging obtained via spectral cameras mounted in unmanned aerial vehicles (UAVs), manned spacecraft, and satellites is another recent advancement. Obtaining multi-spectral 3D data via LiDAR scanning, using laser scanners mounted on manned aircraft or UAV, has

become more common recently [131]. Similarly, digital photography is applied in scientific research to obtain spatial and canopy structure information [129].

Together with imaging and sensing, autonomous robotic systems are crucial for rapid phenotyping. Unmanned Ground Vehicle (UGV) robotic systems use similar sensing and imaging techniques to obtain data, which are analyzed via 3D reconstruction, image processing, and machine learning [132]. These robots are controlled using artificial intelligence (AI) technologies such as deep learning, fuzzy logic, and genetic algorithms [133].

All these high-throughput phenotyping platforms have allowed the screening of large populations with high accuracy and low labor input achieved through image analysis, remote control, and automation [134]. Although progress has been made with the introduction of these technologies in the improvement in many crops, some are still behind. Since phenotyping activities of macadamia are costly and laborious, applying rapid phenotyping would highly aid breeding programs by reducing cost and increasing accuracy, thus improving genetic gain.

8. Trait Mining for Low Vigor Trees

Analyzing plant architecture is important for the understanding of plant growth and vigor. While tree vigor refers to the intensity of vegetative growth, plant architecture is a representation of the form, arrangement of its parts, and organization of the plant components in a space that changes over time. It can also be characterized by the shape, size, and spatial arrangement of branches, twigs, leaves, and flowers as a result of the combination of genetic, environmental, and management effects [135].

Different components of tree architecture are associated with whole tree vigor and, therefore, are used to estimate vigor in tree crops (Table 4). The direct association of tree architecture to the light-capturing capacity of trees and the importance of light distribution in tree growth and vigor explains the association of tree architecture with growth and vigor. For instance, widely spaced branches reduce self-shading, which may accelerate height growth in favorable environments [136]. In addition to the light interception, the strong impact of canopy architecture on water transport, transpiration, carbon acquisition, and allocation also explains the association of architectural traits with tree vigor.

Plant vigor in many studies has been described with more generic parameters of tree architecture, like tree height and trunk diameter. Trunk Cross Sectional Area (TCSA), in particular, is generally used as a single variable to objectively evaluate orchard vigor and is believed to provide integrative information on whole tree growth [137]. Studies in tree crops observed positive correlations of TCSA with transportation of photosynthates from source to sink [138], tree crown weight [139], and other architectural traits such as height and canopy volume (Table 4) [36,38]. However, Nesme et al. [137] studied apple tree orchard vigor and did not find any relation between TCSA and the sum of the fruiting branch sectional area. This study concluded that TCSA measurements alone are not sufficient for indication of tree vigor in a diverse orchard population, and thus, more sets of variables are required to obtain an in-depth measurement. In another study, Whiting et al. [140] noted that TCSA was not significantly different between six cherry cultivars for the first two years, but the differences became significant in the following years and increased for another eight years. This states that TCSA measurements for tree vigor may be cumulative with time and cannot be used alone for tree vigor indication.

Another important part of tree architecture is determined by the woody crown architecture, i.e., the branching pattern. In a study by Broeckx et al. [141], tree architecture in *Populus* was heavily associated with spatial branching patterns such as the number of branches, branching degree, branch angle, and orientation; a strong relationship was identified between branch diameter, branch length, and tree vigor. Many studies have shown that horizontally inclined branches grow less vigorously while upright extension shoots grow more vigorously [142]. Similarly, Solar et al. [143] observed that tree vigor of walnut trees correlated positively with uprightness of growth habit and branching density; vigor in this study was calculated on the basis of visual analysis with 1 point (very weak

growth) to 9 points (very strong growth). Many leaf traits, branch and stem traits, and root traits are also reported to be associated with tree vigor in different fruit crops [144,145].

Table 4. Association of architectural and vigor traits in tree crops.

Crop	Traits	Phenotypic Correlations	Source
Walnut (<i>Jugans regia</i>)	Tree vigor and tree height	0.63	[143]
	Tree vigor and trunk diameter	0.79	
	Tree vigor and branching density	0.58	
	Canopy Volume and TCSA		
	Canopy Volume and internode length	0.82	
Macadamia (<i>Macadamia</i> spp.)	Canopy Volume and lateral branching	0.47	[36,38]
	Canopy Volume and branching unit length	0.49	
	TC and tree height	0.52	
	TC and canopy width	0.76	
	TC and canopy length	0.65	
	TCSA and Canopy volume	0.65	
	Tree height and canopy volume	0.828	
Mango	Branching density and canopy volume	0.78	[34]
	Growth unit length and canopy volume	0.466	
		0.505	
Olive	Tree height and trunk basal diameter	0.66	[30]

TCSA, trunk cross-sectional area; TC, trunk circumference.

To provide a clear definition of tree architecture, Halle et al. [146] generated an architectural model to analyze the dynamics of plant development. The tree architectural models developed from this work are classified on the basis of four major features: temporal growth pattern, branching pattern, morphological differentiation of axes, and sexual differentiation of meristems. Halle's architectural model implies that to understand the aspects of tree architecture, different scales, from nodes to the whole tree, should be considered. Similarly, Montesinos et al. [147] classified tree vigor into five architectural traits—trunk length, number of internodes, average internode length, trunk base diameter, and trunk apex diameter—while phenotyping almond orchards for growth traits. In apples, tree vigor was determined by the observation of the average length and node number of primary shoots and number of spurs [148]. Terminal shoot growth, particularly on the canopy periphery, has also been given special attention as an indicator of tree vigor because of its capability to strengthen young branches by extending and expanding the canopy [149].

Integrating small growth units and moving to a whole tree scale, Toft et al. [26], in a recent study in macadamia, phenotyped fifteen macadamia genotypes for different scales of architecture, including tree height, TCSA, within- and between-row width of the canopy and BCA at the largest scale, and traits like length, angle, and number of nodes at the growth unit scales. This study suggested that canopy size is determined by complex interactions between architectural traits at different growth scales. However, measuring all these traits in breeding programs is highly time and cost-consuming; therefore, an efficient architectural model with a set of morphological traits that accurately models plant vigor is required.

9. Rootstocks Affect Scion Growth and Vigor

Total plant size is an important scion feature that is influenced by rootstock. Specific rootstocks can be used to regulate scion size and architecture, and rootstocks for different crops have been classified as dwarf, semi-dwarf, vigorous, and highly vigorous based on

their effect on the scion cultivar [150]. Since rootstock effect on scion vigor depends on the type of rootstock used, scions grafted on dwarf rootstocks produce a dwarf combination while the same cultivar grafted on vigorous rootstock would grow highly vigorously.

Rootstock genotype can influence scion vigor, for example by reducing tree height, trunk circumference, and canopy volume [150]. A study in macadamia concluded that rootstock and rootstock-scion interactions accounted for 23% of the observed variance in tree height [151]. The ‘Clementine’ scion, when grafted onto several different citrus rootstocks, showed varied tree height, canopy diameter, and tree volume [152]. A study on apricot trees by Sitarek and Bartosiewicz [153] identified that scions grown on standard rootstocks had the largest TCSA in comparison to the most dwarfing rootstock. Rootstock genotype was also responsible for an up to 2.5-fold variation range on the trunk of peach [154].

Small-scale architectural measurements such as stem extension growth, internodal length, and no. of nodes are strongly affected by rootstocks, which results in visible effects on the scion canopy. Webster [61] studied 9-year-old apple trees grafted on dwarf M.27 rootstocks, and the final mean shoot length was only 25% of that of trees grafted onto vigorous rootstocks. In another study by Weibel et al. [59], varying shoot length growth was observed between different peach rootstocks. While examining rootstock-mediated dwarfing in sweet cherry, TCSA, final shoot length, and final node number were significantly affected by different rootstocks [155]. Similarly, in a study of a dwarfing rootstock of apple, shoot length and node number of all shoots were seen to be reduced along with the number of axillary shoots formed on the main axis [148]. While exploring the rootstock effect on peach shoot growth, in comparison to trees on robust rootstocks, dwarfing rootstocks were observed to produce scion with a lower stem extension growth [156].

Branching angle is another important component of vigor that is influenced by rootstock genotype. Wider branching angles have been observed in scions of fruit trees grown onto a dwarfing rootstock [157]. The limb angle growing from the trunk was altered by rootstocks in apples [158] and in sweet cherries [159]. However, Weibel et al. [59], in their study on two peach cultivars, did not observe any significant difference in branching angle between different rootstocks, suggesting that the effect on branching angle might not be a direct rootstock effect but indirectly related to increased precocity due to the rootstocks.

Rootstock effect on scion growth and vigor is dependent on interactions with the scion. Tworowski and Miller [150] conducted a study on six apple scions with different growth habits, which were grafted on rootstocks with different dwarfing tendencies, and reported that there were interactions between the dwarfing potential of rootstocks and the growth habit of scions. They concluded that rootstock could control growth, but the degree of control depends upon the scion cultivar that is grafted onto the rootstock. Research on rootstock-scion interaction in apples showed how rootstock influenced scion dry weight and growth rate, but the duration of growth was influenced more by scion [160]. Apart from rootstock and scion interactions, other factors influencing the rootstock effect on scion could be the training system, planting density, and different biotic and abiotic stresses. Studying the rootstock effect, and other factors affecting rootstock-scion interactions, may assist in identifying suitable rootstocks for improved orchard efficiency.

10. Physiological Traits Associated with Tree Vigor

10.1. Plant Water Potential and Hydraulic Conductivity

The water status of a tree is an important factor that determines vegetative growth and vigor in fruit trees. Plant water content is the major contributor to almost every plant physiological process, e.g., cell enlargement, photosynthesis, stomatal control of transpiration, and CO₂ absorption [161]. Water potential (Ψ), a measure of the free energy status of water, is the common characterization of plant water status. The rate of water movement via stem or branch tissue/cells is indicated by hydraulic conductivity (k), which is the ratio of water flow to its known driving force [162]. Therefore, the capacity of transporting water throughout the tree can be determined via the measurement of k [156]. It

is well established that the relationship between tree water status and stem Ψ is determined by k of plants [163].

Growth reduction induced by grafted rootstocks has been correlated with lower leaf and stem Ψ in trees grafted onto them. This relationship between Ψ and tree vigor is associated with the reduced ability of dwarfing rootstocks to transport water from rootstock to scion [164], which reduces the Ψ and k . Supporting evidence was provided by Solari et al. [156], who reported that peach cultivars grafted on dwarfing rootstocks had lower midday stem Ψ values in comparison to the trees grafted on vigorous rootstocks. Similar results were obtained in apple, pear, and peach [164–166].

Reduced k causes reductions in canopy water status, gas exchange rates, and subsequent growth [167]. Lower k was observed in low-vigor cherry plants [168], along with many scion cultivars grafted on a dwarfing rootstock. Martinez-Cuenca et al. [60] reported that whole plant k was 40% higher in citrus trees grafted onto dwarfing rootstocks with respect to trees grafted onto a vigorous rootstock. Studies in many crops, including apple [169], pear [165], peach [164,170], olive [171], and grapes [172], demonstrated that scions grafted onto dwarfing rootstocks had a lower k than those grafted onto vigorous rootstocks. Leaf k in dwarfing peach rootstocks was observed to be lower than vigorous rootstocks, and it is also directly linked to the rootstock k [156]. Increased hydraulic resistance was observed either in the root system [171] or in the graft union region [162]. Lauri et al. [173] performed Quantitative trait loci (QTL) analysis on 90 apple genotypes and identified 16 traits related to k that co-localized with traits identified for tree growth and fruit production. Thus, lower k in size-controlling rootstocks can be linked to the limited water absorption capacity of those rootstocks, which in turn is a function of plant water potential.

10.2. Xylem Anatomy

Hydraulic conductivity in plants is a function of the xylem anatomy. Hagen-Poiseuille law states that k of a tube is a function of the diameter of the tube raised to the fourth power [174], which is still used to calculate k of plants theoretically. This law exerts importance on xylem vessel diameter as an important part of xylem anatomy that determines plant k . The number and size of functional xylem elements were hypothesized to decrease during callus differentiation to vascular tissue, which leads to lower k , which is the phenomenon that usually occurs in grafted combinations utilizing dwarfing rootstocks [162].

The differences in k between rootstocks in fruit trees are related to xylem vessel characteristics, with dwarfing rootstocks having smaller xylem vessel diameters [170,172]. Vascular anatomy between low- and high-vigor rootstocks was studied in cherry and peach trees, and positive correlations between calculated k , vessel diameter, and vigor were observed. Similar relationships were observed in a range of tropical rainforest tree species, which was explained as a need for higher k in taller trees to prevent drought-induced embolism [175]. Furthermore, additional evidence relating hydraulic conductance with the diameter of xylem vessels has been provided in citrus rootstocks [176]. This explains that the number and diameter of xylem vessels may influence scion vigor as these characteristics are directly linked to k and shoot growth.

It has been pointed out that the anatomical measurements of xylem vessels might be useful for the prediction of rootstock vigor and early identification of dwarfing rootstock cultivars in rootstock breeding programs [177]. A study in three cultivars of macadamia by Toft et al. [178] identified a positive relationship between tree vigor and xylem vessel area and suggested that anatomical and hydraulic traits associated with vigor may be used for the selection of low-vigor cultivars at the seedling stage. Understanding this relationship in rootstocks would highly speed up rootstock improvement for preferable vigor imparting capacity, yield efficiency, water-use strategies, and stress resilience.

10.3. Stomatal Conductance, Size, and Density

Stomatal size and density are altered by plants by actively adjusting the guard cell turgor pressure, which controls the rate of CO_2 uptake and water loss using a process called

stomatal conductance [179]. Stomatal conductance, an indicator of plant water status, is the degree of stomatal opening and can also be defined as the rate of CO₂ entrance and water vapor exit from stomata [161]. During evolution, modification of stomatal traits has aided the adaptation of plant species to new environments with major importance in drought tolerance and water use efficiency [180].

Stomatal conductance (g_s) is related to Ψ through a feedback mechanism where reductions in Ψ further reduce g_s by reducing transpiration, and a reduction in g_s results in stomatal closure, thus lowering the Ψ [161]. Stomatal conductance declines with decreasing k under ideal conditions to maintain a constant value of leaf Ψ . Many studies have found a positive relationship between tree vigor and leaf stomatal density, and rapid growth has generally been linked to high stomatal conductance [181].

While studying the relationship between stomatal characteristics and tree growth, the relationship between stomatal traits and the vigor-inducing capacity of rootstocks has been identified in tree crops. As vigor-controlling rootstocks have a reduced capacity to transport water [164], a water deficit condition develops in the scion, which reduces the Ψ . This reduction in Ψ induces partial stomatal closure to prevent cavitation in xylem vessels and damage to the hydraulic system, with a consequent reduction in photosynthesis exerting an adverse effect on shoot growth [161]. This explains the size-controlling phenomenon by rootstocks.

Supporting this phenomenon, a study by Martinez-Alacantara et al. [182] in citrus concluded that lower leaf Ψ and low k during the time of maximum evaporative demand resulted in decreased g_s in scions grafted onto size-controlling rootstocks. Similarly, lower midday leaf Ψ of apple trees grafted on size-controlling rootstock M9 was associated with lower g_s in comparison to the trees grafted on intermediated-vigorous rootstocks [166]. Beakbane and Majumder [183] studied the effect of seven apple rootstocks on scion vigor and suggested stomatal density measurements as a rapid method of rootstock assessment for growth potential. Recently, preliminary research in macadamia by Wakefield et al. [184] identified a significant effect of rootstock genotype on scion stomatal density while studying three different rootstocks grafted onto a common scion. However, simultaneous evaluation of vigor traits was lacking, and therefore, the relationship between stomatal traits and vigor traits could not be established. Further research to investigate this relationship in diverse germplasm may provide useful information for future breeding programs.

10.4. Net Photosynthesis and Gas Exchange

Photosynthesis is a basic biological process in plants for energy production, used to convert light energy into chemical energy. This converted energy is stored in the form of carbohydrates and used to power metabolism. Photosynthetic efficiency is usually considered the major physiological trait for evaluating plant growth and biomass accumulation [185]. Various studies have shown that rootstocks directly affect the photosynthetic and gas exchange characteristics of grafted scions [186].

The photosynthetic characteristics of grafted plants are highly correlated with plant vigor. Plants grafted onto dwarfing rootstocks of apples had lower photosynthetic rates compared to vigorous rootstocks [187]. Qureshi et al. [188] documented a positive correlation of canopy volume with photosynthetic activity and g_s in citrus trees grafted onto rootstocks with varying degrees of vigor.

Several attempts have been made to compare tree water status and leaf gas exchange in scions on different rootstocks. As mentioned above, water stress can have serious effects on g_s , which in turn exerts a negative effect on the net photosynthetic assimilation rate. Solari et al. [156] reported that hydraulic limitations in dwarfing peach cultivars caused photosynthetic reductions via stomatal limited mechanisms. Stomatal limitations due to water stress in peach trees significantly affected net photosynthesis [189]. Similarly, sweet cherry trees grafted on dwarfing rootstocks had lower stem Ψ , g_s , and net CO₂-exchange rates. Photosynthesis was highly correlated with g_s and transpiration rate when roots were pruned for vigor control in pear trees [190]. Therefore, photosynthetic characteristics

may be used as an indicator of rootstocks' dwarfing ability, and the effect of rootstocks on photosynthesis may explain the impacts of rootstocks on scion growth.

10.5. Leaf Area and Leaf Area Index

Leaves are the major site of water vapor loss during evapotranspiration, which helps to maintain a negative water potential and allows water uptake by the roots. Leaf area has an influence on the light interception pattern and photosynthesis [191]. Leaf area with sufficient light interception across the canopy produces maximum carbon assimilation, as a leaf with a broader area contains more chloroplasts and has higher stomatal density on the leaf surface. This is crucial for gas exchange in photosynthesis [192].

Leaf Area Index (LAI), measured as half the area of all leaves per unit ground area, is one of the most important parameters to measure leaf activity. As an important indicator of light and precipitation interception, water status balance, and photosynthesis, LAI is used in many horticulture studies as an indicator of growth [193]. Vigor-controlling rootstocks and their effect on leaf area development have been studied in several fruit crops. Dwarfing rootstocks are known to direct a higher fraction of dry weight into fruit production, thus reducing the vegetative growth; this is related to a reduced leaf area, which therefore produces less photo assimilate [14]. Scions grafted on high-vigor apple and kiwi rootstocks had higher leaf area per tree as compared to low-vigor rootstocks [191,194].

Leaf area reduction in tree species has also been explained as a mechanism for coping with water stress. Under low water conditions, plants tend to decrease leaf area to reduce the transpiration surface and thus avoid the extreme reduction of water potential [195]. Leaf area reduction by vigor-controlling rootstocks may be related to this mechanism, as vigor-controlling rootstocks have a reduced capacity of transporting water to the scion, which reduces Ψ . These simulations suggest that leaf area is a trait directly influenced by size-controlling rootstocks, which relates to reduced photosynthesis and dry matter accumulation.

11. Mechanisms of Rootstock Mediated Vigor Control

While knowing which traits are affected by the rootstock is important, understanding the underlying mechanisms of vigor control by rootstocks is vital to controlling these traits in production systems and finding the most beneficial rootstock genotypes for a given crop in each planting system. Several hypotheses have been generated and tested in order to identify the underlying mechanisms to date, and most of the hypotheses are generated on the assumption that roots and shoots have complementary and dependent functions and influence each other strongly [186]. Described below are some theories and hypotheses explaining widely studied dwarfing mechanisms in tree crops.

11.1. Semi-Incompatibility and Production of Phenolics

Graft incompatibility is usually defined as the inability of rootstock and scion to bind together to form a successful bud union. One of the oldest theories, the semi-incompatibility theory of rootstock-induced dwarfing, suggests that specific rootstock-scion combinations have partial incompatibility, which alters the production and transport of water, nutrients, and hormones across the graft union [142]. It may also lead to the production of phenolics or other chemicals that can inhibit plant growth. While graft incompatibility may lead to early necrosis and even premature death, some incompatibilities show minor symptoms and do not result in sudden dysfunction [196]. However, these symptoms may become more prevalent over several years after grafting, affecting different phases of growth and development. The appearance of abnormal morphological or developmental traits in graft union vascular structures, such as depletion of xylem sap constituents, smaller and fewer vessels, swirling pattern of vessels, presence of senescent tissues, and necrotic area, are thought to be involved in creating the incompatibility [162,197]. Phenolic productions at the graft union can also play an important role in the incompatibility of grafted trees by

influencing the decarboxylation of Indole-3-Acetic acid (IAA), whereas the synthesis of phenols is thought to be regulated by auxin [142].

11.2. Rootstock-Scion Water Relations

Another well-studied theory of rootstock-induced vigor control is the water relations theory, which was first proposed by Beakbane [198]. Explaining rootstock and scion water relations, this theory states that inefficient water supply from the root systems to the scion through the graft union affects plant growth as a consequence of water stress caused by size-controlling rootstocks (Figure 4). The reduced flow could either be caused by the restricted amount of water transported from the root system or due to the presence of anomalies in the graft union that restrict water transportation to the scion [186]. The theory was later experimentally confirmed by Giulivo and Bergamini [199], who reported that apple trees grafted onto dwarfing rootstocks had lower stem water potential than vigorous rootstocks.

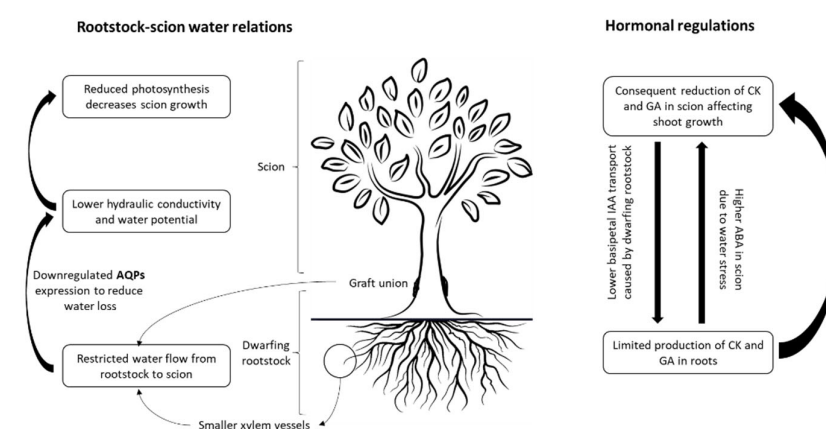


Figure 4. Figurative representation of possible mechanisms of rootstock-mediated vigor control in tree crops.

This theory further postulates that dwarfing rootstocks have smaller xylem vessels, which leads to reduced water flow to the scion. Hydraulic conductance, a major parameter representing tree water status, has been given particular attention as a central component of rootstock-scion water relations. It has been suggested that the dwarfing effect could be a result of reduced k . This relationship between hydraulic conductivity and vigor control by rootstocks has also been demonstrated in a range of fruit crops, including apple [169], pear [165], peach [170], olive [171], grapes [172], and citrus [60], where low-vigor rootstocks showed lower k than that of vigorous rootstocks. As a result of reduced k , gs , net CO_2 assimilation, and photosynthesis are reduced, leading to lower biomass accumulation and low vigor [182].

11.3. Molecular Mechanisms (Protein Transport and Differential Gene Expression)

The transport of proteins and mRNAs plays an important signaling role in plant growth [200]. This transport has also been identified to occur across the graft union in many tree species, affecting several rootstock and scion attributes [201]. Xu et al. [202] reported that *Gibberellic acid insensitive* (*GAI*) transcripts traveled in both directions (upward and downward) via the graft union; *GAI* negatively regulates responses to gibberellic acid, which is an important plant hormone regulating plant growth and development. A previous study showed that scions grafted onto *GAI*-overexpressing transformants showed dwarfing effects, which could be explained by the movement of *GAI* [203].

Gene expression studies have been used to elucidate the molecular mechanisms of rootstock-induced vigor control in several fruit trees. Rootstocks trigger distinct scion gene expression patterns, which could also be due to the movement of particular RNA molecules from the rootstock to the scion [202]. Differential gene expression in dwarfing rootstocks,

according to Foster et al. [204], is linked to five biological processes: primary metabolism, cell wall construction and modification, secondary metabolism, hormone signaling and response, and redox homeostasis. Genes that promote the biosynthesis of amino acids, lipids, and cell walls had decreased expression in size-controlling rootstocks, while genes that promote the breakdown of these compounds were highly expressed.

Several studies have identified differential gene expression in dwarfing and vigorous rootstocks to explain the molecular aspects of rootstock-induced dwarfing (Table 5). For example, in sweet cherry, a number of genes were found to be differentially expressed before, at, and after the differential cessation of terminal growth in dwarfing and vigorous rootstocks [205]. In a study of grafted apple trees, Jensen et al. [206] identified one hundred sixteen genes whose expression levels were correlated with plant size; sorbitol dehydrogenase, a homeobox leucine zipper protein, and a hevein-like protein were strongly correlated with trunk cross-sectional diameter. Upregulation of some flowering genes (e.g., *FLOWERING LOCUS T*) in the vascular system of dwarfing apple rootstocks (M9) was observed by Foster et al. [207]. In addition to increased flowering, these genes were involved in early shoot termination, which was speculated to be a part of the underlying dwarfing mechanisms. In the same study, genes involved with responses to biotic and abiotic stress were also found to be upregulated in dwarfing rootstock.

Table 5. Differential gene expression involved in rootstock-induced dwarfing in fruit trees.

Genes/Proteins	Crops	Findings	Source
<i>PYL4</i> (Absciscic acid receptor) and <i>Absciscic acid-insensitive mutant 5 (ABI5)</i>	Apple	Upregulated in dwarfing rootstocks	[207]
<i>WRKY</i> transcription factor family	Apple	Responsible for dwarfing phenotype in ‘M26’ rootstock of apple	[208]
<i>MdAUX1</i> and <i>MdLAX2</i> (Auxin influx transporters)	Apple	Down-regulation of these genes, together with an increase in flavonoid concentration, led to reduced auxin movement, which correlated with dwarfing effect of rootstocks.	[204]
<i>PIN1</i>	Apple	Gene expression decreased in trees with dwarfing genotypes used as an inter-stock.	[209]
<i>Isopentenyl transferases (IPT)</i>	Apple	<i>IPT3</i> expression was correlated with plant vigor	[209]
<i>Sorbitol dehydrogenase (SDH)</i>	Peach	<i>SDH</i> activity in shoot tips of peach were related to shoot growth rate.	[210]
<i>Tiller Angle Control 1 (TAC1)</i>	Peach <i>Prunus</i> Poplar	Gene expression promotes the outer lateral shoot growth.	[211]
<i>Gibberellin Insensitive Dwarf 1 c (GID1c)</i>	Apple	The expression of <i>GID1c</i> was comparatively lower in dwarfing rootstock.	[212]
99 transcripts (transcription regulation, brassinosteroid signaling, flavonoid metabolism, and cell-wall biosynthesis)	Sweet cherry	Differentially expressed in dwarfing and semi-vigorous rootstocks	[205]
<i>GID1c</i>	Plum	Silencing of <i>GID1c</i> led to a dwarf phenotype	[213]
5049 differentially expressed genes	Breadfruit (<i>Artocarpus altilis</i>)	Upregulation and downregulation of genes in scion stems were associated with rootstock-induced dwarfing	[214]

11.4. Aquaporins in Vigor Control Mechanism

Aquaporins (AQPs) are a group of proteins present in the plasma membrane that play an important role in plant water relations and facilitate the movement of water between plant cells [215]. AQPs are divided into five sub-classes on the basis of homology sequence: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), Nod26-like intrinsic proteins (NIPs), small and basic intrinsic proteins (SIPs) and unclassified X intrinsic proteins (XIPs) [216]. Exact numbers of AQP genes have been identified through genome sequencing in some plants, including 35 in *Arabidopsis* [217], 79 in olive [218], 42 in apple [219], and 55 in poplar [220].

The major function of AQPs in plants is to regulate water transport via membranes in critically low water flow conditions or in situations where the flow of water needs to be adjusted. Root water uptake in plants occurs via two pathways: flow within a cell and flow from cell to cell (C-C). As cell walls must be crossed in C-C water flow, the water transport efficiency of this pathway is thought to be regulated by the activity, density, and location of AQPs, with obvious consequences for plant growth [221]. AQP expression is observed to be highest in areas of high cell division and expansion [222]. PIPs are considered to be more important than other intrinsic proteins in regulating water uptake by roots because of the much lower plasma membrane permeability of PIPs [223].

Recent molecular investigations have demonstrated that AQPs play an important role in plant vigor and water relations in several species. AQPs, especially the PIPs, are also known for partially controlling change in the k of a plant [224]. AQPs are specifically expressed in different plant tissues, although root and stem AQPs are found to be more important in regulating plant k [225]. A study in grapes by Gambetta et al. [172] concluded that the expression of several *VvPIP2* isogenes was significantly greater in high-vigor rootstocks. As a type of membrane protein, AQPs were found to be involved in the regulation of transpiration rates, plant vigor, photosynthesis, and shoot growth rate in tobacco [226]. One of the few studies relating AQP gene expression and rootstock effects in perennial woody plants reported that *OePIP1* and *OePIP2* gene expression in olive was higher in scions grafted onto dwarfing clones than the vigorous clones [221].

A study in citrus by Rodriguez-Gamir et al. [227] investigated AQP expression and its significance in the water stress tolerance mechanism. This study concluded that low AQP expression in the roots of “Cleopatra” mandarin resulted in lower root k , which facilitated water maintenance in the cells, leading to an optimum level of leaf Ψ and photosynthesis in plants under water stress. Hence, down-regulated AQP expression, leading to loss of vigor, could be a water stress tolerance mechanism in citrus. Further investigation of AQP expression in relation to plant physiological processes, such as hydraulic conductivity, stem water potential, stomatal conductance, and photosynthesis, is warranted.

11.5. Hormonal Regulation of Vigor

Phytohormones are known to regulate all phases of plant growth and development, including the physiological processes and differences between the rootstock and scion. Some studies have suggested that endogenous hormones may be involved in rootstock-mediated control of the scion [186]. In addition, hormonal signals can also contribute to rootstock-induced dwarfing via the modification of gene expression patterns, as described in the previous section. The hormonal theory of scion vigor control is based on the assumption that the translocation of plant hormones from source to sink influences cell growth and differentiation [228]. This theory is also well connected with the ‘hormone message concept’, which states that the production site and action site of hormones are different, and hormones are transported in between these sites [229]. The role of different phytohormones in rootstock-induced dwarfing is described in the following sub-sections.

11.5.1. Auxin and Cytokinin

Auxin is known to be the principal regulator of cell growth and differentiation, and other hormones adjust their activity based on auxin signaling [230]. It is believed that

auxin stimulates root initiation, and cytokinin (CK) is responsible for cell division, shoot growth, and leaf expansion [148]. A study demonstrated that grafting disturbs this auxin-CK balance [231]. Several studies have reported that rootstocks control scion vigor by reducing the basipetal transport of Indole-3-Acetic Acid (IAA) from scion to root, limiting the production of CK and Gibberellic acid (GA) in roots. Consequently, the amount of CK and GA supplied to the scion xylem vasculature is reduced, affecting shoot growth [232]. A different study found an inverse relationship between IAA diffusion rate and xylem CK concentration [233].

Auxin concentrations in the scion were found to be lower when grafted onto dwarfing rootstocks in apples and peaches compared to vigorous rootstocks [234]. Smaller vessel diameters were also observed by Soumelidou et al. [197] in dwarfing apple rootstocks compared to semi-dwarfing rootstocks, with altered auxin transport at the graft union of the dwarfing rootstock. Hooijdonk et al. [148], in their study, injected an IAA transport inhibitor N-1-naphthylphthalamic acid (NPA) into the stem of vigorous apple rootstocks, which resulted in reduced shoot length and other architectural modifications resembling the dwarf M9 rootstock. According to Li et al. [209], the expression of an auxin transporter gene (*PIN1*) was lower in scions grafted onto a dwarfing rootstock, which led to a low supply of IAA to the roots and a dwarfing phenotyping in the trees. Growth potential in both grafted and ungrafted peach trees was positively associated with the transport rate of cytokinin in the xylem [234]. Similarly, in orange, lower cytokinin activity was observed in less vigorous rootstocks in comparison to vigorous rootstocks [235]. Together, this evidence suggests that dwarfing rootstocks have reduced IAA levels from the scion, which reduces the amount of CK produced in the roots, thus altering scion growth and structure.

11.5.2. Gibberellin

Gibberellins (GA) are fundamental plant growth regulators that influence tree architecture [236]. Changes in GA metabolism play an important role in controlling scion size, and this theory is proved by several studies. Explaining the role of GA in apple shoot growth, Bulley et al. [237] reported that suppressing the expression of a gene encoding the GA biosynthetic enzymes *GA3-oxidase* and *GA20-oxidase* reduced the amount of GA in a scion variety of apple resulting in significant height reductions. Scion cultivars grafted on dwarfing rootstock also had lower *GA₁₉* in root and xylem exudates [230]. Similarly, El-Sharkawy et al. [238] observed increased levels of a GA catabolic gene, *GA2-oxidase*, which resulted in shorter internodes and reduced stem elongation along with reduced scion growth in a dwarf hybrid plum. However, Hooijdonk et al. [233] reported that *GA₁₉* concentrations were similar in xylem sap collected throughout the growing season from scions grafted onto different apple rootstocks.

11.5.3. Absciscic Acid

Absciscic acid (ABA) is a phytohormone that regulates shoot growth and development. ABA can inhibit auxin translocation and thus reduce xylem development in dwarfing plants [197]. ABA is found to affect several growth attributes, such as leaf abscission, stomatal closure, and root growth inhibition [239], and it is also capable of inducing dwarfism in higher plants. ABA has also been shown to regulate tolerance responses to different plant stress factors [230]. Elevated ABA has been observed in scions on dwarfing apple rootstocks [240] and associated with reduced scion growth in citrus growing on a dwarfing rootstock [241]. Furthermore, Moghadam et al. [242] studied sweet cherry rootstocks and observed that the ratio of ABA/IAA was lower in vigorous rootstocks compared with dwarfing rootstocks, and they also found a close relationship between ABA concentrations in shoot bark and plant vigor. According to Atkinson et al. [243], a possible explanation could be the abnormal xylem configuration at the graft union, which reduces hydraulic conductance and increases ABA concentrations in the xylem stream. A high level of xylem ABA in grafted trees may play an important role in vigor control and water stress tolerance.

12. Conclusions

Excessive vigor reduces orchard efficiency and productivity, while effective vigor control facilitates high-density plantations and reduces management costs in modern orchards. Low-vigor cultivars and dwarfing rootstocks serve as the primary basis of vigor control in several tree species. As a newly domesticated crop, macadamia dwarfing rootstocks and low-vigor cultivars are not commercially available yet, although research toward novel cultivar selection has begun. The selection process in macadamia is lengthy and laborious due to a long juvenility phase, large tree size, and a long generation gap. Recent advancements in genomics have allowed rapid and early selection of cultivars in many crop species via GWAS, GS, and QTL mapping. Incorporation of these high throughput technologies in tree crops may accelerate the selection process of low-vigor cultivars. Understanding the mechanisms of rootstock-mediated dwarfing is another strategy to accelerate the process of rootstock selection through the identification of morpho-physiological, anatomical, or molecular traits associated with vigor. Rootstock effects on tree crops, including macadamia, are widely known; however, the physiological and molecular mechanisms underlying these effects are not clearly understood. Current evidence from studies in other tree crops suggests that the rootstock and scion have a complex interaction affecting several rootstock and scion attributes, including water relations, hormonal balances, anatomical features, and gene expression, which collectively affect tree vigor. Emerging technologies for accelerated selection along with better understanding of the physiological basis of vigor control will generate improved methods of rootstock and scion breeding for reduced vegetative vigor.

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