REVIEW

Rootstock scion somatogenetic interactions in perennial composite plants

Tyson Koepke · Amit Dhingra

Abstract   The ancient plant production practice of grafting which instantly imparts new physiological properties to the desirable scion still remains shrouded in mystery. Yet, grafting remains a widely used technique in the production of several horticultural species. In a composite grafted plant, rootstocks control many aspects of scion growth and physiology including yield and quality attributes as well as biotic and abiotic stress tolerance. Broadly, physical, physiological, biochemical and molecular mechanisms have been reviewed to develop an integrated understanding of this enigmatic process that challenges existing genetic paradigms. This review summarizes the reported mechanisms underlying some of the economically important traits and identifies several key points to consider when conducting rootstock scion interaction experiments. Study of the somatogenetic interactions between rootstock and scion is a field that is ripe for discovery and vast improvements in the coming decade. Further, utilization of rootstocks based on a better understanding of the somatogenetic interactions is highly relevant in the current agricultural environment where there is a need for sustainable production practices. Rootstocks may offer a non-transgenic approach to rapidly respond to the changing environment and expand agricultural production of annual and perennial crops where grafting is feasible in order to meet the global food, fiber and fuel demands of the future.

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Introduction

Grafting is a widely used technique in the production of several horticultural species. For many economically important woody perennials producing fruits and nuts, modern production systems are primarily dependent on grafting of a variety on a rootstock that may belong to the same or different species to control important traits including root-related diseases, plant size and yield (Fig. 1). These advantages have recently been leveraged in the production of herbaceous dicots. The combination of genotypes in a single composite plant has produced a unique and interesting biological model that transcends the known genetic paradigms. The instant physiological modification of desirable traits in the scion, that mimic genetic changes, is mediated by several hypothesized agents derived from the rootstock. Trait improvement bypasses the reproductive cycle eliminating years of selection during breeding and is a result of cellular or genetic interactions in the somatic cells. We have used the phrase 'somatogenetic interactions' to represent this phenomenon in composite plants.

The first verifiable report of grafting was in 412 BC when grafting appeared to have already been a common technique though some believe that grafting has been documented as early as 1800 BC (Mudge et al. 2009). Traits such as disease resistance and cold tolerance have been linked to rootstock control and provision of these traits was the primary use of rootstocks as discussed by Rogers and Beakbane (1957). There are many more scion traits that are controlled, at least in part, by the rootstock and they are detailed in the first section of this review.
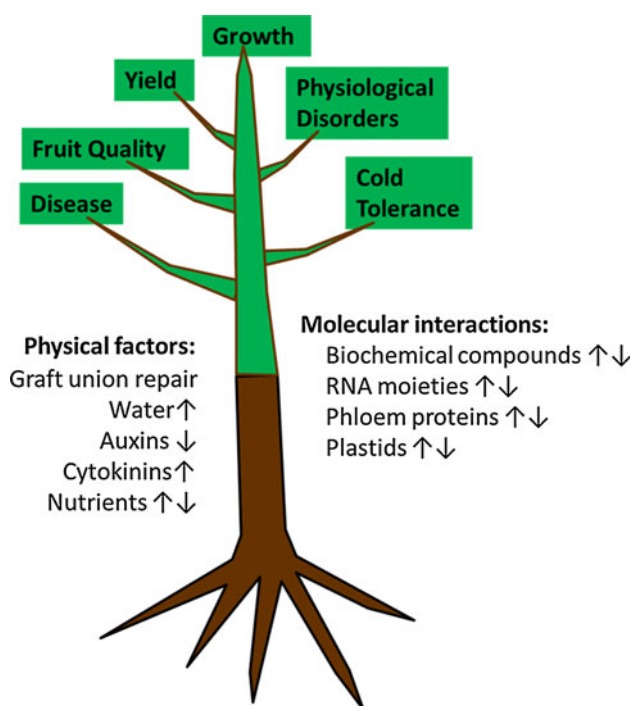
Fig. 1 Overview of review topics. On the top portion of the figure, major categories of rootstock-controlled scion traits are displayed in green boxes. Major physical and molecular factors involved in rootstock–scion interactions are shown on the sides with the general direction of transport when provided in the text

While knowing which traits are affected by the rootstock is important, unraveling the underlying mechanisms for rootstock–scion interactions is critical to control these traits in production systems. Rogers and Beakbane (1957) also discussed the 'hypothetical' mechanisms of rootstock–scion interactions being driven by nutrients, transport, and growth regulating substances. A recent review by Webster (2004) discusses many of the potential changes in scion growth that can result in the dwarf phenotype but focuses on the previously reviewed potential mechanisms indicating lack of change in knowledge since 1957. Several other recent reviews on herbaceous plants examine the physical interactions of the rootstock and scion (Martinez-Ballesta et al. 2010), and the hormonal crosstalk between the two genotypes (Aloni et al. 2010). These reviews form the foundation for the examination of the physiological mechanisms explaining the rootstocks' control of scion traits in perennial crops. This aspect is discussed in the second section of this review.

Lastly, molecular aspects of these interactions are beginning to emerge and they are examined in the third section. Gene expression and protein function underpin the changes in physiology. Identifying which genes and proteins are at the core of the physiological changes would considerably improve our understanding of how grafting

works. These interactions include global changes to gene expression, protein activity changes, and even transport of RNA molecules across the graft union. The effect of RNA transport across the graft union has been reviewed (Harada 2010). The connections between the physiology, gene expression, and protein function in these composite plants and how the rootstock modifies each of these is a very intriguing field with many insights in recent years.

Most of the recent reviews on rootstock–scion interactions have been focused on herbaceous dicots, specifically grafting in vegetable production (Table 1). The woody perennial nature of the species focused here presents challenges and opportunities that are different to those encountered in herbaceous dicots (Fig. 1). Comparisons across the two types of composite plant systems are discussed.

Physiological traits

Growth (tree size)

There are several key aspects of scion growth and physiology that are mediated by the rootstock. Though these traits are quite diverse, they are all connected to the composite plant's vigor. While high or low vigor is desired based on the species and location, low vigor typically corresponds to scions on dwarfing rootstocks that are precocious, flowering earlier and in higher numbers, while scions on vigorous rootstocks generally contain larger amounts of vegetative growth with lower production (Table 2).

Total plant size is a major scion trait that is controlled by the rootstock and has been shown in many plant families. In 2004, Santos et al. (2004) examined rootstock mediated dwarfing in sweet cherry and found that trunk cross-

Table 1 Recent reviews

Title	Citation
Stock scion relations	Rogers and Beakbane (1957)
A history of grafting	Mudge et al. (2009)
Vigour mechanisms in dwarfing rootstocks for temperate fruit trees	Webster (2004)
Physiological aspects of rootstock–scion interactions	Martinez-Ballesta et al. (2010)
Hormonal signaling in rootstock–scion interactions	Aloni et al. (2010)
Grafting and RNA transport via phloem tissue in horticultural plants	(Harada (2010))
Grafting for disease resistance	King et al. (2007)

Table 2 Physiological traits

Traits	Vigor/tree size	Yield	Quality	Disease resistance	Physiological disorders	Cold tolerance	Citation
Clementine	X	X	X				Bassal (2009)
Grape	X						Koundouras et al. (2008)
Sweet cherry	X						Santos et al. (2004)
Pistachio	X						Gijon et al. (2010)
Peach	X	X	X			X	Tsipouridis and Thomidis (2005)
Sweet cherry	X	X	X				Whiting et al. (2005)
Sweet cherry	X						Robinson et al. (2006)
Grape	X	X					Soar et al. (2006)
Pear	X	X	X				North and Cook (2008)
Rubber tree		X					Cardinal et al. (2007)
Mango		X					Smith et al. (2003)
Apricot		X	X				Hernández et al. (2010)
Pear		X	X				Bertelsen and Callesen (2001)
Pear		X					Sugar et al. (2005)
Grape			X				(Main et al. 2002)
Grape			X				Koundouras et al. (2009)
Sweet cherry			X				Gonçalves et al. (2006)
Grapefruit			X			X	McCollum et al. (2002)
Tobacco			X				Ruiz et al. (2005)
Kiwi			X		X		Thorp et al. (2007)
Avocado				X			Willingham et al. (2001)
Pear				X			Seemüller et al. (2009)
Apple				X			Jensen et al. (2003)
Annuals				X			King et al. (2007)
Cucurbita				X			Edelstein et al. (1999)
Citrus					X		Ritenour et al. (2004)
Pistachio						X	Epstein et al. (2004)
Pecan						X	Smith et al. (2001)

sectional area (TCSA), final shoot length and final node number were significantly affected by different rootstocks. Similarly, the rootstock demonstrated up to a 2.5-fold variation range on the girth of peach trunks (Tsipouridis and Thomidis 2005). Whiting et al. (2005) examined the growth characteristics of the sweet cherry variety 'Bing' on a standard, a semi-dwarfing, and a dwarfing rootstock. TCSA varied significantly among the rootstocks starting in the fourth year after planting and expanded to almost twofold variation range by the end of the 9th season. Likewise, 7-year-old Heldelfinger sweet cherry on three different rootstocks showed significant differences in TCSA (Robinson et al. 2006).

Grafting of a clementine scion onto several interspecific citrus rootstocks significantly varied tree height, canopy diameter, circumference and tree volume (Bassal 2009). The clementine trees on the most dwarfing rootstock were ∼10 % smaller in each of these measures. Gijon et al.

(2010) showed that pistachio leaf area and leaf and stem dry weights varied based on the rootstock used. In pear, the number of growth points and total length of annual growth varied based on the rootstock used (North and Cook 2008).

Pruning weight and specific leaf area (leaf area per gram of dry mass) are indicators of plant vigor that are controlled by the rootstock in the wine grape scion Cabernet-Sauvignon (Koundouras et al. 2008). Also, the grape variety Shiraz (Syrah) showed significant differences in pruning weight in each of the 2 years tested (Soar et al. 2006).

Yield

The leading agronomic property controlled by the rootstock is to control yield. Yield is controlled by the rootstock in various perennial species including the rubber tree (Cardinal et al. 2007), grape (Soar et al. 2006; Main et al. 2002), peach (Tsipouridis and Thomidis 2005), mango

(Smith et al. 2003), sweet cherry (Whiting et al. 2005; Robinson et al. 2006), pear (Bertelsen and Callesen 2001; North and Cook 2008), clementine (Bassal 2009) and apricot (Hernández et al. 2010). The changes in yield varied up to 6.5-fold (Whiting et al. 2005) depending on the crop and the type of rootstocks examined. The underlying traits included in the overall yield of these perennial species are: precocity, flower number, fruit set and biennial bearing.

Rootstocks can influence the early initiation of the reproductive phase of growth in the scion and such rootstocks causing earlier maturity are termed to be precocious. Precocious rootstocks were observed by Smith et al. (2003) in mango where early production contributed to the 2.4-fold cumulative yield variation of 'Kensington Pride' mango on nine different rootstocks. In pears, Bertelsen and Callesen (2001) identified precocity and biennial bearing effects of the rootstock with the 'Clara Frijs' scion. Higher flower and fruit densities correlated with higher overall yields but with negative correlation for fruit weights. The precocity effects of the rootstocks is also true in apricot. The cumulative yield data support the conclusion that some rootstocks caused earlier production though the yearly production data was not provided to support this conclusion (Hernández et al. (2010).

Whiting et al. (2005) have shown control of productivity and precocity on sweet cherry scions by the rootstock. Robinson et al. (2006) also showed that differences in yield of three sweet cherry scions was controlled by the rootstock but fruit size showed no significant differences. Since fruit size was not determined to be a contributing factor to the differences in yield, fruit number must have been the cause. Whiting (personal communication) later found that floral bud number was linked to the nearly tenfold yield differences found on Bing sweet cherry on multiple rootstocks.

In grapes as examined by Soar et al. (2006), rootstocks producing greater canopy surface area and pruning weight correlated to greater yield. This could be due to pruning of each plant to the same number of buds during winter allowing more vigorous rootstocks to be more productive. Also in grapes, Main et al. (2002) discovered that rootstock control of Chardonel berry weight contributed to changes in cluster weight and eventually yield differences on the different rootstocks. The impact of berry weight on yield in this study was further supported by the observations that the number of clusters per vine was the same for all rootstocks. Likewise, Bassal (2009) showed that clementine fruit weight was unchanged by the rootstock, leaving fruit number to be inferred as the cause for the yield differences.

Control of pear yield on a per tree basis based on the rootstock used was demonstrated by North and Cook (2008). The yield correlated strongly with the number of fruit per tree while fruit weight did not correlate to overall yield. Also in pear, Sugar et al. (2005) identified significant differences in fruit set based on the rootstock though yearly variation was also found to contribute significantly. Yield was not directly reported but presented as yield efficiency based on trunk cross-sectional area which varied greatly by rootstock so the changes in efficiency could be solely due to TCSA differences. Similarly, bloom and crop densities per TCSA were controlled by the rootstock.

In contrast, Russo et al. (2007) demonstrated that the tested apple rootstocks significantly controlled fruit weight and yield though fruit number were not reported. Similar to this result, peach fruit weight and total yield were controlled by the rootstock and fruit number were not shown (Tsipouridis and Thomidis 2005).

In cotton, Dong et al. (2008) showed that the yield was significantly controlled by the rootstock. The rootstock also had significant effects on the boll weight and number of bolls per plant seed cotton per plant which accounts for the changes in yield.

In summary, the aforementioned examples illustrate the role of the rootstock on reproductive traits underlying yield in perennial plants. In combination, it is clear that flowering, fruit set, and fruit quality aspects related to yield are somatically modified by the rootstock. Flowering is known to be controlled by many environmental variables including water and nutrient stress. The interaction of the rootstock on these variables as well as hormone levels could control these reproductive traits and are discussed in detail later in this review. It is also important to consider that yield based assessments are commonly confounded by "typical" pruning and management practices. These may prevent the identification of more productive varieties that may not work well in the typical management system. This is clearly seen in Whiting et al. (2005) work on sweet cherry where the rootstock had a significant effect on yield, but the training system was also quite essential to the productivity of the system.

Quality

Fruit quality is one of the most economically relevant traits which are influenced tremendously by rootstocks in many different species. As the types of products from perennial composite plants vary greatly, so do the associated quality attributes of interest.

Fruit weight was widely noted to be controlled by the rootstocks in many species including peach (Tsipouridis and Thomidis 2005), grape (Main et al. 2002; Koundouras et al. 2009), grapefruit (McCollum et al. 2002), pear (Bertelsen and Callesen 2001; North and Cook 2008; Sugar

et al. 2005), apricot (Hernández et al. 2010), and sweet cherry (Whiting et al. 2005). Contrary to the aforementioned studies, Bassal (2009) found no change to most fruit qualities such as fruit size, weight, juice %, and peel thickness of clementine. Instead it was found that acidity was strongly controlled by the rootstock while firmness and ascorbic acid content showed differences, though the environment exerted greater control than the rootstock.

Likewise, the acidity of grapefruit, along with the brix were reported to be regulated by the rootstock in 'Marsh' by McCollum et al. (2002). Brix, pH, and titratable acidity were also found to be significantly effected in rootstock trials of the grape variety Chardonel fruit and the resulting wine (Main et al. 2002). Koundouras et al. (2009) found no differences in skin anthocyanins, flavan-3-ol monomers, or phenol-free glycosyl-glucose (PFGG) in Cabernet-Sauvignon tested on different rootstocks. Further, Tsipouridis and Thomidis (2005) found total acids along with soluble solids and flesh firmness in peach fruit to be rootstock influenced traits.

Fruit firmness and color of sweet cherry fruits were reported to be primarily controlled by the scion though rootstock did have a significant effect in the study by Gonçalves et al. (2006). However, Whiting et al. (2005) identified a significant effect of the rootstock on fruit size. Flesh color, soluble solid concentration and flesh firmness were also identified as rootstock controlled in kiwifruit by Thorp et al. (2007). In apricot, Hernández et al. (2010) found flesh firmness, fruit shape, pulp thickness, stone weight, percent pulp, soluble solids, and fruit color were all rootstock effected though only 1 year of data were presented leading to the possibility that these traits may be highly environmentally regulated as seen in studies on other crops. Similar to these results, North and Cook (2008) identified a significant role of the rootstock on fruit firmness, background color, and total soluble solids of pears. Also, Sugar et al. (2005) showed russeting, a physiological alteration of the pear epidermis that becomes corky, was effected by the pear rootstock.

A much more drastic example of quality modification by the rootstock is presented in tobacco grafted on tomato where the production of nicotine is nearly eliminated (127 µg/g dry weight) compared to self-grafted tobacco plants (6,720 µg/g dry weight) (Ruiz et al. 2005). This suggests that the amount of control of scion qualities documented to date in perennials may by a small fraction of the potential impact rootstocks may have on these traits.

Interestingly, in majority of the studies on fruit quality characteristics reviewed above, fruits were tested based on same date of harvest. This practice ignores the likely possibility that the rootstock may affect maturation and, fruits at different maturity would display significant quality differences. For better utility of these studies, it may be desirable to assess quality traits with fruit harvested based on maturity rather than the same date of harvest.

Disease resistance

The ability to control disease is one of the primary historical advantages of rootstocks. The vast majority of the disease resistance, however, is based on soil-borne diseases that infect the roots. These diseases can kill the plants before they come into production or cause significant damage and yield reductions. While resistance and tolerance to these diseases is critical, it does not depend on the interplay between the rootstock and scion and are therefore not covered in detail in this review. This includes the reported fire blight resistance in apple (Russo et al. 2007) where the resistance prevents the rootstock from dying but does not aid the scion's resistance, and pear decline (Seemüller et al. 2009) where the rootstock genotypes varied in amount of symptoms but did not prevent scions from obtaining the causal bacteria. Still, there are a couple of examples where the rootstock influences the disease characteristics of the scion.

While Russo et al. (2007) were unable to show a rootstock effect on fireblight resistance, Jensen et al. (2003) report a difference in 'Gala' apples on several rootstocks. The difference is noticed only when the canker length is expressed as a percentage of the shoot length. This study represents dwarfing rootstocks as more susceptible on a percent of shoot length basis while the mean canker lengths are not significantly different among any of the rootstock scion combinations. The distribution of canker length, however, does support the conclusion that there is a difference among the rootstocks ability to control fireblight though more extensive examination will need to be completed to fully assess this trait.

A second example is anthracnose development in avocado. Anthracnose is a serious post-harvest fungal disease in avocado that leads to rotting of the fruit. Willingham et al. (2001) tested 'Hass' scion on two rootstocks for their influence on anthracnose. After post-harvest ripening at 22 °C, fruit from 3.5- and 8-year-old trees demonstrated significant differences in both disease incidence and severity. Most importantly, the ~30 % decrease in disease incidence and severity on one rootstock corresponded to about 50 % more acceptable fruit. To understand the mechanism for this resistance, the authors examined diene concentrations in the same trees and fruits since several dienes had previously been linked to fungal disease resistance. A significantly higher amount of dienes was found in the leaves of trees on the more resistant rootstock suggesting a role of the dienes in fruit resistance to anthracnose infection of avocado. Leaf nutrient levels were also

examined and showed significant differences thereby suggesting that scion nutrition may be the trait indirectly contributing to anthracnose resistance.

Vegetable grafting for disease has also been recently reviewed by King et al. (2007) though, as seen in perennials, there are few examples of non-soil-borne, scion diseases. Most of the resistance to these non-soil-borne, non-root-related diseases was explained simply as an effect of vigor allowing the plants to sustain production. The contrary example mentioned in the review by King et al. (2007) is a study reported by Edelstein et al. (1999) that showed spider mite resistance was imparted to *Cucurbita* sp. scions from the rootstock.

in summary, these are several examples of the rootstock controlling scion diseases though they are not near as prevalent as would be expected based on the role of grafting to control root diseases. The total effect of rootstocks on scion and fruit diseases may be larger than currently documented.

Physiological disorders

In addition to controlling diseases, physiological disorders of the fruit borne by the scion are influenced by the rootstock. Because these disorders are not caused by an external organism, they are reviewed here separately.

Ritenour et al. (2004) examined stem-end rind breakdown (SERB) in citrus where the fruit develops a very narrow ring of unaffected tissue immediately around the stem surrounded by a dark, irregularly-shaped region of collapsing peel tissue, a condition known to be influenced by both pre- and post-harvest conditions. For 'Valencia' oranges in 2001, Ritenour et al. (2004) found SERB ranged from 56 to 21 % of fruit after 41 days at 70 °F (21 °C) and 54–26 % after 101 days at 38 °F (3 °C). In 2002, SERB was far less severe but still displayed significant differences with the same rootstock being worst in both years. Rootstock was also shown to affect SERB in navel oranges. 'Ray Ruby' grapefruit, however, showed no significant SERB while a rootstock effect on post-harvest decay was shown. 'Oroblanco', a hybrid of pummel and grapefruit, displayed similar results to 'Ray Ruby' with SERB being insignificant and the rootstock having a large impact on post-harvest decay.

In kiwifruit, differences in physiological pitting, sun damage, and stem-end browning were observed on a single scion variety on eight different rootstocks (Thorp et al. 2007). Thorp et al. (2007) continued to test these differences which ranged from 76.6 % for sun damage to 34.5 % for stem-end browning but were unable to find a significant factor. Physiological pitting, however, ranged up to 45 % based on rootstock and was negatively correlated to Mg concentrations though it is unclear if Mg levels significantly differed among rootstocks.

As alluded to by Thorp et al. (2007), physiological disorders may results from any nutrient differences caused by the rootstocks. Additionally, these disorders could both be drastically controlled by the physiological characteristics of the fruit which, as mentioned in fruit quality section, can be modulated by the rootstock. These physiological disorders may be caused by abiotic stresses, such as water shortage and nutrition, which are covered in detail in the physical mechanisms section.

Cold tolerance

Cold hardiness is an economically vital agronomic trait. Cold hardy rootstocks have enabled production of many perennial crops in severe climates and rootstock cold hardiness is a critical evaluation trait for rootstock selection. While it is clear that different genotypes have different cold hardiness attributes, being able to impart differential cold hardiness on the scion is of particular interest.

Tsipouridis and Thomidis (2005) found that the rootstocks effected the frost resistance of 'May Crest' peach ranging from 0 to 55 % of flowers that were damaged from frost. Similarly in Pecan, Smith et al. (2001) found that trees on some rootstocks were more susceptible to early frost damage than others. Data were pooled for all cultivars on the rootstocks making the rootstock scion interaction unrecognizable, possibly diluting the effect of the rootstock on more susceptible varieties. The differences ranged from 0 to 9 % of trees on a given rootstock that were expected to die due to the extensive damage resulting from the early frost. In pistachio, Epstein et al. (2004) found that frost killed 41 % of pistachio trees with 72 % of the surviving trees being damaged while another rootstock showed zero death and only 4 % of trees were damaged. Chilling injury was also markedly controlled by rootstock for the grapefruit variety 'Marsh' (McCollum et al. 2002).

These chilling injury studies are the result of actual environmental anomalies making them nearly impossible to reproduce. Controlled growth rooms and greenhouses, however, could be used to simulate these types of rapid temperature changes at distinct stages of growth to determine if these effects are maturity based susceptibility. Care would need to be taken to modify the air temperature without significantly changing the soil temperature below the surface to more fully replicate the environmental shock the perennial composite plants experience in the field.

Summary

These results show the widespread effect of rootstocks on scion physiology. While these traits were presented

individually, they are very integrated and changes in one are likely to alter others. This is most obvious when considering fruit number and weight where excess crop load leads to decreased fruit size. Additionally, reductions in plant vigor are associated with decreased growth and increased reproductive characteristics. Rootstock-controlled plant vigor may be related to resistance to disease (King et al. 2007) and physiological disorders of perennial scions. The interactions of these traits, however, are also likely modulated through one or more of following physical mechanisms.

Physical mechanisms

As has been clearly established, there are many traits in perennial species scions that are controlled by the rootstock they are grown on. To understand how the rootstock causes these effects, many experiments have been conducted examining multiple facets that can control plant growth and physiology (Table 3). These range from microscopic analyses of the graft unions to testing nutrient and water passage to the scion. The following subsections detail research on the mechanisms for the rootstock to control scion physiology in the areas of: graft union repair, water usage and transport, hormones, and nutrition.

Graft union repair

In herbaceous plants, the formation and function of the graft union has become a point of interest. The healing and formation of the graft union was also described in *Arabidopsis* to have four stages by Flaishman et al. (2008). Recently, Yin et al. (2012) expanded this to six major steps: (1) Wound-induced response, (2) Cell debris clean, (3) graft union cellular communication, (4) auxin accumulation and responses, (5) graft union cell division and differentiation, (6) vascular reconnection. Yin et al. (2012) also identified that mRNA signals are changed in the graft union within 1 day after grafting. By the second day after grafting, auxins had stimulated cell division and differentiation. On the third day after grafting, transport was already functioning across the graft union.

As with herbaceous composite plants, the development of a functioning graft union in perennial crops is a critical stage during the production of a composite plant. Many studies to document and understand the development of the graft union in perennials have been completed including grafting of woody tissues as well as micro-grafting of herbaceous tissues of these plant species.

Examining the development of the graft union in olive through hydraulic resistivity and hydraulic conductance experiments, Gasco et al. (2007) showed a much longer period of healing is required for woody perennial plants. Gasco et al. (2007) measured hydraulic resistance in the roots, across the graft union, and in the shoot to determine the percentage that each comprised of the total plant hydraulic resistance. At day 30, the graft union of the heterografts accounted for roughly 85 % of the total resistivity. By day 90, these had dropped to around half of the total hydraulic resistance. Measurements at 360 days after grafting showed that the graft union was a minor part of the total resistance and continued to drop to about 3 % of the total by the 480th day after grafting. In addition, there were no distinguishable differences in graft union hydraulic resistance when comparing the reciprocal grafts of a dwarfing and vigorous variety. These data provide evidence that in olive, and probably most perennials, the timing of graft union examination and hydraulic studies needs to be carefully considered when interpreting the results. This is because in tests soon after grafting the graft union will have a much larger influence than later in the growth of these plants. Also, since the graft union resistivity ended up decreasing in impact to be nearly negligible, the graft union hydraulic properties do not explain sustained dwarfing. This conflicts with the data from herbaceous composite plants where grafts were functional days after grafting. This discrepancy could result from graft union healing effecting the growth of the plant during that year while Gasco et al. (2007) were able to isolate graft healing from the hydraulic measurements over a year after the grafting stress. A report of micro-grafting in peach by Zhang et al. (2012), however, shows that the graft union is functionally transporting RNAs within 4–10 days of grafting suggesting that the stage and type of grafting used can significantly modify the graft union healing process.

In contrast, Atkinson et al. (2003) examined an apple scion on three rootstocks 1 year after grafting for their root and stem hydraulic conductivity. Analysis of percentage functional xylem revealed that the graft tissue and scion stem vasculature varied based on the rootstock. The graft tissue conductivity increased very strongly with vigor and suggested that the graft union vasculature is a critical part of vigor control by the rootstock. However, since measurements were not completed to demonstrate that the graft union had fully healed at the time of the experiment, these results could arise if the graft union was still unhealed.

To understand early vascular development in the grafts in sweet cherry, xylem vessel anatomy was probed in combinations of the Rainier scions and non-dwarfing, semi-dwarfing, and dwarfing rootstocks 6 months after bud grafting (Olmstead et al. 2006a). Through self-grafts of the rootstocks and the Rainier/rootstock heterografts, these experiments clearly demonstrated that graft union xylem vessel number increased and lumen volume decreased with increasing dwarfing nature of the rootstock. Changes in the

Table 3 Physical mechanisms

Species	Graft union repair	Water usage and transport	Hormones	Nutrition	Citation
Arabidopsis	X				Flaishman et al. (2008)
Arabidopsis	X				Yin et al. (2012)
Olive	X	X			Gasco et al. (2007)
Peach	X				Zhang et al. (2012)
Sweet cherry	X				(Olmstead et al. (2006a)
Sweet cherry	X				Olmstead et al. (2006b)
Peach	X				Tombesi et al. (2010)
Peach		X			Basile et al. (2003)
Apple		X			Atkinson et al. (2003)
Kiwi		X			Clearwater et al. (2007)
Grape		X			Koundouras et al. (2008)
Olives		X			Nardini et al. (2006)
Peach		X			Solari et al. (2006)
Peach		X		X	Solari and DeJong (2006)
Review			X		Aloni et al. (2010)
Apple			X		Kamboj et al. (1999a)
Apple			X		Kamboj et al. (1999b)
Peach			X		Sorce et al. (2002)
Grape			X		Soar et al. (2006)
Apple			X		Tworkoski and Miller (2007)
Apple			X		Van Hooijdonk et al. (2010)
Apple			X		Li et al. (2012)
Cotton			X		Dong et al. (2008)
Peach				X	Tsipouridis and Thomidis (2005)
Apple				X	Fallahi et al. (2001)
Mango				X	Schmutz and Ludders (1999)
Lemon				X	Almansa et al. (2002)
Orange				X	Papadakis et al. (2004)
Avocado				X	Mickelbart and Arpaia (2002)
Pear				X	Okubo et al. (2000)
Tomato				X	Martinez-Rodriguez et al. (2008)
Sweet cherry				X	Gonçalves et al. (2006)
Pear				X	North and Cook (2008)

scion tissue were also discovered though they are much less drastic than the graft union differences. The amount of wounding induced callus at the graft union was also found to differ among the combinations. Olmstead et al. (2006a) continued to note that the formation of xylem rays in the graft union callus tissue was abnormal, forming non-linearly, thereby reducing the continuity of xylem across the graft union.

Using intact 2-year-old nursery budded trees extracted from the field, dye uptake and transport was correlated with decreased vigor studies of the sweet cherry scion Lapins on a non-dwarfing and dwarfing rootstock (Olmstead et al. 2006b). The dye showed a decreased flow into the graft union and was reduced further in the scion just above the graft union in plants grafted on the dwarfing rootstock

compared to those grafted on vigorous rootstock which was associated to xylem vessel size as seen in their work on Rainier grafting combinations.

Tombesi et al. (2010) also examined xylem characteristics in composite plants using peach as their model including vigorous, semi-dwarfing and dwarfing rootstocks. Xylem was analyzed above and below the graft union(s) of 6- and 7-year-old trees and demonstrated that higher rootstock xylem conductance correlated with higher vigor. Additionally, the rootstock genotype did not significantly modify the xylem characteristics of the scion and the vigorous/dwarfing/scion combination displayed an intermediate phenotype. Tombesi et al. (2010) data lead to the conclusion that xylem restriction in the root, stem, or scion may have resulted in a dwarf phenotype in peach.

Several of the studies above examined the repair of the graft union directly through dye uptake and microscopic examination while the others utilized the resistance to water transport across the union. It appears that the state of the tissue when the graft is performed, being herbaceous or woody, may have a significant effect on the rate of graft union healing and subsequent effects on the growth and physiology of the composite plants. Though they are separate sections in this review, the connections between water usage, hydraulic conductivity and healing of the graft union are nearly impossible to separate.

Water usage and transport

Water usage is commonly examined through the measurement of water potential, hydraulic conductivity, or hydraulic resistivity in the grafted plants. Intuitively, increases in hydraulic resistance lead to decreases in hydraulic conductivity which, when severe, can display as drought-like symptoms.

Peach tree daily growth and stem water potential were examined by Basile et al. (2003). The stem relative extension rate was found to be significantly different for many time points during many of the days examined. Stem water potential showed more time points with significant differences and followed the general trend of decreasing as relative extension rate was increasing and vice versa. Additionally, a strong correlation of daily relative extension rate was shown with the mean air temperature.

Clearwater et al. (2007) examined kiwifruit rootstocks of different vigor for their water pressure in various parts of the plants. Through these experiments, Clearwater et al. (2007) identified that spring root pressure increases were rootstock dependent and a delay in increasing pressure was correlated to reductions in scion vigor. Root pressures also correlated to scion leaf pressures suggesting that these vigor reducing rootstocks of kiwifruit acted by causing water deficit stress in the scion. Koundouras et al. (2008) compared the water potential of the grape varietal Cabernet-Sauvignon on two different rootstocks and identified a significant effect of the rootstock in non-irrigated conditions but not with deficit or full irrigation. The supply of deficit or full irrigation reducing the significance of the rootstock on water pressure suggests an important role for grape rootstocks in drought or low water conditions.

In olives, Nardini et al. (2006) tested reciprocally grafted and ungrafted dwarfing and vigorous scions. Using the dwarf as a rootstock lead to a reduction of leaf surface area by about half and correlated with the 2.5-fold decrease in hydraulic conductance of the dwarfing root system. Additionally, they identified that the root system accounted for 60–70 % of total plant hydraulic resistance while the graft union had a negligible effect on hydraulic resistance. This finding is congruent with the graft union healing presented in the previous subsection since the olive plants were tested at 360 and 450 days after grafting, when the graft union of olive is nearly and fully functional, respectively, according to Gasco et al. (2007).

Solari et al. (2006) showed that hydraulic conductance in the rootstock, scion and graft union of peach trees were all controlled significantly by the rootstock with the larger conductance on the larger trees. The graft union, however, only account for 10 % of the overall hydraulic resistance on each 1 year post grafting peach tree, also consistent with the results in olives by Nardini et al. (2006) and Gasco et al. (2007).

In a similar publication on water relations of rootstock–scion interactions, Solari and DeJong (2006) used exogenously increased root hydraulic pressure on composite peach trees. By pressurizing the roots, they were able to demonstrate increases in growth rate, leaf transpiration and conductance, and net CO_2 exchange of peach on both dwarfing and vigorous rootstocks. In both rootstock types, increases in pressure resulted in the mentioned measures in a very linear fashion. Additionally, by recording the relative shoot extension rate during root pressurization, the increased root pressure caused a short-term stimulation of growth that was nearly 500-fold increased within minutes which the authors partially attributed to the elastic expansion of cells. This response then decreased to a new steady state within 15 min after the pressure treatment started and the steady state rate of elongation for the pressurized plants was higher than that of non-pressurized control plants. These results clearly demonstrate that root hydraulic pressure is a significant controlling factor of scion vigor in peach.

Since observations consistent with this finding have been present in many of the perennial species employing grafting, it is likely that root hydraulic pressure is a major player in vigor control of each of these species. Similar experiments testing the hormone and nutrient concentrations of the increased hydraulic flow would be interesting to identify if there is a change in absolute amounts being delivered to the apical meristems for growth during the pressure events.

Hormones

Understanding the hormonal relationships among the rootstock and scion are critical to develop a concept of how these genotypes interact. A recent review of hormonal relationships of grafted crops discussed in detail the effect of exogenous auxins and cytokinins on the establishment of the graft union vasculature (Aloni et al. 2010). Auxins, cytokinins, ethylene, reactive oxygen species, and abscisic acid (ABA) were also discussed with regard to graft

incompatibility, senescence, and salt stress. Aloni et al. (2010) continued to dissect the role of hormones on many of the physiological traits discussed previously in this review from the perspective of herbaceous composite plants.

Hormone examinations have also been reported in perennial grafted plants. Kamboj et al. (1999a) analyzed grafted and ungrafted apple cultivar 'Fiesta' shoot xylem sap for amounts of zeatin and zeatin riboside, the two primary cytokinins in apple xylem. In the ungrafted root-stock varieties, xylem sap cytokinin levels did show a correlation with vigor; however, the role of the scion in the interaction muted these differences to where statistical significance was not found. Additionally, the more vigorous rootstock contained a higher proportion of zeatin riboside than zeatin and the opposite was true for the two dwarfing rootstocks though the authors state that this observation could be a result of differential timing of growth between the rootstocks. Using a similar design, Kamboj et al. (1999b) analyzed ABA and the auxin indole acetic acid (IAA) in the xylem sap of non-grafted root-stocks as well finding small correlations but also lacking statistical significance. These correlations were that mean ABA concentrations decreased with increasing vigor of the rootstocks. The differences in IAA were not significant among the tested population of rootstocks. While these results are primarily within non-grafted rootstocks, they demonstrate differences in the natural state of the rootstock genotypes on their hormone production.

Scion levels of zeatin riboside and IAA amounts were significantly controlled by the rootstock when Sorce et al. (2002) examined the hormone interactions of the peach variety 'Armking' on three interspecific rootstocks. Vigor of the plants was positively correlated to zeatin riboside concentrations and negatively with IAA concentrations. Due to the examination of ungrafted control rootstocks, the authors were able to note that IAA and zeatin riboside were balanced in the ungrafted plants. That balance was not seen in the grafted plants suggesting the hormonal conversation between the rootstock and scion was altered which could explain the changes in vigor.

Soar et al. (2006) examined ABA levels to correlate ABA with differential drought responses of the wine grape Shiraz (Syrah) on seven different rootstocks. The sap levels of ABA did not provide significant evidence that xylem transported ABA was controlling the different drought characteristics imparted by the tested rootstocks. Although there was a negative correlation of ABA concentrations with stomatal conductance, the correlation varied greatly between the 2 years. While ABA levels did not completely explain the differences, the authors recommended that sap screening of ABA concentrations may be a rapid screen to assess relative drought tolerance of rootstocks.

Additionally, Soar et al. (2006) state that differences in water stress may have produced the changes in ABA rather than ABA causing the observed changes in water stress.

Several studies in apple have been reported to examine the hormonal landscape produced by different rootstocks. Tworkoski and Miller (2007) showed that increased auxin to cytokinin ratios corresponded to decreased bud break in removed branches from mature trees. Similarly, Van Hooijdonk et al. (2010) showed that exogenous GA_{4+7} had an equilibrating effect on final node number of stems of apple scions on dwarfing and vigorous rootstocks during the first 7 months after grafting. On 1-year-old apple trees, Li et al. (2012) found that increased zeatin in leaves and roots of scions correlated with increased vigor. IAA content was decreased in the scions by the dwarfing rootstock with significant differences found in leaves, branch bark, and the roots. The graft union, however, was found to have no obvious influence on hormone transport as the IAA interaction was within one genotype and not the graft union.

Dong et al. (2008) described the effects of self and reciprocal grafting on early and late senescence lines of cotton. Higher ABA concentrations were found in plants on the early senescing rootstock and their chlorophyll content and net photosynthesis decreased. Concentrations of the cytokinins zeatin + zeatin riboside, dihydrozeatin + dihydrozeatin riboside, and isopentenyl + isopentenyl adenine were consistently higher in the leaves of plants on the late senescing rootstock. Overall, this work shows that both ABA and cytokinins are controlled by root genotype and both control senescence.

The role of the rootstock in hormone manipulations in the scions has been shown for auxins, cytokinins, and ABA directly and GA_{4+7} indirectly. The crosstalk between auxins and cytokinins seem to control scion vigor with auxins being negative regulators and cytokinins contributing positively. Interestingly, differences in the type of cytokinin, specifically the ratios zeatin versus zeatin riboside (Kamboj et al. 1999a), were implicated to control vigor though the absolute amounts of each were not reported so it may be a change in one that significantly disturbed the reported ratios. Additionally, higher cytokinins were correlated to decreased senescence which could be a mechanism for explaining how high cytokinins can increase overall growth. The role of ABA inducing senescence is consistent with previous examinations of this hormone. The origin of ABA is likely to be from the root as Dong et al. (2008) showed export of ABA from the roots through the xylem sap and is consistent with the recommendation of Soar et al. (2006) to test rootstocks for xylem ABA levels as a preliminary drought tolerance screen. Combining the information from Dong et al. (2008) and Kamboj et al. (1999b) showed that ABA was negatively

correlated to vigor which could result from earlier senescence. ABA caused decreases in stomatal conductance which yields decreased photosynthesis and may promote the onset of senescence. The interplay of all of these hormones in rootstock–scion interactions needs to be examined more fully. By measuring the levels of these hormones in multiple graft combinations across the developmental time course, these interactions may be more fully revealed enabling more concrete assertions to their roles in rootstock-scion interactions to be made.

Nutrition

Since the roots are responsible for absorbing vital minerals and nutrient for the entire plant, it is expected that the rootstock would have a significant contribution to the nutritional status of the composite plant. Here, the results of analyses of scion mineral content, salt stress tolerance, and photosynthesis are reviewed.

Scion mineral composition

'May Crest' peach was grown on 14 different rootstocks and the leaf mineral composition was analyzed. P, Ca, Mg, Mn, Cu, and Fe all showed 2- 6-fold differences in the % composition (Tsipouridis and Thomidis 2005). North and Cook (2008) found that percentage of dry mass of N, P, and Mg in pear leaves was rootstock-controlled, whereas K and Ca did not change. Similarly, Fallahi et al. (2001) examined leaf mineral content on a percent dry weight bases of bc-2 Fuji apples on three rootstocks. Ca, Mg, K, Fe, Zn, Cu, and Mn varied by year and N concentrations were only significant based on rootstock for one of the 2 years of the experiment. Since each of these tests based the amount of each mineral on the total % composition, a significant change in one ion may have influenced the total percentages of all of them. The authors also noted differences in fruit production during the sampling year which could have significantly contributed to the differences in leaf nutrient levels.

Cl, K, Ca, and Mg were all found to accumulate at different rates in leaves of mango when two rootstocks were reciprocally grafted by Schmutz and Ludders (1999) while Na assimilation was not affected by the rootstock genotype. Almansa et al. (2002) also examined the contribution of the rootstock to accumulation of minerals in lemon leaves during salt stress finding that the absolute amounts of K, Na, Fe, Mn, Cu, and Zn did not change due to the rootstock. Meanwhile the Cl levels were changed significantly by the rootstock, especially in the high salt treatment, which correlates with Schmutz and Ludder's findings suggesting that chloride and not sodium may be the toxic element of salt stress.

Papadakis et al. (2004) examined the effect of two rootstocks on 'Naveline' orange and their responses to high boron concentrations. They found that the rootstocks absorbed the boron at different rates which lead to the differences in scion boron concentration and subsequent boron toxicity levels. The effects of the rootstock were most apparent on the higher concentration of boron though the resulting physiological changes were not significant between the rootstocks.

Salt stress

Schmutz and Ludders (1999) showed that the rootstock genotype was significant for the salt tolerance phenotypes of mango. By reciprocal grafting of two rootstocks and applied salt stress, reductions in total growth, CO_2 assimilation, and transpiration were identified. Mickelbart and Arpaia (2002) tested three avocado rootstocks in combination with salt stress to examine the effects on the 'Hass' avocado scion. The rootstocks had significant effects on the amount of Na^+ and Cl^- ions in the leaves which were most extreme at the highest tested level of salinity. The amount of accumulated ions also correlated with decreases in shoot extension and individual leaf area which were rootstock related traits in this experiment. Additionally, leaf necrosis occurred at the highest level of salinity though primarily in the rootstock that accumulated the highest amount of ions. Chlorophyll content also decreased with increases in NaCl accumulation demonstrating an interaction of salt tolerance with photosynthetic capacity.

Examination of Chinese, Japanese, and European pears on two Asian pear rootstocks allowed Okubo et al. (2000) to show that the rootstock had large differences in salt tolerance. Fresh weight, scion defoliation and mortality each had significant differences based on the rootstock. The differences in the traits being tested were even more significant at higher salt concentrations. Some of the scions also clearly showed differences in chloride concentrations and smaller changes in sodium concentrations. These results correspond well with Martinez-Rodriguez et al. (2008) which showed that leaf sap Na concentrations were significantly different based on the tomato rootstocks.

Photosynthesis

In addition to leaf mineral composition, Fallahi et al. (2001) found that net photosynthesis was slightly affected by the rootstocks on bc-2 Fuji apple and was not correlated to overall vigor. Stomatal conductance, intercellular CO_2 concentration, transpiration, and leaf area were all significantly controlled by the rootstock though yearly variation was also apparent for transpiration and leaf area. Averaging measurements for three sweet cherry varieties, net CO_2

assimilation, stomatal conductance, and intracellular CO_2 concentration were lower on dwarfing rootstocks than on semi-dwarfing and vigorous rootstocks (Gonçalves et al. 2006). Conversely, intrinsic water use efficiency was higher in these dwarfing rootstocks compared to the others.

Summary

As seen in this section, the physical mechanisms also interact and overlap significantly. Hydraulic pressure is expected to modulate the amount of hormones and nutrients moved into the scion. This will be affected by the graft union during the healing process. Nutrient levels, water content and hormones also control the photosynthetic capacity and subsequently the growth rate of the composite plant. Though these mechanisms can explain a significant amount of the rootstock-controlled traits, little is understood for how these mechanisms are developed and controlled by the rootstock at the molecular level which forms the basis for the final section of this review.

Molecular mechanisms

The previously discussed studies have revealed many of the physiological aspects that control rootstock–scion interactions including several mechanisms with very strong supporting evidence. It is critical to learn what genetic differences underlie those physical mechanisms and how those physical mechanisms further manipulate the gene expression in the scion and ultimately alter the traits in a grafted scion. Recently, biochemical analyses on protein functions and activity have shed some light on a couple of important pathways in composite plant growth and development. In the past decade, there have been drastic improvements to molecular techniques improving the ease of use, ease of analysis, throughput, and overall costs of gene expression experiments. These advances have moved expression studies from cDNA–AFLP and differential display to microarrays and now to RNA sequencing. Through expression analyses of composite perennial plants, genes putatively controlling plant physiology have been revealed. The following section details molecular analyses to understand the somatogenetic interactions of the rootstock and scion in perennial composite plants (Table 4).

Biochemical analysis

Examining lemon tree leaves on different rootstocks, Almansa et al. (2002) showed that the superoxide dismutase (SOD) activity in the leaves was rootstock-controlled, and varied more by rootstock than the salt levels tested. Another protein activity study was reported by Agbaria et al. (1998) on grafted rose plants. Two scion varieties were grown reciprocally grafted and one interspecific rootstock was also used for both varieties. The activities of glutamine synthetase and nitrate reductase in the leaves were significantly modulated by the rootstock though total nitrate levels did not differ significantly because of the rootstock. As discussed in the disease section, increased production of dienes by Hass avocado led to decreased anthracnose infection of the avocado fruit demonstrating an ability of the rootstock to the control biochemical composition of a scion and its fruit (Willingham et al. 2001).

Expression analysis

One way the rootstock can control many aspects of scion growth including protein activity is through the modulation of scion gene expression. As mentioned in the hormone section, Li et al. (2012) found significant differences in kinetin in 2-year-old scions. Additionally, they found that root expression of IPT3, an important gene in cytokinin

Table 4 Molecular analyses

Species	Biochemical analysis	Expression analysis	Graft union transport	Citation
Lemon	X			Almansa et al. (2002)
Rose	X			Agbaria et al. (1998)
Apple		X		Jensen et al. (2003)
Sweet cherry		X		Prassinos et al. (2009)
Apple		X		Jensen et al. (2010)
Apple			X	Kanehira et al. (2010)
Apple			X	Xu et al. (2010)
Pear			X	Zhang et al. (2012)
Review			X	Harada (2010)
Tobacco			X	Kasai et al. (2011)
Cucurbit			X	Golecki et al. (1998)
Tobacco			X	Stegemann and Bock (2009)

synthesis, correlated with increased cytokinin levels and vigor. Separately, expression of *PIN1*, a gene known to be involved in polar auxin transport in model plants, was far decreased in the trees with a dwarfing genotype used as an interstock. This led to the hypothesis that basipetal IAA transport is significantly reduced in the dwarfing genotype leading to whole plant hormonal alterations and potentially causes the dwarfing effect.

Global gene expression patterns of 3-year-old 'Gala' apple scions on dwarfing and semi-dwarfing rootstocks were investigated by Jensen et al. (2003). Their cDNA–AFLP approach to examine shoot tip gene expression identified 43 bands up-regulated on the semi-dwarfing rootstock correlating to 36 unique genes and 95 bands up-regulated on the dwarfing rootstock relating to 56 unique genes. Of the 92 genes, ontologies from 26 % were transcription/translation related and 31 % had no homology or no known function. Many of the identified genes showed potential roles consistent with physiological differences in photosynthesis, tree size, stress tolerance, and flowering. Jensen et al. (2003) discussed many of the identified genes and there potential roles including a gene with similarity to the *Arabidopsis* BAK1 [brassinosteroid insensitive I (BRI)-associated protein kinase gene] which was up-regulated in the dwarfing rootstock. Additionally, a sorbitol-6-phosphate dehydrogenase (SDH) was up-regulated in the scion on the dwarfing rootstock. Several identified gene expression differences were confirmed with qRT-PCR; however, no functional gene analysis was performed.

The mechanism of rootstock-induced dwarfing in sweet cherry was examined by Prassinos et al. (2009) using 'Bing' on dwarfing and semi-dwarfing. Their results show that the dwarfing rootstock caused shoot elongation of 1- and 2-year-old scions to stop earlier than that of scions on the semi-dwarfing rootstock. As a result of earlier growth cessation, the number of nodes and total length of the branches were decreased in the dwarfing rootstock as was trunk cross-section area. Prassinos et al. (2009) then used cDNA–AFLP and microarray approaches to examine the gene expression in multiple tissues from the 1-year-old scions. RNA was extracted below the graft union, at the graft union, and above the graft union before, during and after the differences in growth cessation. From the cDNA–AFLP experiment, 49 and 136 transcript derived fragments (TDFs) were differentially expressed in the graft union or scion stem between scions on different rootstocks. When examined further, most of the genes these fragments were regulatory proteins with unknown roles. The microarray analysis identified that 99 of the 1,040 TDFs were differentially expressed in the shoot and 56 more in the graft union. These included many transcription factors. Up-regulation of BAKI in the dwarf combination was found which is consistent with the results in apple on dwarfing

rootstocks reported by Jensen et al. (2003) suggesting a possible role of brassinosteroid signaling in the dwarfing control of scions.

Following up on their earlier cDNA-AFLP work on rootstock-related gene expression, Jensen et al. (2010) leveraged microarrays to examine expression of 55,230 apple transcripts with the 'Gala' scions on seven different rootstocks. RNA from actively growing shoots tips of young and mature plants were examined via microarray and the results identified 116 transcripts that were correlated to tree size. In this experiment, sorbitol dehydrogenases were expressed at higher levels in the larger trees as expected since apples utilize sorbitol as a transport molecule rather than sucrose as is seen in many other species. While the expression of SDH explained 34 % of the tree size differences, these results contrast with the 2003 results where higher expression of SDH was linked to the dwarfing rootstock (Jensen et al. 2003). This suggests possible differences in the experimental design that may have changed the way these plants responded, especially if the samples for RNA analysis were taken at different stages of growth between the two experiments which is unclear. Additionally, one-third of the putative SDH transcripts in apple were similarly expressed though they did not meet the filtering criteria (Jensen et al. 2010). This suggests that it may be allelic expression differences that led to the up-regulation of one form seen from the dwarfing rootstock in the 2003 experiment.

Graft union transport

RNA

Using tissue culture grown and grafted apples, Kanehira et al. (2010) sequenced cDNAs from the phloem cells. Analyzing these phloem cell cDNAs from non-grafted plants, they identified multiple sequences that were known to be expressed in phloem especially metallothionin-like genes. In addition, an ABA biosynthetic gene and an auxin/aluminum responsive protein were captured in the phloem. In vitro grafting, or micro-grafting, of the apple scion 'Fuji' onto an interspecific rootstock allowed the expression of gibberellin acid insensitive *(gai)*, a gene whose mRNA is known to be phloem transported. Due to sequence differences between the two grafted species, a rootstock specific allele was tested through nested RT-PCR. The results clearly show that the RNA from the rootstock allele is being transported across the graft union and into the phloem of the scion which was confirmed via in situ hybridization.

Likewise, Xu et al. (2010) inspected *gai* mRNA expression in the roots and shoots of interspecific grafts of tissue cultured apple. As with Kanehira et al. (2010), the

rootstock form of *gai* was found in the shoots. Additionally, the scion form of *gai* was identified in the roots. The transfer of these mRNAs was also seen after the fourth day after grafting while the mRNAs were not found in the xylem even at the 35th day after grafting.

Furthermore, Zhang et al. (2012) used the same approach to perform experiments in Chinese pear micrografted onto a wild pear rootstock. Their results are exactly as seen in apple with the mRNA of *gai* able to transfer across the graft union by the fourth day after grafting and no presence identified in the xylem. Additionally, they examined 2-year-old grafted trees finding the transport of the rootstock *gai* mRNA up to 40 cm above the graft union. From the data provided, it appears that the 2-year-old trees were grafted conventionally and not micro-grafted which would lead to an increased period of healing prior to transport of mRNAs across the graft union. It would be interesting to examine older trees and perform the same experiment to identify if there is a limit to the distance the mRNAs from the rootstock can travel in the scion or if this was an artifact of the slowly healing graft union in woody grafts. Leveraging the knowledge that *gai* is transported through the phloem, Zhang et al. (2012) furthered their experiments by testing the expression of NPTII and GAI:NPTII in transgenic tobacco. These transgenic lines were used as rootstocks and showed that the *Pyrus gai* transcript enabled the phloem based transport of NPTII into the scion while NPTII mRNA expressed without *gai* was not mobile.

RNA transport in composite plants was reviewed by Harada (2010) with a specific focus on RNA molecule transfer between the rootstock and scion. The experiments reviewed utilized Arabidopsis, tobacco, tomato, potato, melons, pumpkins and apple to demonstrate the long distance transport of RNA molecules, being mRNA, miRNA, or siRNA, through the graft union into the scion. Harada (2010) continues to discuss the potential mechanisms for RNA molecules being moved through the phloem including RNA-binding proteins and chaperone proteins as well as some potential conserved motifs in the RNA sequences.

Kasai et al. (2011) have since showed that post-transcriptional gene silencing of scion genes by the rootstock is possible in tobacco, opening another regulatory window for rootstock–scion interactions. They also showed much stronger results utilizing a phloem specific virus, commelina yellow mottle virus (CoYMV), promoter than when the cauliflower mosaic virus (CMV) promoter is used.

Other

Golecki et al. (1998) studied the ability of structural phloem proteins to be transmitted across the graft union of Cucurbits. The appearance of the proteins from the grafted

individual depended partly on the graft method where more direct alignment of the phloem yielded faster translocation as expected. This transfer requires that the vasculature across the graft union to be repaired prior to the translocation of the phloem proteins. Some genotypes were identified as acceptors of the proteins while others were donors.

Stegemann and Bock (2009) were able to also show the transfer of either large portions of plastid DNA or entire plastids through the graft union of tobacco, though the transfer was only successful over several cell layers at the graft union. The transfer of DNA across the graft union, even if only a few cell layers, may produce unique cells in the composite plant that could result in the production of unique products and physiology.

Summary

These reports documenting differential scion gene expression, molecular transport, and biochemical activity have started to reveal the foundation for rootstock–scion interactions. Since many transcription factors have been shown to be modulated, large changes of global gene expression could easily result causing the physiological differences noted previously. The transport of RNA molecules, proteins and potentially plastids from the rootstock into the scion is a critical development in the understanding of these somatogenetic interactions. It remains to be shown how the expression of the transported RNA molecules in the scion can modulate global gene expression. Nodes, internodes, graft unions and growing shoot tips represent general plant organs where the impacts of grafting are observable; however, it is not clear what cell types participate in receiving the signal from the rootstock and modify scion traits.

Conclusions and future prospects

It is clear that our observations of rootstock–scion interactions have expanded and the underlying mechanisms largely remain elusive. The control of scion traits by the rootstock has been highly documented in almost every area of perennial plant growth and physiology.

One major concern must be the evaluation of traits while the plants are not at the same stage of growth or maturity. Clearwater et al. (2007) showed in kiwi that the rootstock controls the timing of sap flow which in turn can lead to altered in-season maturity. While common horticultural practice assumes that the fruit from a scion will be exactly the same on any rootstock, it is likely that the rootstock can affect harvest maturity and therefore all aspects related to harvest date. The role of the rootstock controlling fruit

maturity in other species should provide critically important information when assessing varieties.

While much evidence had suggested the role of the graft union as the critical point of rootstock–scion interactions, the recent work by Gasco et al. (2007) along with concurring evidence from Clearwater et al. (2007), Nardini et al. (2006), Solari and DeJong (2006), and Solari et al. (2006) refute the role of the graft union restricting nutrient and water flow to the scion after the graft union is fully healed. In the annual composite plants, the role of graft union healing may be more critical because the separation of the wound and healing response from the other characteristics is not possible. This is also seen in the micro-grafting experiments performed on the herbaceous growth of the perennial plants where the graft union heals and becomes functional much more rapidly than seen in typical wood based grafting techniques; however, hydraulic conductance experiments on micro-grafted plants need to be completed to prove this unequivocally. Examining these processes in perennials allowed the disjunction of the healing from the overall control and is a critical advantage of studying perennial grafted plants. From the differences in these works, the strict requirement for future research to document the time from grafting is critical to enable the studies to be compared.

Since the graft union is not causing the sustained differences in plant growth control, the clear effect of root hydraulic pressure is very intriguing when considering vigor and the other aspects of the composite plant. Higher amounts of water pressure would be expected to transport nutrients and hormones more effectively to the scion. Experiments either examining the natural correlation among these measures or manipulating the root pressure as Solari and DeJong (2006) did would reveal another layer of the complex interaction between these genotypes. Additionally, improved water content would significantly improve photosynthetic efficiency leading to better carbon fixation and faster growth rates. As in the report by Prassinos et al. (2009), analysis of gene expression at different hydraulic pressures and different periods of growth will significantly improve the knowledge of the gene expression pathways involved in these interactions. Understanding the full relationship between water requirements, hydraulic conductance, and the effects on scion traits will become more critical as climates continue to change and irrigation water becomes less available.

The recent findings that multiple types of RNA molecules are actively transported across the graft union provide a putative mechanism for the rootstock to control scion gene expression, and vice versa. However, it is unclear that the mere movement of RNA molecules in the phloem across the graft junction has any regulatory role. Higher-throughput gene expression studies utilizing RNA sequencing approaches may enable the identification of gene networks involved in the complex pathways controlled by the interaction of the genotypes. Examination of gene expression changes in micro-grafted plants from day 4 onward, as shown by Kanehira et al. (2010), Xu et al. (2010), and Zhang et al. (2012) to be the start of RNA transport into the scion, may help reveal some of the core genes and transcription factors that then trigger the larger, widespread alterations to physiology.

The vast effects of rootstocks on scions may also be important to consider during breeding efforts for both rootstock and scions. Complementation of the combination may 'rescue' seemingly suboptimal genotypes into excellent production partners. This would apply to both rootstocks and scions and the genetic information from DNA markers and DNA sequencing may be useful for predicting these optimal combinations.

Developing worldwide research standards will be critical as the field of rootstock–scion interactions matures. These standards must include the age of the graft union due to its previously stated significance. Also, phenotyping fruit at specific maturities for each species would enable more direct comparisons across sample sites and among different studies. While sampling at specific maturities will place a larger burden on the researcher, the added value to the data obtained would be significant enough to warrant this change to research methodology. Another consideration for future projects is that most existing studies include one genotype of each vigor class whereas a full range of rootstocks may more effectively reveal useable differences. This approach would be similar to bulked segregant analysis where expression levels of many genes are changed and few are consistent across the various rootstocks. This can help reduce the initial gene set for further investigations. In conclusion, the field of rootstock–scion interactions in perennial and annual plants has made major headway yet many courses for future discovery remain.

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