Review

An overview of grafting re-establishment in woody fruit species

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ABSTRACT

The formation of a successful graft includes a series of biological steps involving immediate responses to the wound, such as callus and functional vascular system formation between graft partners. However, grafts are not always successful when different genotypes (plant species) are grafted, resulting in tissue union and regeneration problems—popularly known as graft incompatibility. Numerous studies on graft union formation and graft compatibility between scion–rootstock plants have tested several scientific hypotheses related to the physiological and molecular mechanisms underlying scion–rootstock union at the early and late growth stages following the grafting of herbaceous plants. However, due to long juvenile periods, long generation times, and large plant sizes, few studies have focused on the different growth stages of grafts using woody fruit plants due to inherent difficulties in their study. In the present review, a scientific analysis of existing studies promotes a discussion of scion–rootstock grafts. If such grafts exhibit a certain level of success in their re-establishment immediately following grafting, they are termed "graft compatible." However, if the scion–rootstock union becomes graft incompatible immediately after grafting, this is called "incompatibility," while "late graft incompatibility" occurs when the union dies within three to five years.

1. Introduction

Grafting has been used in agriculture for over 2000 years. Historical records indicate that grafting was practiced by the ancient Chinese (1560 B.C.), the ancient Greeks, and during early Christianity (Melnyk and Meyerowitz, 2015). These cultures were the first to utilize the technique of grafting plants with different species within the genus *Citrus* (family Rutaceae) and olives (family Oleaceae) (see for review Hartmann et al., 2011; Melnyk and Meyerowitz, 2015). Currently, the use of grafted plants is commonplace in orchards, greenhouses, and gardening, and its applications extend beyond horticultural contexts.

The technique for grafting involves the union of two parts of living plants: a root system (rootstock) and a shoot system (scion). Through tissue regeneration, the assembly of these two parts from different species constitutes a new plant (Martínez-Ballesta et al., 2010; Cookson et al., 2014). Grafting is widely used in several woody plants such as the rubber tree (*Hevea brasiliensis* Muell. Arg.) (Prabpreea et al., 2018), pecan [*Carya illinoensis* (Wang.) K.] (Mo et al., 2018), orange (*Citrus*

spp.) (Caballero et al., 2013; He et al., 2018), apple (*Malus* spp.) (Adams et al., 2018; Atkinson et al., 2003), pear (*Pyrus communis* L.) (Hudina et al., 2014; Yang et al., 2017), grape (*Vitis vinifera* L.) (Moreno et al., 2014), atemoya (*Annona* x *atemoya* Mabb.) (Baron et al., 2016), *Prunus* spp. (Zarrouk et al., 2010; Pina and Errea, 2008a), and olive (*Olea europaea* L.) (Fabbri et al., 2004). Although this plant propagation technique has been used for many centuries in evergreen plants—mainly fruit, forest, and ornamental trees—it remains unclear how physiological mechanisms act in the re-establishment of tissues at the graft junction. During the 20th century, the widespread use of grafting for vegetables plants—mainly species belonging to the Cucurbitaceae and Solanaceae families—led to significant advances in knowledge related to the physiological aspects of grafted herbaceous plants (Goldschmidt, 2014).

Grafting can also be used to assist plants in the adaptation to biotic stress, such as resistance to pathogens (Ramírez-Gil et al., 2017) and abiotic stress conditions including drought (Zhou et al., 2018), salinity (Mehdi-Tounsi et al., 2017), and mineral deficiency (Jimenes et al.,

2018). In addition, another goal of grafting is to increase precocity at the beginning of production in order to avoid the juvenile state of the scion plant. The scion grafted onto a rootstock species will maintain its current growth stage, including the capacity to immediately produce fruits, which provides the possibility of reduced spacing in commercial orchards by using dwarf rootstock species that confer a smaller size on the grafted plant.

In addition, grafting can be used as a tool for the study of different biological processes. In particular, grafting on *Arabidopsis thaliana* L.—a plant widely studied as a "model-plant"—has greatly contributed to existing knowledge on many issues related to plant physiology, including the transmission of floral stimuli, proteins, and long-distance RNAs in the phloem tissue (Notaguchi et al., 2009).

In particular, one factor believed to trigger graft mortality is the natural incompatibility reaction between scion–rootstock species known as "incompatibility between partners". However, it should be considered that additional factors can produce graft failure; for instance, pathogens active within the phloem vascular tissue, inadequate environmental conditions (temperature and/or humidity), and the inability to align the cambial tissues of both graft partners (Martínez-Ballesta et al., 2010; Goldschmidt, 2014).

However, even without the interference of abiotic factors, some scion–rootstock combinations are truly incompatible, either immediately following grafting or over time, indicating several physiological, biochemical, and molecular mechanisms being involved in scion–rootstock interaction. Thus, biochemical-molecular and/or anatomical processes (Hartmann et al., 2011) represent some of the factors collaborating in the re-establishment of plants. Nevertheless, the interrelation of these interactions remains poorly understood.

Thus, the present review provides an overview of the current state of the art for grafting woody plants based on several review articles, research articles, and book chapters, each emphasizing a specific aspect of the procedure, such as grafting techniques or the vascular alignment of cambium tissues (Pina et al., 2012; Lima et al., 2017), candidate gene expression (Chen et al., 2017), phenolic compounds (Zarrouk et al., 2010; Irisarri et al., 2016; Prabpreea et al., 2018), or phytohormones (Wang et al., 2014; Xu et al., 2015). In addition, several reports following the restoration of the vascular connection focus on scion–rootstock interaction in relation to leaf gas exchange (Xu et al., 2015; Baron et al., 2017), ion accumulation (Moreno et al., 2014; Samuolienė et al., 2016), production, and vigor, among other factors. As such, we have presented a number of botanical characteristics that may have a direct influence on the re-establishment of vascular connections. Correct anatomical tissue positioning between scion and rootstock triggers the genetic potential necessary for the regeneration of injured tissues and the production of secondary metabolites. Phenolic compounds and phytohormones induce the re-establishment and survival of the grafted plant. A compatible combination will transport and translocate nutrients, carbohydrates, promoters, and phytohormones for normal plant development (Fig. 1).

2. Taxonomic identification of grafted plants

Several biotic and abiotic factors involved in the re-establishment of plants are reported in the literature, though botanical characteristics are scientific debatable regarding "what" can be considered graft compatible or not graft compatible. Notably, no precise definition of "graft compatible" exists, and published research generally refers to this as the establishment of a successful graft, involving prolonged survival and proper functioning of the composite plant (late compatibility) (Goldschmidt, 2014). Among the possible botanical characteristics involved, taxonomic affinity is presumed to be a prerequisite for graft compatibility.

The taxonomic proximity of scion and rootstock species is essential to the success of re-establishment of both graft partners, though current understanding of incompatibility in woody plants remains insufficient.

A homograft occurs when intraspecific grafts are used, with scion and rootstock belonging to the same botanical species, the members of which are presumably always compatible. The more general approach of heterografting occurs when grafts are interspecific, with scion and rootstock belonging to different species of the same genus. In addition, interfamilial grafts are rarely compatible, thereby typically graft incompatible.

The importance of taxonomic identification is particularly evident when analyzing studies of species belonging to the family Annonaceae. Some plants in Annonaceae are erroneously recognized as "graft incompatible" when certain species are used as rootstocks. For instance, this occurred for Brazilian producers and rural technicians who erroneously concluded that the native plant popularly known as *biribá* [*Annona mucosa* (Bail.) H. Rainer] is incompatible with atemoya when used as a plant rootstock. However, a number of botanical species exist with the popular name "biribá" that are perfectly serviceable as rootstocks plants. Notably, "biribá" is commonly used in different regions of Brazil to refer to soursop (*Annona muricata*), araticum-do-brejo (*Annona glabra*), wild-soursop (*Annona montana*), and wild-sweetsop (*Annona reticulata*), which are reportedly graft incompatible as rootstock with atemoya scions (George and Nissen, 1987; Sanewski, 1991).

The importance of correct identification of plants involved in the grafting process can also be verified in *Prunus*. For example, Reig et al. (2018) studied the (in)compatibility reactions between apricot (*Prunus armeniaca* L.) cultivars and 21 different *Prunus* rootstocks before releasing these rootstocks on the market. Reig et al. (2018) affirmed that apricot exhibited perfect unions (the line of the union between bark and wood is hardly visible) with plum rootstocks (*P. insititia*); however, several discontinuities in the bark, with breakage of the tree at the graft union in the nursery or orchard being observed in some plum rootstocks including "Miral 3278 AD" (*P. cerasifera* × *P. amygdalus*), "Ademir", and "Myrobalan 713 AD" (both *P. cerasifera*). Curiously, the plant species popularly known as 'plum' invoked both success and 'failure' as a rootstock. As a result, correct taxonomic identification is necessary to avoid mistakes and provide both nurseries and fruit growers with valuable information regarding the risks of weak unions.

This situation is increasingly aggravated by the number of exsiccates of studied species deposited in herbariums being low, making it difficult to identify the correct taxa of a species. Thus, the characteristics of certain species that are graft incompatible are generalized among all botanical species that present the same popular nomenclature, resulting in misunderstandings when obtaining scion and rootstock seedlings.

3. Grafting technique and professional skills

Grafting plants has been an important tool for improving olericulture and fruticulture, and will likely maintain this position as an important agronomic and agricultural production technique over the long term. The necessity for nursery growers to select rootstock species with ample advantages, along with the ability to reproduce it on a large scale, makes grafting an attractive strategy for increasing yields and growing fruits or vegetables in environments where it was not previously possible.

Furthermore, the professional skills required for the grafting technique are fundamental to obtaining a good bud "take" and determining whether certain scion–rootstock combinations are truly incompatible, that is, whether failure was triggered by anatomical mechanisms or simply by inexpert grafting practice. Nursery growers responsible for the production of commercial rootstocks and scions generally use a slight physical pressure, using either a clip or wrapping tape around a graft tissue joint to promote successful formation.

In addition, nursery growers must graft rootstock and scion stems of a similar size, which allows for the proper alignment of tissue known as the vascular cambium. These tissues are popularly known as "plant stem cells", and give rise to the phloem and xylem during secondary plant growth, when the roots and stems thicken (Melnyk and
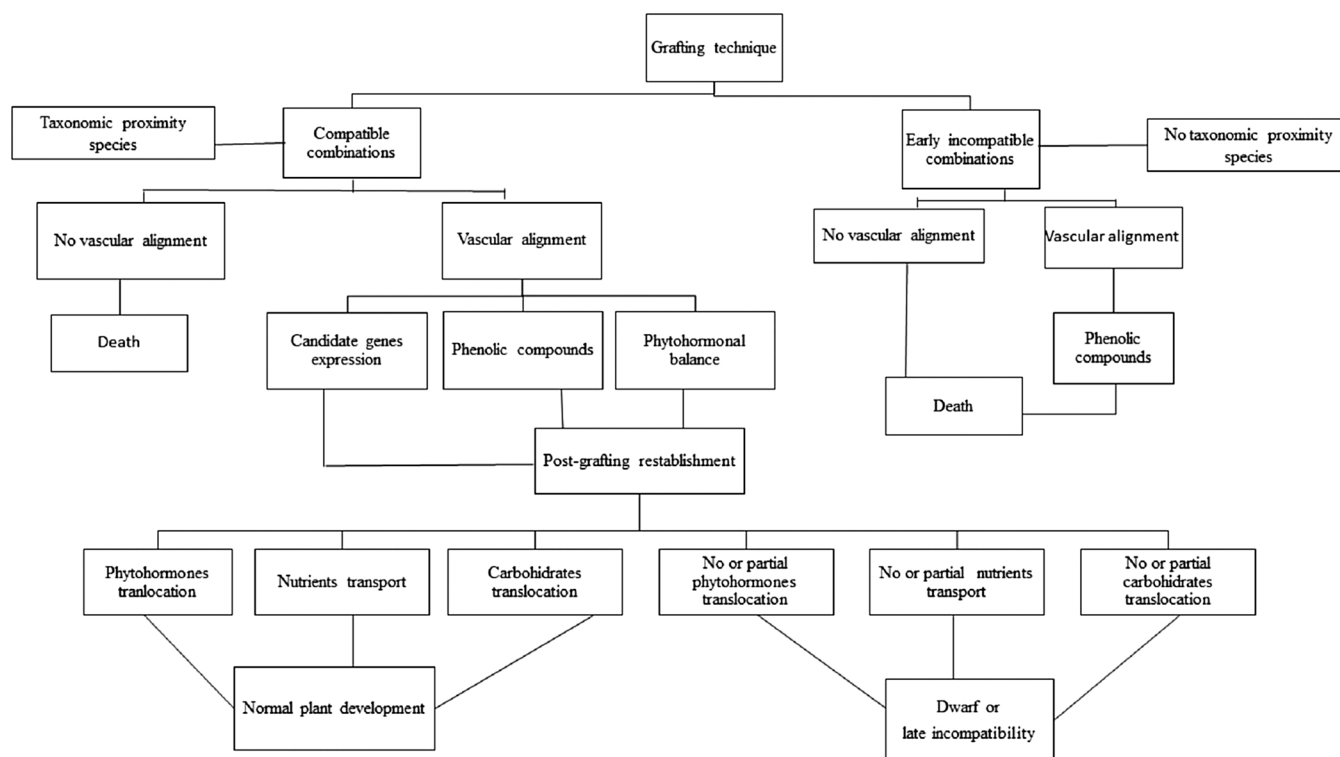
Fig. 1. Schematic representation of the physiological mechanisms of interaction in grafting plants.

Meyerowitz, 2015). For a successful graft, a coincidence between the tissues near the cambium are necessary to form a continuous connection, since the meristematic tissue between the xylem and the phloem is continuously dividing and forming new cells (Kümpers and Bishopp, 2015). Moreover, the influence of abiotic factors must also be considered, such as appropriate environmental temperature (24–27 °C) and relative humidity (equal to or above the saturation point) (Hartmann et al., 2011), which should be properly maintained subsequent to grafting.

4. Vascular alignment of anatomical tissues in grafted plants

Five biological steps have been described to occur during graft union formation: (i) alignment of vascular cambium tissue between the scion and rootstock; (ii) response to the wound; (iii) formation of the callus bridge; (iv) repair of the xylem by the differentiation of the vascular cambium tissue through the callus bridge; and (v) production of the secondary xylem and phloem from the new vascular cambium tissues of the callus bridge (Hartmann et al., 2011). Initial tissue cohesion between the scion and the rootstock is produced as a result of the deposition and subsequent polymerization of cell wall materials in response to the wound. Nevertheless, vascular connections are established in woody fruit plants at early stages of development—even in cases considered as graft incompatible (Espen et al., 2005).

Incompatibility responses in woody plants have been associated with changes in cell/tissue organization at the moment of grafting. In peach grafts, Zarrouk et al. (2010) confirmed the existence of differences in the structural organization of graft compatible combinations compared to graft incompatible ones. Similarly, some authors have reported that the arrangement of cambium tissues is less organized in incompatible unions of apricot (*Prunus armeniaca* L.) grafted on different *Prunus* rootstocks (Errea et al., 2001; Pina et al., 2012), which supports the idea that cellular disorganization in the cambium is an early indicator of graft incompatibility.

Another line of research suggests that the existence of a binding factor in one of the graft partners alters cell-to-cell communication in the union of the other partner, and that this factor can originate from a simplistic pathway through the newly formed plasmodesmata. Therefore, the initial growth stages of graft development (prior to the re-establishment of vascular connections) may be critical for diagnosing future compatibility responses in woody plants (Pina et al., 2012).

Furthermore, incompatibility between scion–rootstock species may induce a decrease in shoot growth and water transport, which may lead to the death of the plant (Davis and Perkins-Veazie, 2008). Graft incompatibility generally occurs at the early stages of graft development when vascular connections are forming; however, symptoms may manifest at later growth stages such as low plant development related to physiological differences in stem diameter, which impairs the normal flow of photoassimilates and the lignification of grafted tissues (Souza et al., 2018), thereby decreasing the hydraulic conductivity of the graft union (Solari et al., 2006; Gascó et al., 2007; Tworkoski and Fazio, 2015). These symptoms appear during the plant fruiting period, when the plant is subject to a high demand for water transport (Martínez-Ballesta et al., 2010).

Another possible explanation for the re-establishment of vascular connections is that the wound site has an efficient wound repair mechanism in response to damage sustained from herbivory, wind, or rain (Minibayeva et al., 2015; Hilleary and Gilroy, 2018; Houmani et al., 2018). Nevertheless, the molecular mechanisms governing graft formation remain unknown, and no genes required for this process have been identified to date (Melnyk et al., 2018) since it is not easy to separate transcripts specific to the graft union development with those specific to the spring reaction of the cambium in woody plants (Cookson and Ollat, 2013).

Recently, Reig et al. (2018) presented the present state of the art for two types of graft incompatibility known as 'translocated' and 'localized'. These two types of incompatibility can present jointly in the same graft combination, and neither of them is privative of any particular species. The translocated type is characterized by yellowing leaves and premature defoliation, with earlier cessation of tree growth and a partially developed radicular system.

The localized type of incompatibility is characterized by anatomical

irregularities at the graft union interface accompanied by anatomical abnormalities of the vascular tissue, as well as breaks in cambial and vascular continuity and poor vascular connections (Errea et al., 2001; Zarrouk et al., 2010; Hartmann et al., 2011). These structural anomalies cause mechanical weakness of the union, which may break over time or following strong wind conditions, which would subsequently lead to major economic losses.

According to Hudina et al. (2014) the localized type of incompatibility can occur when pear is grafted on quince and apricot on other *Prunus* species. A cyanogenic glycoside, prunasin, which is normally found in quince but not in pear, is translocated into the pear phloem. The pear enzymes break down the prunasin in the graft union region, yielding hydrocyanic acid as one of the decomposition products, resulting in anatomical irregularities at the graft union interface.

5. Genes differentially expressed during graft union formation in different species

During the graft union healing process in pear grafted plants, 14 genes of known function were involved in the function of kinase, carbohydrate metabolism, protein metabolism, nuclear metabolism, as well as cell activity and development. These genes may improve our understanding of the molecular mechanisms governing the healing process of grafted plants (Yang et al., 2017).

Currently, despite the widespread use of grafting, the molecular mechanisms of graft formation require further investigation (Melnyk and Meyerowitz, 2015; Pina et al., 2017). According to Pina et al. (2012), the re-establishment of vascular connections may be associated with structural modification of the cell wall. The plant cell wall is a thin, strong, and flexible extracellular layer composed primarily of polysaccharides and proteins divided into non-enzymatic (expansin) and enzymatic (hydrolase) groups (Cosgrove, 2005).

The expansin (EXP) and xyloglucan endotransglucosylase (XET) genes are responsible for encoding expansins and hydrolases (xyloglucan endotransglucosylase), respectively, and have been identified as fundamental precursors for plant tissue (Nishikubo et al., 2011). The expression of these genes results in plant tissue growth, while their suppression decreases plant growth (Zenoni et al., 2004; Buckeridge, 2010). In addition, the uridine diphosphate (UDP)-glucose pyrophosphorylase (UGP) gene is reported to have a direct role in cell wall biosynthesis is, as UGP transcripts were detected in the anatomical region of a successful apricot graft (re-establishment immediately after grafting), making this gene an important target in the study of interactions between grafted plants (Pina and Errea, 2008a).

Notably, the UGP gene is known to encode the enzyme UDP-glucose pyrophosphorylase (UGPase; EC 2.7.7.9), which is considered the major enzyme for nucleotide sugar production and the interconversion of sugar UDP-glucose—a precursor relevant in the biosynthesis of cell wall components such as cellulose, hemicellulose, and pectin (Bar-Peled and O'Neill, 2011). Early increases in the expression of the UGP gene in grafted tissues may indicate the earlier union of plant tissues following grafting. According to Baron et al. (2016), an atemoya scion (*Annona* x *atemoya* Mabb.) grafted onto an "araticum-de-terra-fria" rootstock [*Annona emarginata* (Schltdl.) H. Rainer] is considered a compatible combination, and it exhibits increased UGP gene expression at the graft interface.

Furthermore, the possibility of the genetic expression of UGP homologues in the formation of tissues at the grafting region in woody fruit plants has been reported in the literature, and multiple molecular forms of an enzyme can originate by gene splicing or post-translational modifications. In particular, one explanation could be the existence a greater abundance of UGPase proteins compared to UGP transcripts. UGP genes are under tight posttranscriptional/translational control, and large changes in transcript abundance may only fine-tune protein levels by slowly altering UGPase protein content (Meng et al., 2007, 2009. This suggests that an adaptational advantage must exist to such

regulation, though this mechanism remains unclear. These are important in the regulation of enzymatic activity (Meng et al., 2007, 2009).

UGPase isoforms have been detected in several herbaceous plants, including rice (*Oryza sativa* L.) (Huang and Mu, 2005; Chen et al., 2007), *A. thaliana* (Meng et al., 2009), and potato (*Solanum lycopersicum* L.) (Sowokinos, 2001). Despite the crucial roles of the UGP gene in the metabolism of cell wall structural carbohydrates, little remains known regarding the regulation of UGPase enzyme activity in woody plants.

In cucumber–pumpkin graft combinations, CmRNF5 and CmNPH3L genes were differentially expressed between compatible and incompatible unions, leading to the suggestion that these two genes could be related to graft compatibility/incompatibility responses in plant development (Ren et al., 2018). In *Arabidopsis thaliana* L., the WIND1 gene (wound induced dedifferentiation 1) is strongly regulated after wounding, and its overexpression results in excessive callus formation (Iwase et al., 2011). The same authors reported that root hypocotyls submitted in culture media containing sucrose perform nutrient transport, which is essential for the healing of injured tissue and formation of vascular connections. Nevertheless, this approach has not been described for woody species.

Transcriptomic studies in the grafting interface of homografted grapevine (*Vittis vinifera* L.) revealed differential expression of transcripts associated with phloem and xylem development, cell-wall biogenesis, and secondary metabolism (particularly wounding responses). However, in heterografted grapevine, transcripts associated with stress and defense responses are also accumulated. The cells at the graft interface are capable of detecting the presence of the non-self grafting partner, which may induce an immune-type response (Cookson and Ollat, 2013; Cookson et al., 2014).

However, some studies revealed that plant grafting induced phenotypic variation in anatomy and morphology (Wang et al., 2017; Reig et al., 2018). Endogenous factors have been found to move across the graft union, which is the most convincing explanation for how phenotypic changes are induced by grafts. Several lines of research have investigated long-distance trafficking and phloem unloading of protein signals and mRNA, respectively, in homografted plants; however, the more detailed mechanisms enabling intercellular molecular transport still require further research (Zhang et al., 2016, 2017).

As previously mentioned, researchers have highlighted the regeneration of vascular tissue as a key event in grafted plant re-establishment. However, it remains unclear how graft formation could only be a manifestation of a common mechanism for the formation of vascular tissue in grafting plants. Currently, the source-to-sink transport of phytohormone may be sufficient for generating veins through the cut tissues to graft plants (Melnyk and Meyerowitz, 2015).

6. Phytohormones in plant vascular connections of grafted plants

Many processes involved in plant development are regulated by phytohormones. Two of these secondary hormones, auxins (AX) and cytokinins (CK), are known to serve important roles in vascular tissue formation in grafting plants (Melnyk et al., 2015).

Notably, Kümpers and Bishopp (2015) determined that phytohormones are involved in regulating the complex physiological relationship between scions grafted onto rootstocks in *A. thaliana*. Phytohormones are translocated to the sites of action as signal molecules affecting cell growth and tissue differentiation, especially at the graft interface (Aloni et al., 2010). In this way, they may be considered candidates in the scion–rootstock relationship, signaling both above and below the grafted interface (Kondo et al., 2014).

The involvement of AX in mechanisms of graft incompatibility is highlighted by three observations: (i) the roots and stems of "incompatible" combinations contain higher concentrations of AX than those of "compatible" combinations; (ii) the application of an inhibitor of the AX transporter (2,3,5-triiodobenzoic acid) to the stem of a grafted

plant prevents the death of "incompatible" rootstock species, while having little effect on "compatible" plants; and (iii) the development of the shoot and root parts of a graft-incompatible plant was normal after the blockade of the basin AX transport by partial girdling of the stem. These observations support the theory that AX produced in the aerial part is distributed by basipetal transport soon after the regeneration of vascular tissues when reaching a threshold of phytohormonal concentration initiates processes of cellular degradation, causing root death in the rootstock species (Hartmann et al., 2011).

Additionally, AX affects the production and activity of CK, which is produced at the roots and translocated to the scion, where it controls important plant processes such as shoot growth (Elfving and Visser, 2006). AX/CK interactions affect cell fate, and their roles have been characterized in several founder cell specifications such as vascular connections (Chandler and Werr, 2015). Thus, a strong mutual interaction exists between AX production in the shoots and CK production in the roots, which may be important in regulating the phytohormonal balance between root and shoot growth (Bishopp et al., 2011).

In grafted plants, the phytohormonal balance between AX/CK is reduced; thus, the invigorating properties of the rootstock species in inducing higher graft growth rates may be explained by the increased CK supply to the shoot, whereby the AX supply is decreased (Aloni et al., 2010). In turn, a reduction in AX transport leads to a reduction in the root systems of dwarf rootstock species, which produce less CK. Therefore, lower concentration of CK translocated from the roots to the scion reduces the growth of the scion, thus triggering the "dwarf effect."

In apple tree rootstocks treated with CK (cytokinin 6-benzylaminopurine), stimulation of lateral branch shoot formation was observed, while the application of GA (gibberellic acid) associated with CK (phenylmethyl aminopurine) reduced the proportion of lateral primary and secondary branches, thereby stopping initial growth and increasing its final size and the number of nodes and internodes (Hartmann et al., 2011).

Furthermore, apple (*Malus* sp.) dwarfing rootstocks contain smaller amounts of plant growth promoter phytohormones, though a larger amount of inhibitor phytohormones than vigorous rootstocks of the same species, with high abscisic acid (ABA) levels in dwarfing rootstock stems (Yadava and Dayton, 1972). ABA is one of the main factors responsible for triggering the process of dwarfing in higher species (Tworkoski and Fazio, 2015). While the dwarfing rootstocks of apple plants contain large amounts of ABA (Lordan et al., 2017), we lack a thorough understanding of ABA mechanisms in the re-establishment of vascular connections.

Other hormones may also serve a role in rootstock-induced dwarfing. For example, the high concentration of ABA in the bark of dwarfing apple rootstock compared to vigorous apple rootstock is considered an efficient marker in the selection of rootstocks (Kamboj et al., 1999). Regarding GA, no consensus exists as to whether higher concentrations of ABA are found in vigorous rootstocks. Nevertheless, ABA may support the role of GA in vigorous rootstocks compared to dwarfing rootstocks (Richards et al., 1986; Kamboj et al., 1999).

A complete understanding of phytohormonal control in the re-establishment of grafted plants is far from being achieved. As previously discussed, existing research reflects variations between the botanical species studied. Several scientific hypotheses have been tested to explain graft incompatibility, with the majority of studies referring to the early growth stages of re-establishment of herbaceous systems (Penella et al., 2014). However, few studies have been performed on the early stages of re-establishment in grafted woody plants due to the inherent difficulties in studying plant species that require a longer periods of time to present phenological growth stages suitable for evaluation (Pina and Errea, 2008b; Pina et al., 2012).

7. Phenolic compounds in grafted plant vascular connections

To the best of our knowledge, the most promising studies of the re-establishment of vascular tissues have studied the effects of phenolic compounds (Canas et al., 2015; Pina et al., 2017). More specifically, several studies have indicated that phenolic compounds are involved in lignification. Such compounds are important to the early growth stages of connections between scion–rootstock combinations, as the cell walls of xylem tissues are dynamic structures composed of polysaccharides, phenolic compounds (for example, lignins), minerals, and proteins (Liu, 2012; Herrero et al., 2014). Moreover, the presence of phenolic compounds has been identified as important marker for the evaluation of graft compatibility between scions and rootstocks (Prabpreea et al., 2018).

Phenolic compounds are described as being implicit in the processes of cell division and development. Specifically, these compounds participate in the internal differentiation of new tissues in the grafting region by regulating the synthesis of AIA-oxidase—more precisely, the *p*-coumaric acid precursor of lignin (Errea, 1998; Mn'gomba et al., 2008). These compounds are also responsible for the regulation of peroxidase (PRX) synthesis, which is responsible for the degradation of AX (more precisely, *p*-coumaric acid and hydroxybenzoic acid (monophenols)). These compounds inhibit plant development due to their activation of auxin oxidation, whereas polyphenols (e.g. caffeic acid) inhibit the oxidation of auxins, thereby promoting plant growth.

In grafted *Uapaca kirkiana*, the accumulation of *p*-coumaric acid in grafted tissue may lead to non-differentiation of the tissue and degradation at the vascular tissue of the scion–rootstock interface, indicating graft incompatibility (Mn'gomba et al., 2008). In apricot/plum combinations, a high concentration of phenolic compounds was observed in undifferentiated callus at the scion–rootstock interface of plants previously categorized as incompatible (Errea et al., 2001; Pina at al., 2012). This accumulation reduces AX content, which affects the differentiation of the xylem and phloem vessel elements as well as lignification (Errea, 1998; Liu, 2012), or AX degradation, which interrupts cellular functions and chemical reactions (Hartmann et al., 2011).

The detection of phenolic compounds at the initial growth stages following grafting is of particular interest, as these may block the vascular connection between the vascular cambium tissues of the rootstock and scion species (Errea et al., 2001). Additionally, the accumulation of phenols (anthocyanins, flavanones, *p*-coumaric acid, and hydroxybenzoic acid) has been associated with reduced graft compatibility at both early and late stages in apricot (Errea et al., 2001) and peach grafted plants (Zarrouk et al., 2010).

In plants of the genus *Prunus*, the accumulation of phenolic compounds is related to problems in the differentiation of the callus in the initial growth stages of grafted plants. Additionally, the verification of other phenolic compounds, such as catechins and protocyanidins, may aid our understanding of graft-compatible/incompatible relationships (Errea, 1998). Under stress conditions, the accumulation of flavonoids, which directly affect both metabolism and tissue growth, is inhibited by the biosynthetic lignin pathway (Liu, 2012). Although phenolic biosynthesis inhibits the activity of AIA-oxidases, details regarding such molecular mechanisms—such as the control of the AIA production pathway—are not fully understood.

8. Leaf gas exchange in grafted plants

Leaf gas exchange in grafted plants is directly affected by the rootstock (Xu et al., 2015; Baron et al., 2017), as the graft partner can modify the vigor and productivity of the scion species. This indicates a direct role in graft compatibility between both graft partners, as evidenced by responses in chlorophyll content (Rouphael et al., 2008) and the efficiency of photosystem II (PSII) (He et al., 2009).

Notably, when forming the callus bridge at the scion–rootstock interface, grafted plants enable water flow from the rootstock to the scion; however, insufficient connection between them leads to a decrease in water flow and, consequently, losses in carbon assimilation and stomatal conductance (Magalhães-Filho et al., 2008).

Photosynthetic processes in grafted species belonging to the genus *Annona* have been widely investigated, and have aided our understanding of gas exchange under different abiotic stresses, such as tolerance to water stress due to an excess or scarcity of water (Mantoan et al., 2016). Additional studies have also been conducted to evaluate water use in grafted plants involving different species, with water economy becoming even more complex because, in addition to factors related to plant–soil interaction, transport through the stem xylem can present problems related to the connection of vessels and dimensioning between the aerial part of the plant and the root system.

According to Núñez-Elisea et al. (1999), a scion of wild-sweetsop (*A. reticulata*) grafted onto a pond apple (*A. glabra*) rootstock exhibited a similar carbon assimilation rate to the ungrafted pond apple. However, the study affirmed that the physiological mechanisms that promoted the improved performance of the grafted plant (compared to the freestanding one) under flood conditions were unclear. Although our literature review did not uncover any studies that explored the direct relationship between the biochemical mechanisms governing leaf gas exchange and the re-establishment of grafted plants, reconnection of the vascular system is of greater importance due to no transport of water and nutrients between the organs being possible without it.

9. Ion accumulation in grafted plants

In grafted plants, the role of the rootstock and scion on root and leaf ion accumulation can affect plant growth and development in different ways (He et al., 2009; Nawaz et al., 2016). The quantification of nutrients in the leaves is another relevant factor in the study of graft compatibility (Rouphael et al., 2008), as the rootstock is responsible for absorbing water and soil nutrients, thereby directly influencing the mineral content in the shoot of the graft (Baron et al., 2017). Several studies have reported the accumulation of macro- and micronutrients in young plants of rootstock species, though few studies have focused on the concentration of mineral elements in the foliar and root tissues of grafted plants, as well as their interaction and regulation of the processes involved in the re-establishment of woody plants following grafting. The uptake of mineral elements at the biochemical level signals the shoot, which then governs the demand for nutrients (Savvas et al., 2010; Marschner, 2012).

In *Citrus* grafted plants [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] and Cleopatra mandarin (*C. reshni* Hort. ex Tanaka), different K transporters have been identified in modifying ion uptake and transport to the scion, suggesting that rootstocks possess efficient high-affinity K^+ uptake systems that supply K^+ for growth—even at very low K^+ concentrations (Caballero et al., 2013).

Tomasi et al. (2015) affirmed that grape rootstocks (Chardonnay and Sauvignon Blanc cultivars) improved nitrate uptake by affecting the low-affinity nitrate transporter gene activity. In pear (*Pyrus betulaefolia*) rootstock plants, Li et al. (2016) reported the presence of high-affinity nitrate transporter genes; nevertheless, the roles of these transporters in grafted plants remain unclear.

Furthermore, some studies (Savvas et al., 2009; Nawaz et al., 2016) indicate that grafting may limit the absorption and toxicity of heavy metals, thereby increasing the uptake efficiency of essential mineral elements (Colla et al., 2010); however, it is not possible to determine the tolerance of grafted plants in an environment where the scion species could not survive, such as saline or alkaline soils (Edelstein et al., 2011).

Nevertheless, Brumós et al. (2010) reported that *Citrus* rootstock exhibited an adaptive response to salinity, and that the tolerant genotype reduces Cl^- accumulation. Specifically, the *C. sinensis* × *P. trifoliata* rootstock shows a number of uncharacterized membrane transporter genes differentially expressed in poor Cl excluder and efficient Cl excluder *C. reshni* hort. ex Tanaka rootstocks. Furthermore, Li et al. (2013) reported that, in apple rootstock (*Malus* sp.), overexpression of a Na^+/H^+ anti-porter gene improves the salt tolerance of dwarfing apple

rootstock, reducing Na and Cl loading and transport to the scion while allowing small osmotic potential with a low energetic cost.

10. Concluding remarks

Based on the existing literature, no consensus exists on the definition of the terms "graft compatibility" and "graft incompatibility", which predominantly reflects the notion that so-called "compatibility" entails survival immediately subsequent to grafting at the nursery stage, and over time following transplantation to the field.

Conversely, graft partners classified as "graft incompatible" may not survive immediately following grafting, or do not survive over time following transplantation to the field. It is observed that the evaluation of grafting presents various levels of success: re-establishment immediately following grafting is called "graft compatibility"; however, if the grafted plant becomes immediately incompatible after grafting, this is called "graft incompatibility," whereas a situation in which a grafted plant dies between three to five years after grafting is referred to as "late graft incompatibility."

In the present review, a scientific analysis of the compiled studies facilitates a discussion of advances in this field, and amplifies recent evidence related to the possible physiological mechanisms active in the re-establishment of woody fruit plants subsequent to graftingin addition to underlying graft compatibility.

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