

Long-Distance Signaling: What Grafting has Revealed?

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Abstract Grafting has been a long-standing technique used to improve crop production and provide tolerance to biotic stresses, including soil-borne pathogens, as well as tolerance to abiotic stresses, such as cold, salinity, drought, and heavy metals. Currently, beyond its use in horticultural production, grafting has received great prominence as an important research tool, mainly regarding signaling mechanisms related to root–shoot communication. Most of the emphasis from recent studies has been on detection of the long-distance transport of molecules and signaling factors, especially phytohormones, RNAs, peptides, and proteins. Through these studies, there have been significant breakthroughs about the underlying mechanisms implicated in the regulation of developmental and stress responses at the whole plant level. Because of this, the aim of this paper is to provide an overview of grafting as an important tool for plant science. Thus, in the following sections, we will summarize what is most recent and relevant in the use of grafting in plant research.

Keywords Grafting · Long-distance signaling · Phytohormones · Root-to-shoot communication · Rootstock

Introduction

Grafting is an ancient technique used for a long time for plant propagation and crop production improvements (Lowe 2010; Melnyk and Meyerowitz 2015). Although it is not

clear when exactly its use was started, there are accounts about the use of grafting in woody plants from 3000 years ago (Miguel 1997; Mudge and others 2009). Whereas the first record about the use of vegetable grafting occurred as recently as 1920 in Japan, when watermelon (*Citrullus lanatus* L.) plants were grafted onto bottle gourd [*Lagenaria siceraria* (Mol.) Stand.] to prevent *Fusarium* wilt (Kawaide 1985). Grafting basically consists of the union between parts of two plants. Ordinarily, the shoot piece is known as the scion and the root piece is called the rootstock (Turnbull 2010; Goldschmidt 2014). Although countless factors, such as anatomical, physiological, and biochemical features, are necessary for successful unions (Aloni and others 2010; Martínez-Ballesta and others 2010; Milien and others 2012; Fan and others 2015; Melnyk 2017), it is very common to perform not only intra-specific grafts but also inter-specific or even inter-generic grafts (Maršić and others 2014; Gao and others 2015; Huang and others 2015; Li and others 2015). Furthermore, several studies have demonstrated that grafting is a powerful tool for increasing culture efficiency and to overcome many biotic and abiotic limiting factors to plant production (Louws and others 2010; Albacete and others 2015; Yin and others 2015); because of this, grafting has been practiced in many parts of the world.

Since the influence of the rootstock on the scion and vice versa was noticed, a number of experiments have been performed in an attempt to establish the basis of the transport of molecules and signals between both parties (see review Aloni and others 2010; Martínez-Ballesta and others 2010). In these experiments, grafting has been an important tool in biological research for discovering how long-distance communication can coordinate and modify plant development in response to endogenous and environmental factors. For instance, when growing fruit, it is common to use rootstocks that induce a dwarfed size of the scion. This reduction of

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shoot growth was explained recently by Tworowski and Fazio (2016) as associated with hormonal imbalances between abscisic acid (ABA) and gibberellin (GA) in the scion caused by the rootstock.

Indeed, beyond its use in horticultural production, grafting has currently acquired great relevance as a research tool in plant biology research, especially after establishment of suitable protocols in model plants, such as tomato (*Solanum lycopersicum* L.), *Arabidopsis* (*Arabidopsis thaliana*), and *Medicago truncatula* (Turnbull and others 2002; Kasaw and Frugoli 2012; Marsch-Martínez and others 2013; Turnbull and Lopez-Cobollo 2013; Meyer and others 2017). Thus, grafting has been used to study many inner plant processes, mainly those related to root–shoot communication, such as molecular transport (Ivanchenko and others 2015; Jin and others 2015; Ali and others 2016), signaling between organs (Goldschmidt 2014; Notaguchi and Okamoto 2015; Notaguchi 2015; Notaguchi and others 2015), and nutrient uptake (Bautista and others 2011; Kumar and others 2015; Martínez-Andújar and others 2016).

Most of the emphasis from recent studies has been on the detection of long-distance transport of molecules, especially phytohormones (Dodd and others 2009; Kiba and others 2013; Seto and Yamaguchi 2014; Ivanchenko and others 2015; Regnault and others 2015), RNAs (Bhogale and others 2014; Li and others 2014; Notaguchi and others 2015; Ali and others 2016), peptides (Huault and others 2014; Tabata and others 2014; Okamoto and Kawaguchi 2015), and proteins (Notaguchi and others 2008, 2015; Jin and others 2015; Spiegelman and others 2015). Through these studies there have been significant breakthroughs about the underlying mechanisms implicated in the regulation of development and stress responses at the whole plant level. Thus, in the following sections, we will summarize the most recent and relevant advances achieved with the use of grafting in plant research.

Phytohormones

Plant hormones are important compounds that play key roles as long-distance messengers through which plants regulate many aspects of development and exert a highlighted role in root–shoot communication. Likewise, hormonal transport from the apical meristem of the root and/or the shoot to the grafted region is an important factor to control the differentiation of new vascular tissues during the formation of the rootstock–scion union and consequently determine compatibility or incompatibility between parties (Aloni and others 2010). In addition, the transport of small substances, such as phytohormones, through the xylem and phloem can control several other processes of plant development (Notaguchi and Okamoto 2015; Zadnikova and others 2015; Davière and Achard 2016). This has been unveiled by performing elegant grafting experiments that have confirmed the mobility of

various hormones as well as their precursors towards sink tissues. For example, it is well established that auxin acts as a critical factor in inhibiting axillary bud outgrowth. Beveridge and others (2000), using two pea (*Pisum sativum* L.) mutants with increased branching, *ramosus* (*rms*) 1 and 2, noted that the response to exogenous auxin was massively diminished in decapitated *rms1* and *rms2* mutant plants, but the auxin response was restored when *rms1* or *rms2* were grafted onto wild-type (WT) plants. They found that the genes *Rms1* and *Rms2* regulate a graft-transmissible substance from which auxin response is dependent. Interestingly, a higher auxin content was observed in the shoots from mutants, and cytokinin (CK) levels were reduced in the root sap of WT. These results provide evidence that increased branching is not due to enhanced CKs in the shoot but to some other unknown root-derived compound (Beveridge and others 1994, 1997). Currently, it is known that *rms1* and *rms2* mutants are strigolactone (SL) deficient. SLs are a new root-derived class of hormones involved in many plant processes, and the grafting process was essential to determine their role in plant development. They are able to induce the germination of parasitic plants, behave as signals for the establishment of arbuscular mycorrhizal fungi symbiosis, and inhibit axillary bud outgrowth. Booker and others (2005) carried out grafting experiments in *Arabidopsis* using the *max1* mutant, which is defective in a key enzyme of SL biosynthesis that converts carlactone (CL) to active SL, and *max4* (equivalent to *rms1* in pea) or *max3*, which acts upstream of *max1* in the SL biosynthetic pathway. When *max4* or *max3* were grafted onto *max1* rootstock, the WT phenotype was restored. This revealed that CL accumulated in the *max1* roots is transported to the shoot and converted in active SL controlling axillary bud outgrowth.

Auxin also regulates lateral root formation through its transport from shoots and redistribution in roots, and these responses are dependent on the DIAGEOTROPICA (DGT) protein. DGT seems to affect the localization of PIN-FORMED (PIN) proteins, auxin efflux transporters, and auxin transport is consequently altered; therefore, the *dgt* tomato mutant exhibits reduced lateral root formation (Retzer and Luschnig 2015; Spiegelman and others 2017). Grafting *dgt* roots with WT shoots rescues the normal development of lateral roots, consistent with participation of a mobile signal, but cannot be explained only by DGT modulating auxin transport and requires a more complex way of affecting auxin signaling (Ivanchenko and others 2015). Additionally, Guo and others (2016) used *dgt* and *phyB* tomato mutants in reciprocal grafting with WT to demonstrate that photosynthetic CO₂ assimilation is systemically induced by red light. Red light sensed by phyB in the upper leaves activates the synthesis of auxin that is transported downward, where it induces hydrogen peroxide (H₂O₂) accumulation, which triggers cyclic electron flow

and ATP production. The authors observed that whenever a scion carries a mutant *dgt* or *phyB*, the photosynthesis activated by red light is altered.

In addition, auxin seems to exert an influence on the symbiotic development of nitrogen-fixing nodules. Lohar and VandenBosch (2005) performed reciprocal grafting experiments between *Lotus japonicus* and *M. truncatula* nodulated by *Mesorhizobium loti* and *Sinorhizobium meliloti*, respectively. Although *L. japonicus* roots grafted on *M. truncatula* shoots or self-grafted exhibited normal nodulation by *M. loti*, plants of *M. truncatula* roots grafted on *L. japonicus* shoots were unable to establish symbiosis with *S. meliloti*. These results indicate that symbiosis between *M. truncatula* and *S. meliloti* is dependent on a shoot-derived signal. This signal may be auxin, because its level and transport correlate with nodulation (Noorden and others 2006), although more studies are necessary to determine the exact role of auxin during establishment and development of symbiosis.

Cytokinins (CKs) are phytohormones that play a central role in numerous developmental and physiological processes in plants, such as cell division, root and shoot growth, cambial proliferation, stress and nutritional responses, and senescence (Huang and others 2012; Murai 2014). They are mainly synthesized in root and shoot vasculature tissue and transported both upward and downward to sink tissues, where they coordinate plant growth. The transport of CKs from the root toward the shoot was demonstrated by grafting experiments using mutants of CK-biosynthetic genes. For example, Matsumoto-Kitano and others (2008) used an *Arabidopsis* quadruple knockout mutant of CK synthesis genes, adenosine phosphate isopentenyl transferase (*IPT*) 1;3;5;7 (*ipt1;3;5;7*). They observed that when they grafted *ipt1;3;5;7* onto WT rootstock, both the root and shoot grew normally and CKs levels were restored. The same occurred with the reciprocal grafts. Similarly, after grafting an *Arabidopsis* double mutant of a *trans*-zeatin-type CK synthesis genes (cytochrome P450 monooxygenase *CYP735A1* and *CYP735A2*), Kiba and others (2013) found that the WT acts as a source of *trans*-zeatin (*tZ*) to the mutant and induces its normal growth. Recently, Osugi and others (2017) performed a series of micrografting experiments to confirm that *tZ* and *trans*-zeatin riboside (*tZR*) are the major CK long-distance signal transported by xylem vessels. Therefore, the CK-biosynthetic septuple mutant of *Arabidopsis* (*log1234578*; called as *logS*) was grafted onto WT or *abcg14* (a transportation mutant, as will be explained below). When grafted onto WT, *logS* scions exhibited increased *tZ* content in leaves and recovery of shoot phenotype comparing to non-grafted *logS* and reciprocal-grafted *logS/abcg14* (Osugi and others 2017). Further, *logS*/WT plants also had higher *tZ* and *tZR* contents in xylem exudates, indicating that both *tZ* and *tZR* act as a dual long-distance signal which permits

plants to fine-tune growth in response to environmental cues (Osugi and others 2017).

Grafts have also been extremely useful for studying the mechanisms underlying the transport of CKs, which were not known until recently. The *Arabidopsis abcg14* mutant with loss of expression of an ATP-binding cassette (ABC) transporter subfamily G14 (*ABCG14*) resulted in severe shoot growth retardation and increased CK content in the roots (Ko and others 2014; Osugi and others 2017). However, the grafting of *abcg14* scions onto WT roots rescued shoot growth, whereas the grafting of WT scions onto *abcg14* roots showed reduced shoot growth, similar to the mutant (Ko and others 2014). This result demonstrates that *ABCG14* is a key factor for CK transport to the shoot.

Gibberellin (GA) is also a plant hormone related to plant growth and development that plays critical roles during seed germination, floral initiation, and internode elongation (Hedden and Thomas 2012). Numerous studies have provided evidence of long-distance transport of GAs (Eriksson and others 2006; Shani and others 2013). For example, Katsumi and others (1983) grafted seedlings of WT and dwarf gibberellin mutants (*dwarf₁* and *dwarf₅*) of maize (*Zea mays*) and observed that dwarf scions grafted onto WT showed normal development with rescued internode elongation. Recently, *Arabidopsis* GA-deficient mutants altered at early (*ga1-3* mutant), intermediate (*kaol kao2* mutant), and late steps (*ga20ox1 ga20ox2 ga20ox3* and *ga3ox1 ga3ox2* mutants) of the GA biosynthetic pathway, and their respective WT plants were used to perform a series of micrografting experiments (Regnault and others 2015). This work revealed that WT rootstock was able to restore the WT phenotype in *ga1-3* and *kaol kao2* mutant scions, providing evidence that there is transport of GA from the root to the shoot. However, WT rootstock did not affect the phenotypes of *ga20ox1 ga20ox2 ga20ox3* and *ga3ox1 ga3ox2* scions. These results indicated that GA₁₂, the substrate of GA 20-oxidases, is the graft-transmissible signal (Regnault and others 2015).

In addition to the roles of hormones in plant growth, grafting experiments have been used often for studying plant responses to environmental stresses. For instance, ABA is an important player in adaptive responses of plants to abiotic stress, such as stomatal closure and root hydraulic conductance. In tomato plants, the ABA-deficient mutant (*flacca*) exhibited partial phenotypic reversion when grafted onto the WT rootstock (Dodd and others 2009). However, the partial restoration of growth was associated with normalizing shoot-ethylene relationships and does not diminish plant transpiration rates. Similarly, Holbrook and others (2002) performed several grafting experiments using two tomato ABA-deficient mutants, *flacca* and *sitiens*. They grafted the WT scion onto a split-root system consisting of WT roots grafted to mutant roots, making it possible to withdraw water from one root system while maintaining sufficient

water status in the shoot. They observed that stomata closure occurs when irrigation is stopped, independent of root genotype, but it was scion-dependent. This provided evidence that stomata closure occurs even without loss of turgor in the shoot and does not demand ABA synthesis in roots. There must be other biochemical signal from roots that triggers stomata closure rather than ABA.

Another important plant hormone involved in vegetable responses to both biotic and abiotic stresses are the jasmonates (JA). JA are oxygenated lipids (oxylipins) which regulate, among many others, the responses to wounding (Koo and Howe 2009). Thus, to discover the role of JA signaling at the whole plant level during wounding, the WT and JA-deficient *Arabidopsis* mutant *aos*, defective in a gene encoding a key enzyme of JA synthesis pathway, both containing the *JAZ10p::GUS* reporter were employed in grafting experiments (Gasparini and others 2015). WT self-grafted plants exhibited a strong activation of the *JAZ10p::GUS* reporter in both shoot and root organs in response to leaf wounding (Gasparini and others 2015). On the other hand, self- and reciprocal-grafted *aos* plants (that is, *aos/aos* and *aos*/WT, respectively) were unable to respond to wounding treatment. However, when the WT was used as the scion on *aos* rootstock a strong activation of the reporter throughout the plant after wounding leaf treatment, similar to WT self-grafted plants, was observed (Gasparini and others 2015). These results provide evidence for the existence of a JA shoot-to-root transport in response to leaf wounding.

RNAs

Currently, various experiments have discovered other compounds acting in long-distance signaling. Many researchers have found numerous RNA species, such as small-interfering RNA (siRNA), microRNA (miRNA), mRNA, and viral RNA, in the phloem sap (see review by Ham and Lucas 2017). In addition, studies have indicated that siRNA (Bai and others 2011), miRNA (Bhogale and others 2014; Li and others 2014), mRNA (Harada 2010; Notaguchi 2015; Zhang and others 2016), and viral RNA (Ali and others 2016) are all graft-transmissible. For example, Notaguchi and others (2015), using the heterografting system with *Nicotiana benthamiana* as the scion and *Arabidopsis* as the rootstock, identified 138 transcripts in *Arabidopsis* as mRNAs that moved long distances across the graft junction. Similarly, the reciprocal micrografting of highly divergent ecotypes of *Arabidopsis* Col-0 and Ped-0 resulted in the detection of an incredible number of 2006 mobile mRNA species (Thieme and others 2015). In this experiment, a large part of these transcripts were transported in a shoot-to-root direction by the phloem. However, they also realized a high number of mobile RNAs move from rootstock to specific tissues in the scion (Thieme and others 2015). Interestingly, the proteomic

analysis indicated that mobile RNAs can be translated at their destination tissues and so modulate the plant development (Thieme and others 2015). Further, in an experiment grafting grapevines, more than 3000 mRNAs were identified as being transported directionally or bi-directionally between the rootstock and the scion (Yang and others 2015). Similar results were obtained by Zhang and others (2016); they in turn suggest that mRNA mobility is dependent on tRNA-derived sequences. In contrast, Calderwood and others (2016) verified that mRNA mobility is associated with its abundance and half-life. Actually, this issue remains to be better clarified and should receive additional attention in the coming years.

miRNA has also been shown to be integrated in long-distance signaling systems and participates in many plant responses to internal and external changes, such as phosphate (Pi) homeostasis (Pant and others 2008; Huen and others 2017), drought stress responses (Pagliarini and others 2017), and potato (*Solanum tuberosum*) tuberization (Bhogale and others 2014). For instance, potato tuberization is an event strongly regulated by external and internal factors, including photoperiod and phytochrome B (PHYB), respectively. In addition to the involvement of PHYB, recent studies have demonstrated that microRNA *172* (*miR172*) plays an important role in the control of flowering and vegetative phase change (Jung and others 2007). Potato plants overexpressing *miR172* (*miR172-OE*) exhibit accelerated tuberization, even when grown under non-inductive conditions such as long days. Interestingly, a scion expressing *miR172-OE* grafted onto WT tuberized as early as *miR172/miR172*, providing evidence that the effect of *miR172* on tuberization is transmissible through grafts and that its overexpression in the shoot regulates tuberization (Martin and others 2009). Furthermore, Bhogale and others (2014) observed the presence of *miR156* in the phloem sap of potato plants heterografted between *miR156* and WT. They proposed that *miR156* transport modulates plant architecture and tuberization in potatoes. In a similar experiment, Kim and others (2001) showed that the transcript resulting from chromosomal rearrangements involving the fusion between *LeT6* and *PEP* was responsible for the dominant characteristic in the tomato mutant called Mouse ears (*Me*) and was graft-transmissible. Grafting WT shoots onto *Me* rootstock induced the development of new leaves with *Me* morphology in the shoot; moreover, they detected the *Me* transcript present in the shoot meristem. These findings show that long-distance transport of RNAs from the source organ to the recipient organ controls plant development and morphology.

Likewise, studies on siRNA indicate that they can play a crucial role in systemic acquired resistance (SAR) to pathogens and gene silencing in plants (Molnar and others 2010; Ali and others 2016; Lewsey and others 2016). For example,

Ali and others (2016) performed grafting experiments using non-transgenic tomato scions grafted onto the tobacco Sd1 (a cross between *NiTOM1* and *NiTOM3*) rootstocks, in which the expression is silenced for both the *NiTOM1* and *NiTOM3* genes that are required for tobacco virus multiplication. RT-PCR analysis of the scion revealed reduced levels of mRNA after grafting in both *LeTH3* and *LeTH1*, tomato homologs of *NiTOM1* and *NiTOM3*, respectively; siRNA from both genes was detected in the tomato. In this way, the grafting onto tobacco Sd1 rootstock was able to provide virus resistance to the scion (Ali and others 2016). Frequently, small RNAs moving from the root to the shoot have been identified. For example, Bai and others (2011) used transgenic plants of *N. benthamiana* to observe that siRNA signals translocated across the graft union in both shoot-to-root and root-to-shoot. Indeed, the inhibition of viral infection by siRNA can occur in both short and long distances through phloem sap flow (Patil and Fauquet 2015).

Peptides and proteins

In addition to phytohormones and RNAs, it was recently found that small peptides are transported via the xylem stream from roots to shoots and can control nodule formation and nitrogen (N) starvation responses (Okamoto and others 2013; Tabata and others 2014). As mentioned previously, leguminous plants are capable of establishing rhizobia symbiosis and form nodules on their roots to fix N. However, excessive nodulation inhibits host plant growth because of the large energy cost. Therefore, plants regulate nodule formation through complex root-to-shoot-to-root long-distance feedback loops, known as the autoregulation of nodulation (AON) (Caetano-Anolles and Gresshoff 1991; Huault and others 2014; Notaguchi and Okamoto 2015). Until recently, the molecules involved within those long communications remained unknown. However, recent studies confirmed that oligopeptide transport from roots to the shoots exerted suppression of nodule formation (Lee and others 2012; Tabata and others 2014). For example, the CLE-root signal 2 (CLE-RS2), a glycopeptide, has been hypothesized as the root-derived signal in *L. japonicas*, because its expression in roots is greatly upregulated by rhizobia inoculation (Okamoto and others 2009). CLE-RS2 inhibits nodule formation, and its active form, a glycosylated 13-amino acid oligopeptide, is translocated from the root to the shoot via the xylem stream, where it binds to the HAR1 receptor kinase, an important shoot factor for AON (Krusell and others 2002; Okamoto and others 2013). In this way, CLE-RS2/HAR1 may integrate systemic signaling related to nitrate inhibition of nodulation. Grafting experiments with *L. japonicas* between the *har1* hypernodulation mutant and WT were performed by Okamoto and Kawaguchi (2015) to clarify this question. In a low nitrate environment (0.5 mM

KNO_3), a *har1* scion grafted onto WT (*har1*/WT), showed a hypernodulation phenotype, whereas a WT scion grafted onto *har1* (WT/*har1*) showed the normal nodulation. On the other hand, when grafting combinations were exposed to a high nitrate environment (10 mM KNO_3), known to inhibit nodulation, no reduction in the number of nodules was detected on *har1*/WT, whereas WT/*har1*-grafted plants exhibited high suppression of nodulation. Based on these results, and knowing that *CLE-RS2* expression is regulated by both rhizobial inoculation and nitrates, the authors proposed a model of long-distance signaling where HAR1 is a key factor in nitrate inhibition of nodulation and AON.

Another family of C-terminally encoded peptides, CEP, plays an important role in root-to-shoot signaling in N starvation conditions. Plants are often exposed to unequal conditions of N distribution in soil, and it is necessary to adjust a compensatory system to coordinate root development and improve N uptake. Tabata and others (2014) observed that CEP1 application regulates *NRT2.1* expression, a gene that encodes a N transporter. They used an *Arabidopsis* double mutant defective for CEP receptors (*cepr1-1 cepr2-1*) and WT, and found that the CEP1-upregulated N transporter is dependent on *CEPRs* in the shoot, and the CEP1 signal is graft-transmissible across the graft union. Additionally, *CRA2*, a homolog of the *CEPR1* receptor gene, was identified from *M. truncatula*, and grafting experiments performed between *M. truncatula cra2* mutant and WT showed that *CRA2* controls lateral root development in a local manner by short-communication and nodule formation in a systemic manner by long-distance communication from the shoot (Huault and others 2014).

Studies of phloem stream content have revealed a great number of proteins (Lifschitz and others 2014). However, the importance of protein transport as long-distance signals is well established only for *FLOWERING LOCUS T* (*FT*). In *Arabidopsis*, *FT* is expressed in both cotyledons and leaves under inductive long days (LDs), but it needs to be transported from organs, where it is expressed to the shoot apex to induce target genes, such as *APETALA1* (*API*), and initiate floral morphogenesis (Corbesier and others 2007; Jaeger and Wigge 2007; Notaguchi and others 2008). Despite its importance to flowering initiation, the mechanisms underlying *FT* protein translocation are largely unknown. Recently, it was found that an FT-INTERACTING PROTEIN 1 localized to the membrane of the endoplasmic reticulum plays a critical role in protein loading of *FT* from companion cells into the sieve element of the phloem system (Liu and others 2012). Notaguchi and others (2008) used the *Arabidopsis ft* mutant and WT to perform micrografting experiments and found that the *FT* protein is transported from a scion donor to the apical meristem of the recipient stock. Likewise, *Arabidopsis* has a close homolog of *FT* called *TWIN SISTER OF FT* (*TSF*), and grafting between *ft tsf* scion and

TSF-overexpressing rootstock exhibited only a slight acceleration of floral induction. By contrast, when the double mutant *ft tsf* was grafted onto FT plants, they flowered early, providing evidence that the TSF protein has lower mobility than the FT protein, and its mobility is dependent specifically on the FT protein region II domain (Jin and others 2015).

An interesting case of crosstalk between a protein and a hormone is revealed in tomatoes. The synthesis and translocation of a cyclophilin, SICyp1, is regulated by light. SICyp1 is produced in leaves as FT but is translocated in the downward direction, where it coordinates root architecture changes, influencing growth at the whole plant level (Ivanchenko and others 2015). The response to SICyp1 long-distance communication is mediated by auxin, as shown by grafting tomato *dgt* plants and WT. Mutant plants exhibited limited root and shoot growth; however, WT/*dgt* and *dgt*/WT showed restored root and shoot development, respectively. SICyp1 seems to control plant development by upregulating key enzymes of auxin biosynthesis and signaling, as seen in the heterografted plants (Spiegelman and others 2015, 2017). However, further studies are necessary to completely understand the mechanisms involved between auxin x SICyp1 crosstalk.

Finally, it has been proposed that transgenic rootstocks can induce tolerance to diseases or abiotic stress. For example, expressing polygalacturonases-inhibiting protein (PGIP) in transgenic plants used as rootstock can induce disease tolerance through translocation of the PGIP protein to the non-expressing scion. Tolerance would be induced by the inhibition of polygalacturonases, often the first enzyme secreted during infection, that are responsible for cell separation and tissue degradation (Haroldsen and others 2012).

Oxidative stress molecules

So far, we have seen that numerous molecules such as hormones, peptides, proteins, and RNAs are involved in the perception and signaling of various environmental cues. Moreover, these environmental conditions, mainly adverse conditions, can lead to rapid generation of oxidative stress resulting from high production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), and hydroxyl radicals (HO^\cdot) (Foyer and Noctor 2000). The occurrence of oxidative stress is usually scavenged by a complex antioxidant system involving enzymatic components, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and guaiacol peroxidase (GPOX), as well as non-enzymatic components, such as ascorbate (AsA), glutathione (GSH), and other organic acids (Gratão and others 2005). Most often, the stressor agent is in direct contact with just a small part of the plant or organ, but its negative effects can reach

the whole plant. Thus, a quick and efficient stress signaling system is necessary among the most distant organs and the region in direct contact with the stressor to allow sufficient time for activation of the antioxidant system and plant responses and to minimize the effects of oxidative stress. For instance, Jozefczak and others (2014) found that only 24 h after exposure of *Arabidopsis* roots to cadmium (Cd) stress, there was a clear increase of antioxidant defense systems, both enzymatic, such as APX and GR, as well as non-enzymatic, such as GSH in leaves, despite reduced Cd transport to the shoot and without increased lipid peroxidation in the leaves. The authors suggest the existence of a signal from the roots to the shoot that preventively activates the antioxidant system. Recently, some studies have shown that the use of grafting can be a way to unravel how oxidative stress is signaled to the whole plant. Xin and others (2013) used two distinctive cultivars of water spinach (*Ipomoea aquatica* Forsk.) for Cd accumulation in the shoot: high-Cd ('T308') and low-Cd accumulation ('QLQ') cultivars. They performed reciprocal grafting between both cultivars and verified that Cd translocation root-to-shoot is root-dependent; thus, 'QLQ' rootstock induced lower Cd accumulation in the shoot independent of the cultivar used as the scion. On the other hand, when 'T308' was used as rootstock, an increase of Cd accumulation in the scion in both 'QLQ' and 'T308' was observed. Furthermore, an elegant experiment with tomato plants revealed a clear influence of the rootstock on the scion in response to Cd-stress when plants of cv Micro-Tom, previously exposed to Cd-stress or not, were combined by grafting (Gratão and others 2015). Employment of roots previously exposed to Cd-stress as rootstocks induced an H_2O_2 accumulation in non-stressed scions greater than scions stressed with Cd. In addition, they verified that activity of antioxidant enzymes was greater in the non-stressed rootstock grafted with shoots exposed to Cd-stress than in rootstocks previously stressed but grafted with scions not exposed to Cd (Gratão and others 2015). Moreover, to establish the hormonal basis of Cd-stress signaling between root and shoot, Alves and others (2017) performed a grafting experiment with hormone tomato mutants combined with their wild-type counterpart, Micro-Tom, under Cd-stressful condition. They use ethylene-insensitive (*Never ripe*, *Nr*) and auxin-insensitive (*dgt*) as rootstock for Micro-Tom scion and observed that both mutant rootstocks modulated root-to-shoot signaling, interfering with antioxidant responses to Cd-stress. It was suggested that ROS could act as a main signal of oxidative stress between the parts of the plant. Lately, various experiments have demonstrated that ROS, mainly H_2O_2 , are key mediators of long-distance signaling in plants in response to pathogen infection and wounding (see review by Gilroy and others 2016; Choi and others 2017). In fact, the grafting experiments have outstanding advantages to unveil the underlying mechanism related to oxidative stress

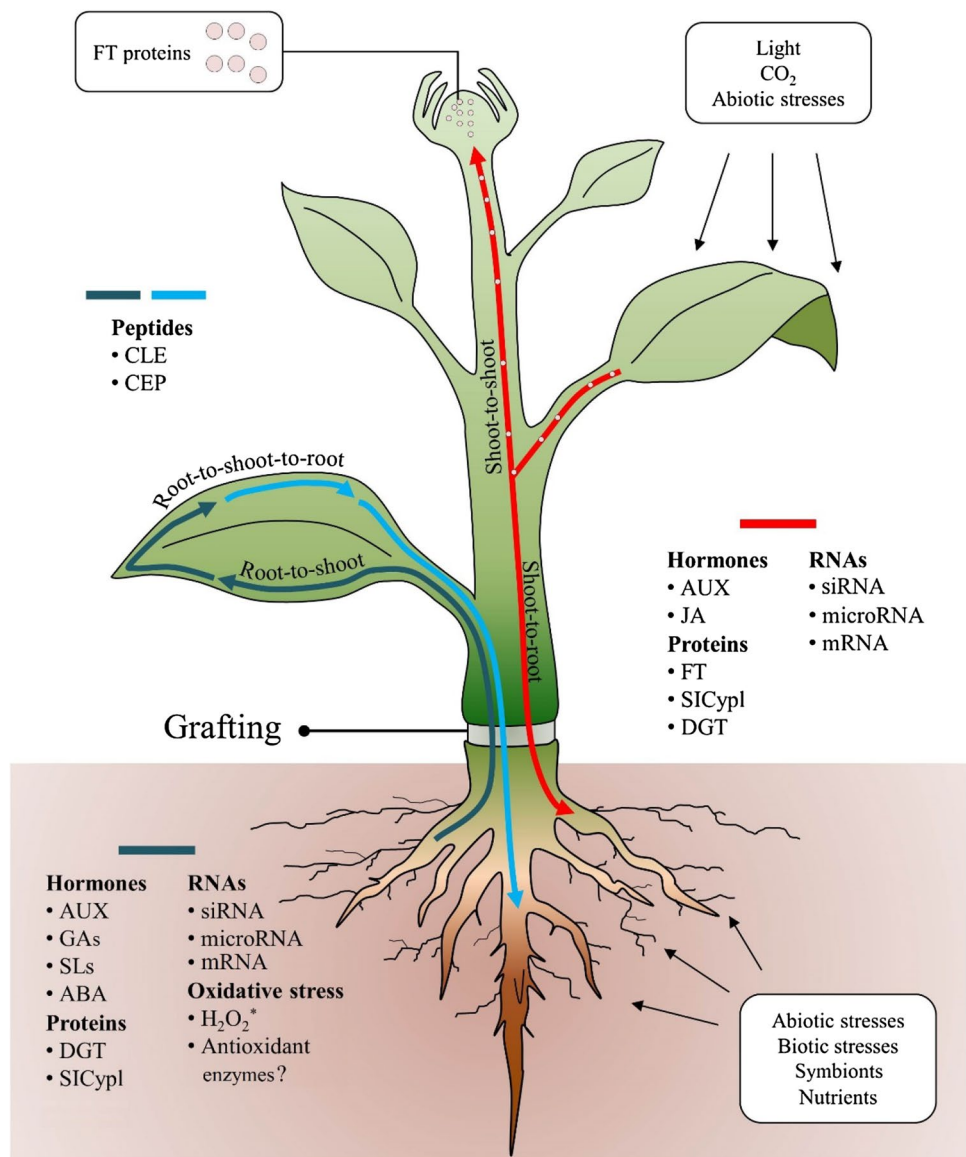
signaling. Despite this fact, little progress has been made to date, leaving a large gap in oxidative stress signaling to be exploited in the future.

Epilogue

Great breakthroughs have been achieved in the knowledge about the long-distance transport of substances and signals controlling several plant processes, such as root development, symbiotic establishment, flowering, and responses to light or abiotic and biotic stress (Fig. 1). These discoveries have occurred through the advances of analytical techniques as well as the generation of mutants and transgenic plants altered in target molecules. In this context, the establishment of suitable grafting protocols for different plant species, especially model plants, have greatly contributed to

the progress of understanding how plants integrate shoot and root growth in response to environmental changes. For example, environmental cues perceived by roots can require signaling molecules to be transported via vascular bundles to shoots for modulating a wide range of responses. Although different factors, such as plant hormones, RNAs, small peptides, molecules of oxidative stress, and proteins, were individually addressed in this review, it is plausible to hypothesize the occurrence of wide crosstalk among them, forming an integrated signaling network of root-to-shoot-to-root long-distance communication. Therefore, further studies using new mutants and transgenic plants for more than one factor and grafting as a tool for biological research allowing union between different parts of plants will certainly contribute to our understanding of the underlying mechanisms of plant development.

Fig. 1 Overview of bidirectional communication between root and shoot. The grafting experiments have provided evidence of long-distance signaling triggered for environmental cues. Thereby, adaptive responses are controlled by compounds such as hormones, proteins, RNAs, and peptides transported via vascular bundles moved from “root-to-shoot,” “shoot-to-shoot,” or “root-to-shoot-to-root” to coordinate modifications in whole plant. *AUX* auxin, *CKs* cytokinins, *GAs* gibberellins, *SLs* strigolactones, *ABA* abscisic acid, *JA* jasmonic acid, *DGT* DIAGEOTROPICA protein, *SICypl* a cyclophilin protein, *siRNA* small-interfering RNA, *CLE* CLE glycopeptide, *CEP* CEP glycopeptide, *FT* FLOWERING LOCUS T, *H₂O₂** hydrogen peroxide (asterisk indicates putative long-distance signal triggered in response to oxidative stress)



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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest regarding the publication of this paper.

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