Journal of Experimental Botany, Vol. 74, No. 8 pp. 2433–2447, 2023
https://doi.org/10.1093/jxb/erad061 Advance Access Publication 27 February 2023

DARWIN REVIEW

Grafting in plants: recent discoveries and new applications

Grégoire Loupit[1,†], Lysiane Brocard[2,†], Nathalie Ollat[1] and Sarah Jane Cookson[1,*]

[1] EGFV, Université de Bordeaux, Bordeaux Sciences Agro, INRAE, ISVV, F-33882, Villenave d'Ornon, France
[2] Université de Bordeaux, CNRS, INSERM, Bordeaux Imaging Center, BIC, UMS 3420, US4, F-33000 Bordeaux, France

[†] These authors contributed equally to this work.
[*] Correspondence: sarah.cookson@inrae.fr

Received 13 October 2022; Editorial decision 31 January 2023; Accepted 14 February 2023

Editor: John Lunn, MPI of Molecular Plant Physiology, Germany

Abstract

Grafting is a traditional horticultural technique that makes use of plant wound healing mechanisms to join two different genotypes together to form one plant. In many agricultural systems, grafting with rootstocks controls the vigour of the scion and/or provides tolerance to deleterious soil conditions such as the presence of soil pests or pathogens or limited or excessive water or mineral nutrient supply. Much of our knowledge about the limits to grafting different genotypes together comes from empirical knowledge of horticulturalists. Until recently, researchers believed that grafting monocotyledonous plants was impossible, because they lack a vascular cambium, and that graft compatibility between different scion/rootstock combinations was restricted to closely related genotypes. Recent studies have overturned these ideas and open up the possibility of new research directions and applications for grafting in agriculture. The objective of this review is to describe and assess these recent advances in the field of grafting and, in particular, the molecular mechanisms underlining graft union formation and graft compatibility between different genotypes. The challenges of characterizing the different stages of graft union formation and phenotyping graft compatibility are examined.

Keywords: Cell wall, grafting, phloem, plasmodesmata, rootstock, scion, vascular connection, xylem.

Introduction

Grafting has been utilized by humans for thousands of years (Mudge *et al.*, 2009). Today, much of the commercial production of fruit (and some vegetables) relies upon grafting with rootstocks to provide resistance to soil-borne pathogens and abiotic stresses as well as to modify scion vigour and performance (Fig. 1D, E). Grafting also occurs naturally; although stem grafts are easy to observe in nature, they are generally rare. However, a few species readily form natural stem grafts, such as English Ivy and strangler figs (Fig. 1A) (Mudge *et al.*, 2009); the high degree of natural grafting in these species could be a consequence of their climbing habits. Natural root grafts (Fig. 1C) are relatively common although hard to observe in nature, and may have both positive consequences for plant growth and development, and negative consequences for pathogen propagation (Lev-Yadun, 2011).

In addition to using grafting to improve horticultural production and scientifically to characterize rootstock and scion genotypes (Fig. 1B), grafting has the potential to generate new

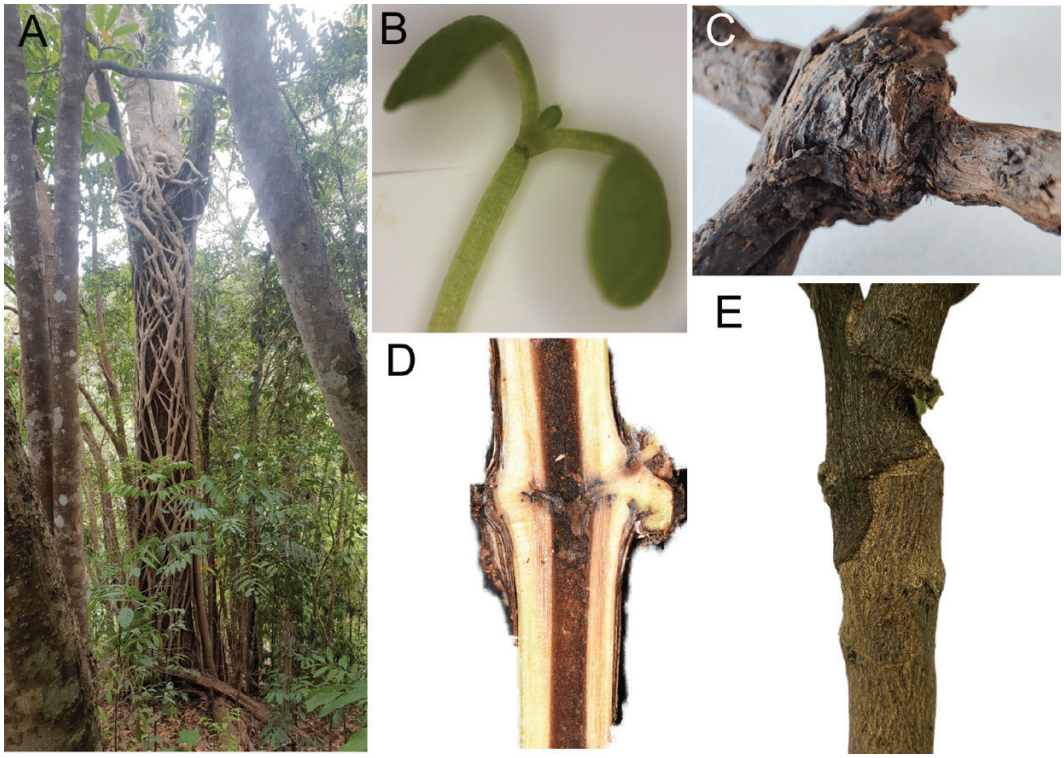


Fig 1. Photographs of natural and artificial plant grafts: (A) strangler fig (photograph courtesy of Rachel Adamson), (B) Arabidopsis hypocotyl graft (photograph courtesy of Clément Chambaud), (C) natural grapevine root graft, (D) 1-year-old artificial grapevine graft cut in half, and (E) mandarin graft.

species resulting from the local horizontal transfer of genetic material between the two genotypes of grafted plants (Stegemann and Bock, 2009; Stegemann *et al.*, 2012; Fuentes *et al.*, 2014; Gurdon *et al.*, 2016). Grafting can be used to produce new combinations of plastid and mitochondrial genomes (Stegemann and Bock, 2009; Stegemann *et al.*, 2012; Gurdon *et al.*, 2016) as well as allopolyploids between distantly related species (Fuentes *et al.*, 2014). This asexual method of producing allopolyploids overcomes many sexual barriers to sexual hybridization (Fuentes *et al.*, 2014). There is also evidence of horizontal gene transfer of mitochondrial DNA from many sources, including epiphytic mosses to the basal angiosperm, *Amborella trichopoda* (Bergthorsson *et al.*, 2004). As epiphytic and parasitic plants are abundant in the natural habitat of *A. trichopoda* and parasitic plants are known to transfer genes to their hosts (Mower *et al.*, 2004), this could be a source of this high level of horizontal gene transfer in *A. trichopoda*.

In this review, we provide an overview of graft union formation, from the initial wounding response to changes in the cell wall and the formation of vascular connections between the scion and rootstock. In addition, we describe the factors affecting successful grafting when different species are grafted together and our knowledge of the causes of graft incompatibility. Genes differentially expressed in response to grafting are discussed, as well as challenges to studying grafting and new potential applications for grafting in agriculture.

Identifying genes involved in graft union formation

Gene expression studies on graft union formation have been done to identify the genes and processes involved in a number of species including both annuals (Yin *et al.*, 2012; Melnyk *et al.*, 2018; Xie *et al.*, 2019; Reeves *et al.*, 2021) and perennials (Cookson *et al.*, 2013, 2014; Qiu *et al.*, 2016). These studies include characterization of gene expression in homo-grafts (Yin *et al.*, 2012; Cookson *et al.*, 2013; Melnyk *et al.*, 2018; Reeves *et al.*, 2021), and compatible and incompatible hetero-grafts covering dicot (Cookson *et al.*, 2014; Assunção *et al.*, 2019; Thomas *et al.*, 2021; Ji *et al.*, 2022), monocot (Reeves *et al.*, 2021), and interfamilial (Notaguchi *et al.*, 2020) grafts. Control samples generally include homo-grafts (for hetero-graft studies), intact plants, wounded tissues, and non-grafted scions and rootstocks. These gene expression studies tend to show that similar genes are differentially expressed during graft union formation in the different systems studied (Xie *et al.*, 2022), and there is even a degree of conservation in the gene expression responses between monocotyledonous and dicotyledonous plants (Reeves *et al.*, 2021). More genes involved in graft union formation are being identified. However, using gene expression analysis to identify genes involved in different aspects of graft union formation is challenging because the graft interface is a heterogeneous, complex tissue, comprised

of different cell types with different responses. For example, those cells that are involved in vascular reconnection may be very sparsely distributed in other tissues present at the graft interface (Thomas *et al.*, 2021). New techniques could be applied to the study of grafting to elucidate the roles of each tissue, such as *in vitro* callus grafting to study plasmodesmata formation at the callus graft interface (Machin *et al.*, 2022) and single-cell transcript profiling (Christiaens *et al.*, 2021).

Wounding responses and cell wall modifications at the graft interface

The speed of graft union formation depends upon the species and environmental conditions, but we assume that there is some degree of conservation of the sequence of events occurring at the graft interface across different species (Fig. 2). The first stage of graft union formation is presumably an initial wound response; damaged cells at the graft interface collapse, and electric and ion flux signals are activated within minutes, followed by the accumulation of reactive oxygen species (ROS) and wound-related hormones (Savatin *et al.*, 2014). The remains of cellular debris and necrotic tissue can accumulate at the graft interface and, in many cases, a necrotic layer forms at least in some areas of the graft interface even in homo-grafts (Moore and Walker, 1981a, b; Flaishman *et al.*, 2008). Recent work suggests that sensing cell wall damage is key to activating wound healing responses and graft union formation (Zhang *et al.*, 2022). Zhang *et al.* (2022) demonstrated that modifying cell walls by enzymatic digestion or genetic modification activates the expression of some transcription factors regulating wounding responses and graft union formation (Zhang *et al.*, 2022).

Within 6 h after grafting, dictyosomes (flattened plates or double lamellae, which are part of the Golgi apparatus) accumulate along the cell walls next to the graft interface of *Sedum telephoides* auto-grafts and appear to fuse with the plasma membrane. These structures potentially extrude their contents at the graft interface (Moore and Walker, 1981a, b). Dictyosomes and Golgi vesicles are also observed at 5 days after grafting (DAG) in *Vicia faba*/*Helianthus annuus* grafts (Kollmann and Glockmann, 1991). There is evidence that the Golgi apparatus is involved in the synthesis and secretion of components of the cell wall in plants (Driouich *et al.*, 2012), suggesting that this could be one of the earliest responses to grafting. The presence of dictyosomes disappears quickly over time in *S. telephoides* auto-grafts. As the presence of dictyosomes occurs before tissue adhesion (which typically occurs with 24–48 h), it is possible that these dictyosomes are involved with generating adhesion between the scion and rootstock. Bead-like structures appear on the surface of cells at the graft interface; these beads stain with ruthenium red and are removed by a 30 min 1% pectinase treatment (Jeffree and Yeoman, 1983), suggesting that they are rich in pectins. This extracellular pectin-rich cement is thought to adhere the scion and rootstock together. A recent SEM study on hypocotyl grafts of Arabidopsis shows that extracellular fibrillar or homogenous material covered the surface of the graft interface (Sala *et al.*, 2019).

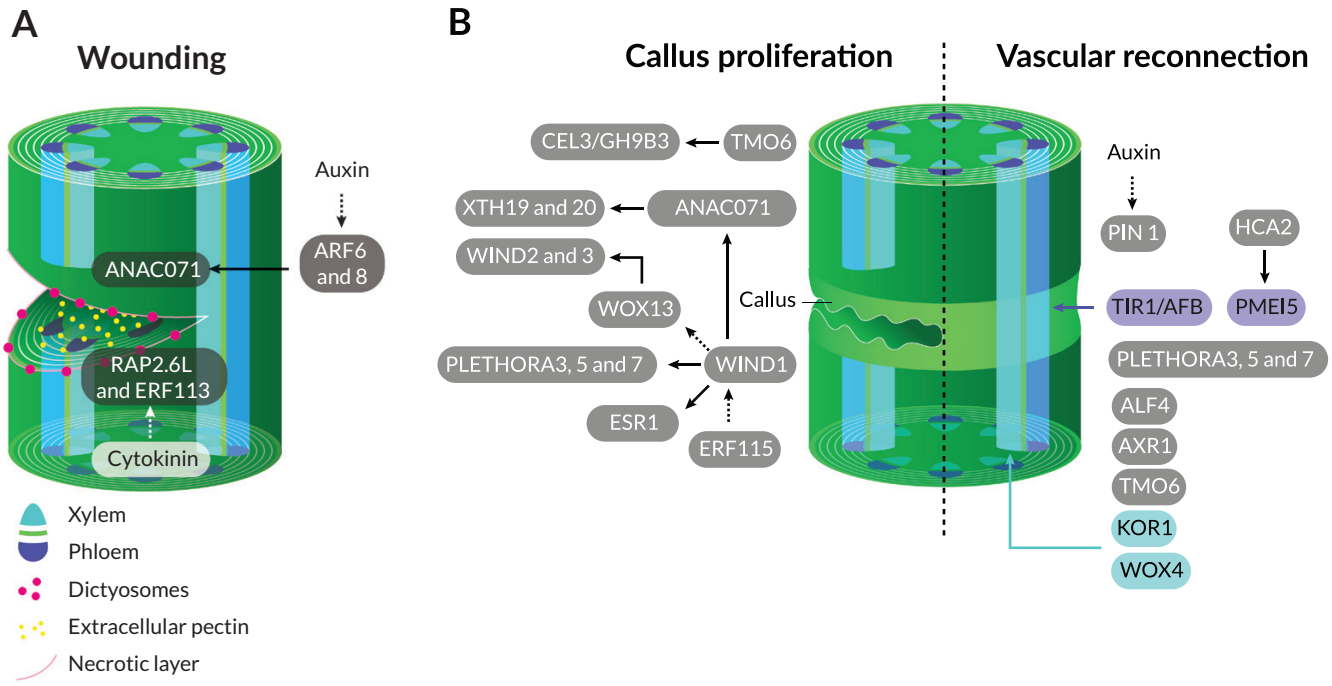


Fig. 2. Schematic diagram of proteins involved in (A) wounding responses and (B) callus proliferation and graft union formation. Proteins involved in xylem and phloem formation during grafting are shown in turquoise and purple, respectively. GH9B3 was identified in tobacco, WOX4 was identified in tomato, and all the other genes were identified in Arabidopsis.

Callus cells had a speckled surface, suggesting that this material has a role in the adhesion between new divided cells. Whereas the identity of these extracellular structures was not fully confirmed, parallel immunofluorescence experiments suggested that they could be composed of unmethylesterified homogalacturonan and extensins (although some weak detection of methlyesterified homogalacturonans was observed on the cut surfaces of the scion and rootstock) (Sala *et al.*, 2019). Another study has used cell wall fractionation, Fourier transform infrared spectroscopy, GC, and immunodot assays to study the cell wall at the graft interface (Frey *et al.*, 2022). Frey *et al.* (2022) reported that both unmethylesterified and methylesterified homogalacturonans accumulate at the graft interface of tomato. These differences could be due to the different species and techniques used. There is evidence from the study of mutants that pectins are important for cell–cell adhesion (Du *et al.*, 2022), but functional studies have not been done to determine whether these materials also contribute to adhesion between the scion and rootstock. It appears that scion/rootstock adhesion is not restricted to compatible grafts and does not involve cellular recognition; this is because a *Sedum* rootstock can adhere to a wooden stick 3 d after 'grafting' (Moore and Walker, 1981b). In addition, some plants produce pectin-rich gels after wounding to block xylem vessels to prevent tissue desiccation and pathogen infection (De Micco *et al.*, 2016); it is possible that there are similarities between these wounding responses and some aspects of tissue adhesion at the graft interface.

In addition to the accumulation of pectins, grafting modifies other cell wall components; xyloglucan (the type 1 hemicellulose in primary cell walls), phenolic compounds (presumably lignin), and arabinogalactan proteins accumulate in the cell wall at the graft interface of tomato (Frey *et al.*, 2022). These compounds may also have roles in cell adhesion. Sala *et al.* (2019) observed, according to the antibody used, the accumulation of extensins at the graft interface under microscopy (using the JIM11 and JIM20 antibodies). They observed no accumulation of extensin with LM1 antibody, in agreement with Frey *et al.* (2022), who found that extensins decreased over time in cell wall fractions using the LM1 antibody. The precise spatio-temporal accumulation patterns of pectins and other cell wall modifications at the graft interface and the molecular mechanisms behind tissue adhesion during grafting remain to be determined. However, Notaguchi *et al.* (2020) recently identified an extracellular β-1,4-glucanase which promotes grafting success; this enzyme probably targets cellulose in cell walls. In agreement with this hypothesis, the exogenous application of cellulase also increases tissue adhesion in *in vitro* stem grafts (Kawakatsu *et al.*, 2020). How digestion of cellulose at the graft interface aids tissue adhesion and grafting success is not known.

Changes in cell division and expansion at the graft interface

Wounding plant tissues can trigger both cell expansion and proliferation (Hoermayer *et al.*, 2020), and both cell expansion and proliferation have been described at the graft interface of hypocotyl grafts of Arabidopsis (Melnyk *et al.*, 2015). In most scion/rootstock combinations, one of the most obvious indications of graft union formation is the proliferation of parenchymal cells at the graft interface to form the callus, which will serve as a bridge between the two tissues. A wide range of genes have a role in callus formation in different contexts including those encoding many transcription factors, enzymes that modify histones, components of auxin and cytokinin signalling, cyclins, enzymes of cell wall synthesis, and mechanosensitive ion channels of the MscS-Like family (Ikeuchi *et al.*, 2019, 2020; Rymen *et al.*, 2019). Several of these transcription factors regulate wound-induced callus formation; the best characterized are the APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) transcription factors WOUND-INDUCED DEDIFFERENTIATION 1–4 (WIND1–4) (Iwase *et al.*, 2011a, 2011b; Ikeuchi *et al.*, 2013). The expression of *WIND1* rapidly increases after wounding; overexpression of *WIND1* induces callus cell formation whereas callus cell formation is dramatically reduced in *WIND1-SRDX* (when *WIND1* is turned into a dominant repressor by the SUPERMAN domain) (Iwase *et al.*, 2011a). Ectopic overexpression of *WIND1* triggers the differential expression of thousands of genes, including genes related to defence responses, callus formation, tracheary element formation, and vascular regeneration (Iwase *et al.*, 2021). WIND1 controls the expression of *ENHANCER OF SHOOT REGENERATION1* (*ESR1*) by directly binding to its promoter; ESR1 drives the cellular reprogramming underlying callus formation and shoot regeneration (Iwase *et al.*, 2017). Another set of AP2/ERF transcription factors, PLETHORA3/5/7, also regulate wound-induced callus formation (Ikeuchi *et al.*, 2017) and vascular regeneration (Radhakrishnan *et al.*, 2020), and their expression is also increased by induction of *WIND1* (Iwase *et al.*, 2021). Recently, another transcription factor, WUSCHEL-RELATED HOMEOBOX 13 (WOX13), was identified as a key regulator of callus formation and organ adhesion. WOX13 is partially regulated by WIND1 and regulates the induction of *WIND2* and *WIND3*, as well as cell wall-modifying enzymes (Ikeuchi *et al.*, 2022). ETHYLENE RESPONSE FACTOR 115 (ERF115), which is upstream of WIND1 (Heyman *et al.*, 2016), also regulates callus formation via WIND1 (Ikeuchi *et al.*, 2017). Although WIND1 has a key role in callus formation (Ikeuchi *et al.*, 2013; Iwase *et al.*, 2021), grafting wild-type scions with *WIND1-SRDX* rootstocks did not reduce grafting success in hypocotyl grafts of Arabidopsis (Melnyk *et al.*, 2015). This could suggest that either WIND1 does not have a role in graft union formation in Arabidopsis hypocotyls or that the presence of WIND1 in the scion is enough to ensure normal graft union formation. However, as WIND1-SRDX blocks petiole grafting when the petioles of two different WIND1-SRDX leaves are grafted together in a silicon tube (Iwase *et al.*, 2021), it would be interesting to determine whether the presence of WIND1 in one grafting partner is sufficient to ensure normal graft union formation in the petiole grafting system.

Recently, the analysis of a quadruple mutant of four DNA binding with one finger (DOF) transcription factor genes, *HIGH CAMBIAL ACTIVITY2* (*HCA2*), *TARGET OF MONOPTEROS6* (*TMO6*), *DOF2.1*, and *DOF6*, showed that these genes also regulate callus formation. Callus formation after wounding was considerably reduced in this mutant, whereas overexpressing *TMO6* increased callus formation under the same conditions (Zhang *et al.*, 2022). This defect in callus cell formation was associated with a reduction in scion/rootstock attachment and in reduced formation of functional vascular connections at the graft interface.

Other genes have been shown to have roles in wounding healing such as *NAC* (*NAM, ATAF1,2 and CUC2*) *DOMAIN CONTAINING PROTEIN 71* (*ANAC071*), which was identified as a regulator of wound healing responses above wounds, and *RELATED TO AP2 6L* (*RAP2.6L/ERF113*) which regulates wounding responses below the wounds (Asahina *et al.*, 2011). ANAC071 is regulated by auxin through AUXIN RESPONSE FACTORS/ARF6 and 8 (Pitaksaringkarn *et al.*, 2014a). Subsequent work has shown that the homologue of ANAC071, ANAC096, has similar functions to ANAC071 and that these genes function in the process of cambialization and contribute to vascular cell formation (Matsuoka *et al.*, 2021). ANAC071 regulates the expression of two members of the xyloglucan endotransglucosylase/hydrolases family, *XTH19* and *XTH20*, which are involved in cell proliferation after wounding (Pitaksaringkarn *et al.*, 2014b). *ANAC071*, *XTH19*, and *XTH20* are amongst the genes up-regulated by *WIND1* overexpression (Iwase *et al.*, 2021). Although RAP2.6L has a role in wound healing in Arabidopsis flowering stems (Asahina *et al.*, 2011), it does not have a role in grafting in Arabidopsis hypocotyls (Matsuoka *et al.*, 2018). This could suggest that wounding and graft healing require different genes and/or that wounding and grafting responses vary in different plant tissues (i.e. in the hypocotyl and the stem).

The formation of plasmodesmata and gaps in the cell wall at the graft interface

Cell to cell communication in plants is mediated via plasmodesmata, nanometric channels that cross the cell walls of adjacent cells to allow the movement of cytosolic and membrane molecules. Plasmodesmata form either during cell division by trapping strands of endoplasmic reticulum in the newly forming cell wall (primary plasmodesmata) or in pre-existing cells walls by an unknown mechanism (secondary plasmodesmata). Secondary plasmodesmata form within a few days between the scion and rootstock at the graft interface (Kollmann *et al.*, 1985; Kurotani and Notaguchi, 2021; Chambaud *et al.*, 2022) presumably to allow development of cell to cell coordination. Plasmodesmata formation at the graft interface is not always successful, and some plasmodesmata, called half- or hemi-plasmodesmata, do not entirely span the cell wall between the scion and rootstock (Kollmann *et al.*, 1985; Chambaud *et al.*, 2022). We found that ~40% of the plasmodesmata at the graft interface of Arabidopsis hypocotyl homo-grafts were hemi-plasmodesmata and that these hemi-plasmodesmata occurred in regions of the cell wall which were thicker than successfully formed plasmodesmata (Chambaud *et al.*, 2022). This suggests that successful plasmodesmata formation at the graft interface requires cell wall thinning; currently we have little knowledge of the processes involved. A recent review described a list of candidate genes involved in plasmodesmata formation that are up-regulated at the graft interface during graft union formation (Kurotani and Notaguchi, 2021). Grafting mutants or transgenic lines altering the expression of these candidate genes could shed light on the molecular mechanisms of secondary plasmodesmata formation, as the graft interface is an excellent model to study this process. Ideally, to avoid pleiotropic effects, using either an inducible promoter or a promotor induced exclusively at the early stages of grafting to drive expression of the RNAi or gene editing inserts would be the best method to perform functional studies on candidate genes involved in plasmodesmata biogenesis at the graft interface. Although plasmodesmata have been known to form at the graft interface for many decades, we do not know the exchange capacities of these plasmodesmata at the graft interface and when they become functional.

It is possible that defective plasmodesmata formation (or functioning) at the graft interface could be responsible for graft incompatibility and poor survival of hetero-grafted plants, but experimental evidence would be laborious to obtain. Plasmodesmata have been shown to form at the graft interface of interfamilial grafts (Kollmann *et al.*, 1985; Kurotani and Notaguchi, 2021), suggesting that plasmodesmata formation proceeds correctly even between distantly related species. However, in one study, cell to cell exchange of a fluorescence dye was reduced at the graft interface of an incompatible compared with a compatible *in vitro* stem graft (Pina *et al.*, 2012), suggesting that plasmodesmata function could be impaired around the graft interface of incompatible grafts. Unfortunately, some homograft control samples were missing to demonstrate that the differences in cell to cell communication were unequivocally linked to compatibility (and not just intrinsic differences in the different genotypes studied) (Pina *et al.*, 2012). Callus cells of different *Prunus* genotypes have been previously shown to have different intrinsic levels of cell to cell connectivity by the use of photoactivatable fluorophores and photobleaching of fluorophores, but it is unknown whether this has any consequence on graft union formation (Pina *et al.*, 2009).

We observed some cases of extreme cell wall thinning (to a thickness of 6 nm) at the graft interface of Arabidopsis hypocotyl grafts and the formation of gaps in the cell wall between the scion and rootstock (Chambaud *et al.*, 2022). These cell wall gaps between the scion and rootstock at the graft interface have also been observed in other studies (Hertle *et al.*, 2021). The gaps in the cell wall at the graft interface are presumably responsible for exchange of organelles between the scion and rootstock which occurs at the graft interface (Stegemann and

Bock, 2009; Stegemann *et al.*, 2012; Lu *et al.*, 2017; Hertle *et al.*, 2021). Both the exchange of organelles (Hertle *et al.*, 2021) and the formation of gaps between the scion and rootstock (Hertle *et al.*, 2021; Chambaud *et al.*, 2022) appear to occur before vascular reconnection (at 3–4 DAG). Live-cell imaging was used to show plastid movement through these gaps, but the lack of 3D imaging and a single-particle tracking method means that unequivocal evidence is still lacking (Hertle *et al.*, 2021). When studying 2D images, it is possible that different plastids move in and out of the focal plane, and what seems like the movement of one plastid from one cell to a neighbouring cell is actually the movement in the third dimension of two different plastids belonging to two different cells. One means to overcome this limitation would be to activate caged fluorescence in one plastid and track its movement from cell to cell, or to use 3D imaging and track particle movement in the three dimensions.

Vascular connection between the scion and rootstock

The graft interface has been studied with various imaging techniques to understand the process of vascular connection; that is, the connection of phloem and xylem as well as the cambium in many species. Dyes of different types have been used to visualize and quantify vascular connections between the scion and rootstock (Olmstead *et al.*, 2006; Melnyk *et al.*, 2015; Deng *et al.*, 2021). Thanks to elegant experiments on grafted hypocotyls of Arabidopsis, this sequence of phloem and xylem connections was resolved (Melnyk *et al.*, 2015). Movements of tracers revealed that functional vascular connections between the scion and the rootstock occurred around 3 DAG for the phloem and 6 DAG for the xylem (Melnyk *et al.*, 2015). Although functional xylem and phloem connections at the graft interface are essential for plant development, it appears that phloem connection is not necessarily associated with xylem connection (Deng *et al.*, 2021); some grafts can have successfully connected phloem and failed xylem connection, suggesting that different molecular mechanisms are involved.

The differentiation of new vascular cells is unevenly distributed across the graft interface, with more rapid new xylem development in the scion than in the rootstock, and more rapid phloem connection in the upper graft interface of a double-grafted plant (Melnyk *et al.*, 2015). The interruption of vascular connections leads to auxin and photoassimilate accumulation above the graft interface (Melnyk *et al.*, 2015); this asymmetry could explain the different kinetics of cell differentiation between the scion and the rootstock. Auxin from the bud is essential for vascular connection after wounding (Sachs, 1968) and presumably grafting. Although wound healing and grafting are different, there are likely to be common mechanisms involved in wound healing and grafting. Responses to auxin increasing above the graft interface could be similar to those above a wound, in which *PIN-FORMED 1* (*PIN1*) polarity changes before the formation of new vascular tissues (Mazur *et al.*, 2016). According to the auxin canalization hypothesis (Sauer *et al.*, 2006), the auxin accumulation above the graft interface would modify *PIN* polarity and permit new vascular generation. In the vascular regeneration process, the TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX (TIR1/AFB) signalling pathway is required for vascular regeneration after wounding (Mazur *et al.*, 2020). Melnyk *et al.* (2015) tested the role of several mutants of the TIR/ARB-mediated signalling pathway in graft union formation by grafting mutant rootstocks. They found that the triple mutant in the TIR/ARB-mediated signalling pathway, *tir1afb2afb3*, dramatically reduced phloem reconnection at 4 DAG, suggesting that the mechanisms of new vascular generation at the graft interface and at the sites of wounds could be similar. The dominant gain-of-function mutations of *AUXIN/INDOLE-3-ACETIC ACID* (*Aux/IAA*), *iaa18* and *iaa28* (which are targets for degradation by TIR/AFB), also reduced phloem connection when grafted as a rootstock. In addition, Melnyk *et al.* (2015) also showed that grafting rootstocks mutated in *ABERRANT LATERAL ROOTFORMATION 4* (*ALF4*) and *AUXIN-RESISTANT 1* (*AXR1*) caused delayed xylem and phloem connections, but that *alf4* and *axr1* scions did not affect vascular connections. The authors concluded that *ALF4* and *AXR1* are required in the rootstock for vascular auxin responses and highlighted the importance of the asymmetric responses occurring at the graft interface. Despite the pivotal role of auxin in vascular development, surprisingly few mutants of individual genes regulating auxin synthesis, signalling, and degradation have obvious graft union formation phenotypes when grafted as a rootstock (Melnyk *et al.*, 2015). This suggests that there is considerable functional redundancy within genes regulating vascular connection via auxin signalling. However, a more detailed characterization of these mutants could reveal subtle phenotypes related to vascular connections and, as there were only two biological repetitions in the study of Melnyk *et al.* (2015), increased repetition could identify statistically significant, but smaller changes in grafting success rates.

Certain transcription factors of the DOF family are involved in graft union formation (Melnyk *et al.*, 2018; Zhang *et al.*, 2022). *HIGH CAMBIAL ACTIVITY2* was shown to delay phloem connection in Arabidopsis hypocotyl grafts in an SRDX line (Melnyk *et al.*, 2018); however, the normal loss-of-function mutant did not affect phloem connection between the scion and rootstock (Zhang *et al.*, 2022). Single loss-of-function mutants of three closely related DOF transcription factor genes, *TMO6*, *DOF2.1*, and *DOF6*, also did not have phloem connection phenotypes, but the quadruple mutant inhibited phloem (and xylem) connection (Zhang *et al.*, 2022). Overexpression of any of these four DOF transcription factors below the graft interface accelerated phloem reconnection in Arabidopsis hypocotyl grafts (Zhang *et al.*, 2022). Many cell wall-related genes were differentially expressed between the quadruple *hca2tmo6dof2.1dof26*

mutant and the wild type, and *TMO6* was shown to directly bind *CELLULASE3* (*CEL3*/*GH9B3*) (Zhang *et al.*, 2022). The tobacco orthologue of *CEL3*, *NbGH9B3*, is involved in cell adhesion and graft union formation, and increases grafting success in heterografts between different species (Notaguchi *et al.*, 2020). In Arabidopsis, homo-grafts of two mutant lines of *CEL3* did not have a grafting success phenotype, but overexpressing the tobacco *NbGH9B3* under a wound-inducible promoter increased grafting success in Arabidopsis homo-grafts (Notaguchi *et al.*, 2020). This could suggest that *CEL3*/*GH9B3* has similar functions in Arabidopsis and tobacco, but that there is more functional redundancy within the *CELLULASE* gene family in Arabidopsis than in tobacco. Zhang *et al.* (2022) showed that two genes that modify cell wall composition also modified xylem and phloem connection. Overexpression of *PECTIN METHYLESTERASE INHIBITOR 5* (*PMEI5*), which increases pectin methylesterification, increased *TMO6* and *HCA2* expression, and grafting with rootstocks overexpressing *PMEI5* increased phloem reconnection rates (Zhang *et al.*, 2022). Zhang *et al.* (2022) present evidence that HCA2 acts downstream of PMEI5. *KORRIGAN1* (*KOR1*), encoding an endo-1,4-β-D-glucanase involved in cellulose biosynthesis, did not affect phloem connection, but mutants of *kor1* have decreased *TMO6*, *HCA2*, and *DOF2.1* transcript levels in wounded hypocotyls and reduced xylem connection in *kor1* homo-grafts (Zhang *et al.*, 2022).

Many genes are known to regulate vascular development (Agustí and Blázquez, 2020) and presumably many of them are essential for graft union formation. One well-characterized transcription factor, WOX4, regulates vascular proliferation redundantly with WOX14 in Arabidopsis (Etchells *et al.*, 2013). Interestingly, *wox4* homo-grafts of tomato have disorganized xylem vessels at the graft interface and mechanically weak graft unions, despite having no major differences in vascular organization in the stem (Thomas *et al.*, 2021). This could suggest that although *WOX4* and *WOX14* act redundantly in normal plant development, WOX4 has specific functions at the graft interface.

We still have much to discover about the processes and genes involved in vascular connection at the graft interface and how these processes differ in different genotypes and tissue types. We still do not know which cell lineages are involved in which processes. The use of the cell lineage tracing tool based on the Cre-lox recombination system, which was used to trace the cell lineage of new divided cells during vascular tissue formation in the root of Arabidopsis (Smetana *et al.*, 2019), could help to address many unresolved questions concerning graft union formation.

Grafting compatibility between different eudicot species

There is no universally accepted definition of graft compatibility (Tedesco *et al.*, 2022); however, for an agricultural perspective, we can assume that a compatible graft has sufficient yield and longevity to be economically viable in the field/greenhouse, and a sufficiently high grafting success rate to be economically viable for nurseries. Broadly, highly compatible grafts develop normally, and have functional xylem and phloem connections, and a graft union able to withstand mechanical stress. Poorly compatible grafts are often described as having more necrotic tissues at the graft interface, fewer vascular connections, mechanically weak graft unions, and reduced plant growth and survival. The lack of consensus on what constitutes graft compatibility/incompatibility is partially due to the fact that assigning a percentage of plant survival determines the threshold for graft compatibility/incompatibility (given that grafting success rates of homo-grafts is often <100% potentially due to technical difficulties or intrinsic healing capacity). In the context of scientific research, we could consider that incompatible grafts have no xylem and/or phloem connections formed or that the accumulation of toxic compounds at the graft interface reduces cell viability to the point that all grafts perish. Except in these cases of extreme incompatibility, it is probably better to describe different degrees of compatibility rather than compatibility/incompatibility.

It was widely believed that graft compatibility is restricted to closely related species (Goldschmidt, 2014), although some (often short-lived) interfamilial grafts are widely cited in the literature: such as *Vicia faba*/*Helianthus annuus* grafts (Kollmann *et al.*, 1985), Arabidopsis/tomato grafts (Flaishman *et al.*, 2008), and the interfamilial grafts used to graft inoculate pathogens [e.g. *Chenopodium quinoa* rootstocks with *Vitis* spp. (Belin *et al.*, 2001)]. More recently, Wulf *et al.* (2020) grafted together a range of leguminous species and concluded that genetic proximity and vascular anatomy are not good predictors of graft compatibility within the leguminous species studied. At the same time, Notaguchi *et al.* (2020) showed that *Nicotiana benthamiana* is capable of grafting with a wide range of angiosperms, therefore overturning the idea that graft compatibility is restricted to closely related species. *Nicotiana benthamiana* was shown to be capable of forming grafts (assessed 4 weeks after grafting) with 73 species from 38 families including magnoliids, five species of monocots, and 65 eudicots including both annual and perennial species (Notaguchi *et al.*, 2020). However, it is difficult to know whether these plants were truly grafted together with function vascular connections; it is known that Arabidopsis/tomato grafts can survive to form flowers and seeds without vascular connections (Flaishman *et al.*, 2008). It is possible that some of the apparently successfully grafted plants in Notaguchi *et al.* (2020) did not have functional vascular connections (as vascular connections were not studied in their paper). Although largely ignored in the recent literature, other studies on interfamilial grafts have been reported in the past (Funck, 1929; Simon, 1930; Nickell, 1948). In 1948, Nickell stated that 'These results clearly show that we may abandon the idea that grafts between unrelated plants cannot be made', yet this conclusion had disappeared from scientific knowledge. The reason that this knowledge did not persist is possibly because the studies were

much less comprehensive than the study by Notaguchi *et al.* (2020) and few details were provided in these papers. In addition, when commercial nurseries and scientists have difficulties in grafting certain scion/rootstock combinations repeatedly, and with no apparent reason, they are likely to ascribe these problems to graft incompatibility, with little evidence. Furthermore, as successful grafting requires the callus-producing tissues near the cambium of the scion and rootstock to be in close proximity, is would seem likely that more distantly related species have more diverse tissue organizations and therefore more difficulty grafting together [however, there is some evidence that this is not necessarily the case (Wulf *et al.*, 2020)].

As previously mentioned, Notaguchi *et al.* (2020) identified a β-1,4-glucanase gene, *NbGH9B3*, that is highly expressed at the cambium tissues at the graft boundary of interfamilial grafts. Notaguchi *et al.* (2020) discovered that NbGH9B3 is involved in cell adhesion and that silencing *NbGH9B3* reduces grafting success and, vice versa, its overexpression increases grafting success rates (Notaguchi *et al.*, 2020). The same group found similar results in *Petunia hybrida*, which also grafts successfully with a wide range of species and expresses *GH9B3* to high levels during both auto- and interfamilial grafting (Kurotani *et al.*, 2022). Because *GH9B3* is highly expressed even in autografts, Kurotani *et al.* (2022) suggested that the strong induction of *GH9B3* during grafting in Solanaceae could underlie the ability of these species to form interfamilial grafts with a wide range of species. In support of this hypothesis, the β-1,4-glucanase inhibitor D-glucono-1,5-lactone was shown to reduce grafting success in *P. hybrida*/Arabidopsis grafts (Kurotani *et al.*, 2022). How this β-1,4-glucanase regulates grafting success is not known. It could have a physical role by facilitating the formation of a functional cell wall between the scion and rootstock, or a signalling role inducing the cellular responses required for the formation of the graft union (such as the induction of wound-related transcription factors as described in Zhang *et al.*, 2022).

Although many different genotypes can be grafted together successfully in the short term, this high level of graft compatibility may not persist in the long term in perennial woody species growing in temperate climates. In these conditions, perennial woody species enter into dormancy during the winter months in which growth and metabolic activity cease. Lack of coordination of dormancy behaviour between the two different genotypes of a grafted plant could have consequences on long-term plant viability and could have a role in the delayed incompatibility seen in perennial crops many years after grafting.

Causes of graft incompatibility

Although recent studies suggest that graft compatibility is more widespread that we previously thought, some cases of graft incompatibility for agronomically important crops are well known. The most famous example is the case of graft incompatibility between pear/quince grafts; this incompatibility response is due to the movement of a cyanogenic glycoside (prunasin) from the rootstock to the scion where β-glycosidases release cyanide and locally poison plant tissues (Gur *et al.*, 1968). Graft incompatibility is widespread in fruit trees, particularly in those of the *Prunus* genus (Hartmann, 2002; Baron *et al.*, 2019), and to date we have not identified the cause of incompatibility (except in the case of pear/quince cited above). Graft incompatibility in fruit trees is challenging to study as it may develop many years after grafting; this is often called delayed incompatibility, a subject that was was recently reviewed by Adhikari *et al.* (2022).

Another well-known incompatible graft, pepper/tomato, has been recently studied in detail by Thomas *et al.* (2021). They found that pepper/tomato incompatibility was associated with the formation of weak graft unions with poor vascular connections between the scion and rootstock. By using gene regulatory networks, they identified hub genes potentially regulating grafting success, and in particular focused on genes potentially regulating vascular formation (Thomas *et al.*, 2021). Their analysis predicted that WOX4 regulates the expression of *VASCULAR-RELATED NAC-DOMAIN* and *NAC SECONDARY WALL THICKENING PROMOTING FACTOR4*, genes involved in xylem differentiation, and they showed that the presence of WOX4 is required in at least one grafting partner in order for the scion and rootstock to connect (Thomas *et al.*, 2021). Determining whether overexpression of *WOX4* in incompatible pepper/tomato or tomato/pepper grafts could increase graft compatibility and whether overexpression of WOX4 could increase graft compatibility in other species should be a priority. It will be interesting to learn what mechanisms regulate *WOX4* and ultimately identify the signal(s) triggering this graft incompatibility response.

A new method of grafting monocotyledons

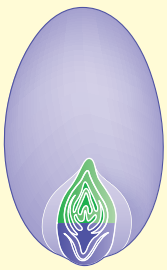
A wide range of grafting techniques have been developed to graft together different herbaceous and woody species (Box 1). Up until recently, it was widely accepted that the absence of vascular cambium and the presence of scattered vascular bundles in monocot stems would prevent monocot grafting. However, Reeves *et al.* (2021) experimentally determined that the embryonic hypocotyl (or mesocotyl in grasses) allows intra- and interspecific grafting in all three monocotyledon groups. These grafts were shown to be functional because fluorescent dyes moved between the scion and rootstock, and because complementation assays were successful for the movement of xylem/phloem mobile hormones. Reeves *et al.* (2021) developed a protocol that could graft together a wide range of monocots including some crop species such as banana, date palm, pineapple, onion, oil palm, and tequila agave, and showed

that grafting ability is conserved across all three monocotyledon groups: the commelinids, lilioids, and alismatids. There had been previous reports of monocot grafting in the literature (Krenke, 1933; Muzik and LaRue, 1952, 1954; Muzik, 1958); few details were given in these early papers, but some authors reached similar conclusions to those of Reeves at al. (2021). For example, it appears that Muzik and LaRue (1954) were correct when they stated, 'a cambium is not an absolute requisite for grafting but a meristem is'. Reeves *et al.* (2021) found that young tissues have a higher rate of fusion than older tissues, but we do not know what molecular mechanisms are involved and what characteristics make young cells easier to graft. It is possible that younger cells are in a less differentiated state than older cells, which could facilitate the more rapid initiation of cell expansion and new cell divisions.

The work described above raises the interesting question of whether the ability to graft is conserved across vascular plants. We now know that monocots and dicots can be grafted, along with gymnosperms (such as conifers; Blada and Panea, 2011, 2012), suggesting that all seed plants are potentially graftable. There is some literature to suggest that pteridophytes can be grafted: the cinnamon fern, *Osrmunda cinnamomea*, was grafted but the grafts were stunted, and no vascular connections were observed at the graft interface (Hicks *et al.*, 1967).

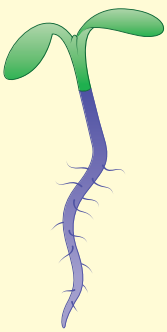
Box 1. Plant grafting methods

Many different grafting techniques are used; manual grafting is technically demanding, but some grafting machines are commercially available. The graft interface is susceptive to dehydration during the early stages of graft union formation so it needs either physical protection or to develop in a humid environment. (In the drawings below, the scion is depicted in green and the rootstock in blue.)



Monocot grafting

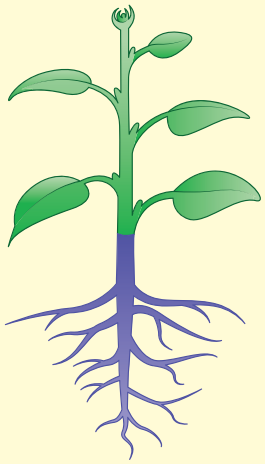
Monocot grafting requires very young tissues such as the shoot–root interface of the embryonic mesocotyl of seeds; the shoot (plumule) of seed is removed and replaced with the plumule of another seed (Reeves *et al.*, 2021). The success of monocot grafting seems to be dependent on the species studied, but presumably this newly developed technique could be improved upon in the future for commercial applications (Reeves *et al.* (2021)).



Hypocotyl grafts

Hypocotyl grafting is widely used in the commercial production of grafted vegetables (Colla *et al.*, 2017) and has been extensively used in scientific research (Bartusch and Melnyk, 2020). Although this technique is technically demanding when done on small plants such as Arabidopsis, it generally has high grafting success rates.

Box 1. Continued



Herbaceous stem grafting

Herbaceous stems are also easily grafted; flowering stem grafts are used for scientific purposes in Arabidopsis (Bartusch and Melnyk, 2020) as well as other species. Some herbaceous stem grafts are also made commercially.

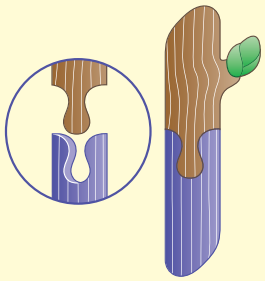
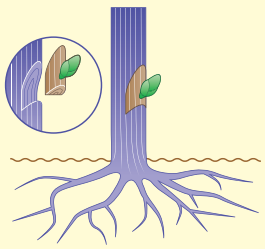


Table-top grafting of perennials

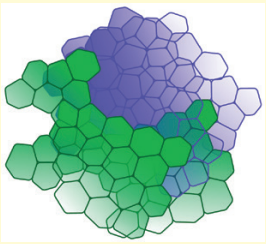
Table-top grafting of woody perennials involves cutting and assembling overwintering branches of the scion and rootstock genotype, for which numerous grafting machines are available. However, unlike other grafting techniques, the grafted plant must produce both a graft union and adventitious roots on the rootstock in order to survive. In species with poor rooting capacity, assessing graft failure is difficult as plant survival depends on both root and graft union development.



Field grafting of perennials

For fruit trees, rooted rootstocks are generally grown in stool beds for 1 year, the rootstock shoot is removed, and the rootstock is top grafted with the scion genotype. The most widely used technique is chip budding, when the scion is only one bud, therefore limiting the size of the wound inflicted on the plant during grafting.

Box 1. Continued



Different simplified grafting techniques

Various simplified 'grafting' techniques have been developed, such as *in vitro* stem grafting (Pina *et al.*, 2012; Kawakatsu *et al.*, 2020), which can be used to phenotype scion/rootstock adhesion and callus cell proliferation at the graft interface, and callus grafting, which has been used to study cell wall changes and plasmodesmata formation at the callus graft interface. (Pina and Errea, 2008; Pina *et al.*, 2009; Hertle *et al.*, 2021; Machin *et al.*, 2022).

How recent developments in the field of grafting could shape the future

The recent advances in grafting research made by Notagushi *et al.* (2020) and Reeves *et al.* (2021) open up the possibility of grafting a wider range of species together than previously imagined for both research and commercial applications. The benefits of grafting with rootstocks that provide disease resistance have been exploited commercially in the production of perennials such as grapevine and citrus trees since the mid to late 19th century (Mudge *et al.*, 2009). Selecting separately for root traits can allow breeders to access a larger gene pool (such as wild and more distantly related species) than available in elite cultivars, while being able to keep the taste that consumers like. For example, in viticulture, using rootstocks tolerant to *Phylloxera* (an insect pest that causes plant mortality by root feeding) has proven to be a sustainable and environmentally friendly solution for ~150 years, while keeping the desired scion cultivars. Hopefully, similar approaches could solve problems related to the production of important monocots such as banana and date palm, which are susceptible to various soil-borne pathogens and pests. However, although bananas are perennial crops, the perennial structures are the rhizome and root system, which produce new suckers or shoots for the next cycle of growth. Presumably, the rhizome is only the rootstock genotype, so this would suggest that commercial grafting of bananas would require regular planting of grafted plants, which may be beyond the reach of small-scale producers. There is probably a higher potential to graft date palm commercially; although suckers are also the main method to propagate date palm today, one shoot can survive and produce fruits for many decades, which could offset a higher plant cost of grafted material.

Challenges of studying grafting in plants

Phenotyping grafting success is challenging as causes of graft failure are multiple and may be different depending on the scion/rootstock combination. Graft failure can be due to technical errors and poor workmanship; seemingly well-aligned scion and rootstocks may actually not be in close enough proximity for the graft union to form. Graft failure can also be due to the presence of (potentially unknown) pathogens and differences in pathogen sensitivity (Rowhani *et al.*, 2017). This is a particular problem for clonally propagated perennial crops. It is important for researchers to graft sufficient plants when calculating success rates, and ideally to perform independent repetitions of the grafting series or with independent batches of seeds or wood/buds. Differences in seed or bud/wood quality could affect grafting success and make characterizing genetic diversity of grafting success erroneous.

In addition to the difficulties associated with phenotyping grafting success, unequivocally demonstrating that a candidate gene has a role in graft union formation is also difficult. Characterizing constitutive mutants or overexpressing lines can result in grafting success phenotypes, which are caused by differences in grafting technique rather than the failure of intrinsic graft healing mechanisms. For example, it can be more difficult to graft plants with thinner or thicker stems or hypocotyls, therefore reducing grafting success rates because of difficulties associated with aligning the stems optimally rather than graft union formation *per se*. For this reason, the functions of genes putatively related to graft union formation are more precisely characterized by changing their expression locally at the grafting site. The function of *GH9B3*, for example, was characterized under the wound-inducible promoter *RAP2.6* in Arabidopsis (Notaguchi *et al.*, 2020). Accurately determining whether a gene regulates one specific process of graft union formation is also challenging due to difficulties in phenotyping the graft union (see Box 2).

Problems of graft union formation could also be due to differences in the kinetics of wounding responses between the different genotypes of a grafted plant. This is potentially more of an issue for perennial grafts in which overwintering tissues are grafted in the spring—differences in bud and vascular phenology between the scion and rootstock could lead to a lack of coordination in graft union formation mechanisms between the two partners. To date, there have been no studies to address these questions.

Box 2. Phenotyping graft union development

Imaging the graft interface. Histological examination of the graft interface in plants began ~100 years ago (Bailey, 1923), and continues today. However, 2D images can be of limited usefulness as the same graft interface imaged at different angles and locations can lead to different interpretations. This is because the graft interface is a mixture of many different tissue types heterogeneously distributed across the graft interface; in some areas, the scion and rootstock can appear fused together, whereas in others there may be little connection. One example of this heterogeneity is cell wall thickness, which varies from 6 nm to >1300 nm (Chambaud *et al.*, 2022), highlighting how difficult it would be to identify a gene regulating cell wall thickness at the graft interface. Because of the difficulty of understanding a complex 3D tissue with 2D images, some studies have used 3D imaging techniques such as X-ray tomography (Milien *et al.*, 2012) and magnetic resonance imaging (Bahar *et al.*, 2010), but these studies have been of relatively low resolution. The development of a high-resolution 3D phenotyping system, which provides quantitative measurements, would be a major breakthrough.

Quantifying vascular connections. Different approaches can characterize vascular connections at the graft interface; some use the movement of dyes (Schoning and Kollmann, 1995, 1997; Flaishman *et al.*, 2008; Notaguchi *et al.*, 2009; Melnyk *et al.*, 2015) or radioactive isotopes (Schoning and Kollmann, 1995, 1997). Quantifying movement between the scion and rootstock is challenging because transport rates depend on many factors, for example xylem transport depends on transpiration rates. Developing a 3D imaging system to quantify functional xylem vessels (labelled with contrast agents or dyes) would be ideal, but has not yet been developed. For xylem connections, scion/rootstock connections can be assessed with measurements of hydraulic conductivity (Atkinson *et al.*, 2003; Solari *et al.*, 2006; Gasco *et al.*, 2007), which has the advantage of being quantitative and does not require expensive equipment to set up. Xylem connections can also be assessed indirectly by placing the grafted plants in environmental conditions with high evaporative demand and assessing plant survival; surviving plants are considered to have sufficient vascular connections to support the scion

Quantifying mechanical strength of the graft union. In nurseries producing perennial crops, one of the criteria to select plants for commercialization is the resistance of the graft union formation to mechanical force (Carrere *et al.*, 2022). For annual plants, the bend test is useful in academic research to assess the biophysical integrity of grafted combination (Thomas *et al.*, 2021). However, these techniques are not quantitative and highly depend on the person performing the test. An *in vitro* method to assess *Nicotiana benthamiana* stem grafts has been developed to measure the adhesion strength up to the breaking point when the two grafted partners separate (Kawakatsu *et al.*, 2020), and a quantitative test has been used to measure the strength of Arabidopsis graft (Melnyk *et al.*, 2018).

Conclusion

Humans have been grafting plants for thousands of years (Mudge *et al.*, 2009), and the use of grafting, particularly for vegetable crops, has increased recently (Colla et al., 2017). Grafting offers the potential to change the root system of shoot crops, and potentially the shoot of root crops. Rootstocks have the potential to adapt crops to different climates (Gregory *et al.*, 2013; Albacete *et al.*, 2015) and potentially mitigate the effects of climate change; however, in order to be used commercially, nurseries need economically acceptable graft success rates. Using wild relatives and related species of crop plants is often cited as a strategy to increase climate resilience (Bohra *et al.*, 2022), and this is also the case for rootstocks (Ollat *et al.*, 2016). Although increasing the genetic diversity of crop species cultivated has numerous benefits, in grafted crops maintaining high grafting success rates is essential. Quantitative genetic studies on grafting have been slow to appear compared with other traits such as biotic or abiotic stress resistance, or fruit quality. This is because of the challenges associated with phenotyping grafting success in large populations as many thousands of grafts need to be produced which is a logistical challenge. In addition, in perennial crops, it could take many years for the plants to produce the quantity of woody stems/buds necessary to perform large numbers of grafts. To add further complexity, in some cases, poor grafting success appears many years after grafting, which rapidly increases plant maintenance and phenotyping costs. Partly for these reasons, there has been intensive research effort to identify proxies or markers of grafting success, markers such as transcripts, proteins, enzyme activities, and secondary metabolites (as reviewed by Loupit and Cookson, 2020); however, this strategy has so far been of limited success and has not yet been applied to quantitative genetics studies. Recently, two studies have been published on *Prunus* grafts which described the genetic variation in anatomical characteristics of the graft union (Irisarri *et al.*, 2019), and tentatively identified quantitative trait loci of graft compatibility/anatomy in a scion mapping population (although the study was limited to only 92 individuals, n=5–10, and heritability was not calculated; Pina *et al.*, 2021).

Transferring knowledge of the molecular mechanisms of graft union formation to breeding programmes in grafted crops has the potential to expand the range of genotypes that can be grafted together in the future. For example, selecting genotypes which intrinsically express high levels of *GH9B3*

in the stem, or the application of GH9B3 or cellulases to the graft interface, could be used commercially to increase grafting success in crop species. Certainly, other molecular mechanisms could be exploited in the future to facilitate both grafting success rates in nurseries and the range of genotypes that can be grafted together commercially.

Acknowledgements

We thank Cyril Hevin, Maria Lafargue, and Nicolas Hocquard for the grafted grapevine, and Jean-Pascal Tandonnet for the root graft. We thank Camille Crouzet for providing access to her garden.

Conflict of interest

No conflict of interest declared.

Funding

The PhD studentship of GL provided by the French Ministry of Higher Education. Research was supported by the European Union INTERREG POCTEFA project Vites Qualitas (EFA 324/19) which is co-financed by the Fonds Européen de Développement Regional (FEDER).

References

Adhikari PB, Xu Q, Notaguchi M. 2022. compatible graft establishment in fruit trees and its potential markers. Agronomy **12**, 1981.

Agustí J, Blázquez MA. 2020. Plant vascular development: mechanisms and environmental regulation. Cellular and Molecular Life Sciences **77**, 3711–3728.

Albacete A, Martínez-Andújar C, Martínez-Pérez A, Thompson AJ, Dodd IC, Pérez-Alfocea F. 2015. Unravelling rootstock×scion interactions to improve food security. Journal of Experimental Botany **66**, 2211–2226.

Asahina M, Azuma K, Pitaksaringkarn W, *et al*. 2011. Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA **108**, 16128–16132.

Assunção M, Santos C, Brazão J, Eiras-Dias JE, Fevereiro P. 2019. Understanding the molecular mechanisms underlying graft success in grapevine. BMC Plant Biology **19**, 396.

Atkinson CJ, Else MA, Taylor L, Dover CJ. 2003. Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). Journal of Experimental Botany **54**, 1221–1229.

Bahar E, Korkutal I, Carbonneau A, Akcay G. 2010. Using magnetic resonance imaging technique (MRI) to investigate graft connection and its relation to reddening discoloration in grape leaves. Journal of Food, Agriculture & Environment **8**, 293–297.

Bailey JS. 1923. A microscopic study of apple graft unions. MSc thesis, Iowa State University of Science and Technology.

Baron D, Amaro ACE, Pina A, Ferreira G. 2019. An overview of grafting re-establishment in woody fruit species. Scientia Horticulturae **243**, 84–91.

Bartusch K, Melnyk CW. 2020. Insights into plant surgery: an overview of the multiple grafting techniques for *Arabidopsis thaliana*. Frontiers in Plant Science **11**, 613442.

Belin C, Schmitt C, Demangeat G, Komar V, Pinck L, Fuchs M. 2001. Involvement of RNA2-encoded proteins in the specific transmission of Grapevine fanleaf virus by its nematode vector *Xiphinema index*. Virology **291**, 161–171.

Bergthorsson U, Richardson AO, Young GJ, Goertzen LR, Palmer JD. 2004. Massive horizontal transfer of mitochondrial genes from diverse land plant donors to the basal angiosperm *Amborella*. Proceedings of the National Academy of Sciences, USA **101**, 17747–17752.

Blada I, Panea T. 2011. Improvement of grafting procedures for the ornmental species: I. *Picea pungens* Engelm. var. glauca Reel. Annals of Forest Research **54**, 185–196.

Blada I, Panea T. 2012. Improvement of grafting procedures for the ornamental species: II. *Abies concolor* (Gord. & Glend.) Lindl. Annals of Forest Research **55**, 25–31.

Bohra A, Kilian B, Sivasankar S, Caccamo M, Mba C, McCouch SR, Varshney RK. 2022. Reap the crop wild relatives for breeding future crops. Trends in Biotechnology **40**, 412–431.

Carrere C, Spilmont AS, Loupit G, Stessels C, Ollat N, Cookson SJ. 2022. Evaluation of criteria to assist the selection of good quality grafted grapevines prior to their commercialization. OENO One **56**, 15–27.

Chambaud C, Cookson SJ, Ollat N, Bayer E, Brocard L. 2022. A correlative light electron microscopy approach reveals plasmodesmata ultrastructure at the graft interface. Plant Physiology **188**, 44–55.

Christiaens F, Canher B, Lanssens F, Bisht A, Stael S, De Veylder L, Heyman J. 2021. Pars pro toto: every single cell matters. Frontiers in Plant Science **12**, 656825.

Colla G**,** Perez-Alfocea F**,** Schwarz D, **eds.** 2017. Vegetable grafting principles and practices. Wallingford, UK: CABI Publishing.

Cookson SJ, Moreno MJC, Hevin C, Mendome LZN, Delrot S, Trossat-Magnin C, Ollat N. 2013. Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. Journal of Experimental Botany **64**, 2997–3008.

Cookson SJ, Moreno MJC, Hevin C, Mendome LZN, Delrot S, Magnin N, Trossat-Magnin C, Ollat N. 2014. Heterografting with nonself rootstocks induces genes involved in stress responses at the graft interface when compared with autografted controls. Journal of Experimental Botany **65**, 2473–2481.

De Micco V, Balzano A, Wheeler EA, Baas P. 2016. Tyloses and gums: a review of structure, function and occurrence of vessel occlusions. IAWA Journal **37**, 186–205.

Deng ZY, Wu HY, Jin TL, Cai TT, Jiang MT, Wang M, Liang DC. 2021. A sequential three-phase pathway constitutes tracheary element connection in the Arabidopsis/Nicotiana interfamilial grafts. Frontiers in Plant Science **12**, 664342.

Driouich A, Follet-Gueye M-L, Bernard S, Kousar S, Chevalier L, Vicré M, Lerouxel O. 2012. Golgi-mediated synthesis and secretion of matrix polysaccharides of the primary cell wall of higher plants. Frontiers in Plant Science **3**, 79.

Du J, Anderson CT, Xiao C. 2022. Dynamics of pectic homogalacturonan in cellular morphogenesis and adhesion, wall integrity sensing and plant development. Nature Plants **8**, 332–340.

Etchells JP, Provost CM, Mishra L, Turner SR. 2013. WOX4 and WOX14 act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. Development **140**, 2224–2234.

Flaishman MA, Loginovsky K, Golobowich S, Lev-Yadun S. 2008. *Arabidopsis thaliana* as a model system for graft union development in homografts and heterografts. Journal of Plant Growth Regulation **27**, 231–239.

Frey C, Manga-Robles A, Acebes JL, Encina A. 2022. The graft framework: quantitative changes in cell wall matrix polysaccharides throughout the tomato graft union formation. Carbohydrate Polymers **276**, 118781.

Fuentes I, Stegemann S, Golczyk H, Karcher D, Bock R. 2014. Horizontal genome transfer as an asexual path to the formation of new species. Nature **511**, 232–235.

Funck R. 1929. Untersuchungen tiber heteroplastische Transplantationen bei Solanaceen und Cactaceen. Beiträge sur Biologie der Pflanzen **17**, 404–468.

Gasco A, Nardini A, Raimondo E, Gortan E, Motisi A, Gullo MA, Salleo S. 2007. Hydraulic kinetics of the graft union in different *Olea europaea* L. scion/rootstock combinations. Environmental and Experimental Botany **60**, 245–250.

Goldschmidt EE. 2014. Plant grafting: new mechanisms, evolutionary implications. Frontiers in Plant Science **5**, 727.

Gregory PJ, Atkinson CJ, Bengough AG, Else MA, Fernández-Fernández F, Harrison RJ, Schmidt S. 2013. Contributions of roots and rootstocks to sustainable, intensified crop production. Journal of Experimental Botany **64**, 1209–1222.

Gur A, Samish RM, Lifshitz E. 1968. Role of cyanogenic glycoside of quince in incompatibility between pear cultivars and quince rootstocks. Horticultural Research **8**, 113–134.

Gurdon C, Svab Z, Feng Y, Kumar D, Maliga P. 2016. Cell-to-cell movement of mitochondria in plants. Proceedings of the National Academy of Sciences, USA **113**, 3395–3400.

Hartmann HT. 2002. Principles of grafting and budding. In: Hartmann HT, ed. Hartmann and Kester's plant propagation: principles and practices. Upper Saddle River, NJ: Prentice Hall, 411–460.

Hertle AP, Haberl B, Bock R. 2021. Horizontal genome transfer by cell-to-cell travel of whole organelles. Science Advances **7**, eabd8215.

Heyman J, Cools T, Canher B, *et al*. 2016. The heterodimeric transcription factor complex ERF115–PAT1 grants regeneration competence. Nature Plants **2**, 16165.

Hicks GS, Steeves TA, Sweeny PR. 1967. A method for grafting fern leaf primordia in vitro. Canadian Journal of Botany **45**, 2232–2236.

Hoermayer L, Montesinos JC, Marhava P, Benková E, Yoshida S, Friml J. 2020. Wounding-induced changes in cellular pressure and localized auxin signalling spatially coordinate restorative divisions in roots. Proceedings of the National Academy of Sciences, USA **117**, 15322–15331.

Ikeuchi M, Favero DS, Sakamoto Y, Iwase A, Coleman D, Rymen B, Sugimoto K. 2019. Molecular mechanisms of plant regeneration. Annual Review of Plant Biology **70**, 377–406.

Ikeuchi M, Iwase A, Ito T, *et al*. 2022. Wound-inducible WUSCHEL-RELATED HOMEOBOX 13 is required for callus growth and organ reconnection. Plant Physiology **188**, 425–441.

Ikeuchi M, Iwase A, Rymen B, *et al*. 2017. Wounding triggers callus formation via dynamic hormonal and transcriptional changes. Plant Physiology **175**, 1158–1174.

Ikeuchi M, Rymen B, Sugimoto K. 2020. How do plants transduce wound signals to induce tissue repair and organ regeneration? Current Opinion in Plant Biology **57**, 72–77.

Ikeuchi M, Sugimoto K, Iwase A. 2013. Plant callus: mechanisms of induction and repression. The Plant Cell **25**, 3159–3173.

Irisarri P, Zhebentyayeva T, Errea P, Pina A. 2019. Inheritance of self- and graft-incompatibility traits in an F1 apricot progeny. PLoS One **14**, e0216371.

Iwase A, Harashima H, Ikeuchi M, *et al*. 2017. WIND1 promotes shoot regeneration through transcriptional activation of ENHANCER OF SHOOT REGENERATION1 in Arabidopsis. The Plant Cell **29**, 54–69.

Iwase A, Kondo Y, Laohavisit A, *et al*. 2021. WIND transcription factors orchestrate wound-induced callus formation, vascular reconnection and defense response in Arabidopsis. New Phytologist **232**, 734–752.

Iwase A, Mitsuda N, Koyama T, *et al*. 2011a. The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in Arabidopsis. Current Biology **21**, 508–514.

Iwase A, Ohme-Takagi M, Sugimoto K. 2011b. WIND1: a key molecular switch for plant cell dedifferentiation. Plant Signaling & Behavior **6**, 1943–1945.

Jeffree CE, Yeoman MM. 1983. Development of intercellular connections between opposing cells in a graft union. New Phytologist **93**, 491–509.

Ji P, Liang C, Yang Y, Wang R, Wang Y, Yuan M, Qiu Z, Cheng Y, Liu J, Li D. 2022. Comparisons of anatomical characteristics and transcriptomic differences between heterografts and homografts in *Pyrus* L. Plants **11**, 580.

Kawakatsu Y, Sawai Y, Kurotani K-i, Shiratake K, Notaguchi M. 2020. An in vitro grafting method to quantify mechanical forces of adhering tissues. Plant Biotechnology **37**, 451–458.

Kollmann R, Glockmann C. 1991. Studies on graft unions. III. On the mechanism of secondary formation of plasmodesmata at the graft interface. Protoplasma **165**, 71–85.

Kollmann R, Yang S, Glockmann C. 1985. Studies on graft unions. II. Continuous and half plasmodesmata in different regions of the graft interface. Protoplasma **126**, 19–29.

Krenke NP. 1933. Transplantation (Umpflanzung, Pfropfungen). In: Krenke NP, Moritz O, eds. Wundkompensation Transplantation und Chimären bei Pflanzen. Berlin, Heidelberg: Springer Berlin Heidelberg, 341–601.

Kurotani K-i, Huang C, Okayasu K, Suzuki T, Ichihashi Y, Shirasu K, Higashiyama T, Niwa M, Notaguchi M. 2022. Interfamily grafting capacity of petunia. Horticulture Research **9**, uhab056.

Kurotani K-i, Notaguchi M. 2021. Cell-to-cell connection in plant grafting—molecular insights into symplasmic reconstruction. Plant and Cell Physiology **62**, 1362–1371.

Lev-Yadun S. 2011. Why should trees have natural root grafts? Tree Physiology **31**, 575–578.

Loupit G, Cookson SJ. 2020. Identifying molecular markers of successful graft union formation and compatibility. Frontiers in Plant Science **11**, 610352.

Lu Y, Stegemann S, Agrawal S, Karcher D, Ruf S, Bock R. 2017. Horizontal transfer of a synthetic metabolic pathway between plant species. Current Biology **27**, 3034–3041.

Machin F, Hasbioğlu Y, Kragler F. 2022. An Arabidopsis callus grafting method to test cell-to-cell mobility of proteins. Methods in Molecular Biology **2457**, 299–312.

Matsuoka K, Sato R, Matsukura Y, Kawajiri Y, Iino H, Nozawa N, Shibata K, Kondo Y, Satoh S, Asahina M. 2021. Wound-inducible ANAC071 and ANAC096 transcription factors promote cambial cell formation in incised Arabidopsis flowering stems. Communications Biology **4**, 369.

Matsuoka K, Yanagi R, Yumoto E, Yokota T, Yamane H, Satoh S, Asahina M. 2018. RAP2.6L and jasmonic acid-responsive genes are expressed upon Arabidopsis hypocotyl grafting but are not needed for cell proliferation related to healing. Plant Molecular Biology **96**, 531–542.

Mazur E, Benková E, Friml J. 2016. Vasc cambium regeneration and vessel formation in wounded inflorescence stems of Arabidopsis. Scientific Reports **6**, 33754.

Mazur E, Kulik I, Hajný J, Friml J. 2020. Auxin canalization and vascular tissue formation by TIR1/AFB-mediated auxin signaling in Arabidopsis. New Phytologist **226**, 1375–1383.

Melnyk CW, Gabel A, Hardcastle TJ, Robinson S, Miyashima S, Grosse I, Meyerowitz EM. 2018. Transcriptome dynamics at Arabidopsis graft junctions reveal an intertissue recognition mechanism that activates vascular regeneration. Proceedings of the National Academy of Sciences, USA **115**, E2447–E2456.

Melnyk CW, Schuster C, Leyser O, Meyerowitz EM. 2015. A developmental framework for graft formation and vascular reconnection in *Arabidopsis thaliana*. Current Biology **25**, 1306–1318.

Milien M, Renault-Spilmont AS, Cookson SJ, Sarrazin A, Verdeil JL. 2012. Visualization of the 3D structure of the graft union of grapevine using X-ray tomography. Scientia Horticulturae **144**, 130–140.

Moore R, Walker DB. 1981a. Graft compatibility:incompatibility in plants. BioScience **31**, 389–391.

Moore R, Walker DB. 1981b. Studies of vegetative compatibility–incompatibility in higher plants. 1. A structural study of a compatible autograft in *Sedum telephoides* (Crassulaceae). American Journal of Botany **68**, 820–830.

Mower JP, Stefanović S, Young GJ, Palmer JD. 2004. Gene transfer from parasitic to host plants. Nature **432**, 165–166.

Mudge K, Janick J, Scofield S, Goldschmidt EE. 2009. A history of grafting. Horticultural Reviews, 437–493.

Muzik TJ. 1958. Role of parenchyma cells in graft union in vanilla orchid. Science **127**, 82–82.

Muzik TJ, La Rue CD. 1952. The grafting of large monocotyledonous plants. Science **116**, 589–591.

Muzik TJ, La Rue CD. 1954. Further studies on the grafting of monocotyledonous plants. American Journal of Botany **41**, 448–455.

Nickell LG. 1948. Heteroplastic grafts. Science **108**, 389–389.

Notaguchi M, Daimon Y, Abe M, Araki T. 2009. Adaptation of a seedling micro-grafting technique to the study of long-distance signaling in flowering of *Arabidopsis thaliana*. Journal of Plant Research **122**, 201–214.

Notaguchi M, Kurotani K-i, Sato Y, *et al*. 2020. Cell–cell adhesion in plant grafting is facilitated by β-1,4-glucanases. Science **369**, 698–702.

Ollat N, Peccoux A, Papura D, *et al*. 2016. Rootstocks as a component of adaptation to environment. In: Geros H, Chaves MM, Gil HM, Delrot S, eds. Grapevine in a changing environment: a molecular and ecophysiological perspective. Chichester: John Wiley & Sons Ltd, 68–108.

Olmstead MA, Lang S, Lang GA. 2006. Examining the vascular pathway of sweet cherries grafted onto dwarfing rootstock. HortScience **14**, 674–679.

Pina A, Errea P. 2008. Differential induction of phenylalanine ammonia-lyase gene expression in response to in vitro callus unions of *Prunus* spp. Journal of Plant Physiology **165**, 705–714.

Pina A, Errea P, Martens HJ. 2012. Graft union formation and cell-to-cell communication via plasmodesmata in compatible and incompatible stem unions of *Prunus* spp. Scientia Horticulturae **143**, 144–150.

Pina A, Errea P, Schulz A, Martens HJ. 2009. Cell-to-cell transport through plasmodesmata in tree callus cultures. Tree Physiology **29**, 809–818.

Pina A, Irisarri P, Errea P, Zhebentyayeva T. 2021. Mapping quantitative trait loci associated with graft (in)compatibility in apricot (*Prunus armeniaca* L.). Frontiers in Plant Science **12**, 622906.

Pitaksaringkarn W, Ishiguro S, Asahina M, Satoh S. 2014a. ARF6 and ARF8 contribute to tissue reunion in incised Arabidopsis inflorescence stems. Plant Biotechnology **31**, 49–53.

Pitaksaringkarn W, Matsuoka K, Asahina M, *et al*. 2014b. XTH20 and XTH19 regulated by ANAC071 under auxin flow are involved in cell proliferation in incised Arabidopsis inflorescence stems. The Plant Journal **80**, 604–614.

Qiu L, Jiang B, Fang J, Shen Y, Fang Z, Rm SK, Yi K, Shen C, Yan D, Zheng B. 2016. Analysis of transcriptome in hickory (*Carya cathayensis*), and uncover the dynamics in the hormonal signaling pathway during graft process. BMC Genomics **17**, 935.

Radhakrishnan D, Shanmukhan AP, Kareem A, *et al*. 2020. A coherent feed-forward loop drives vascular regeneration in damaged aerial organs of plants growing in a normal developmental context. Development **147**, 185710.

Reeves G, Tripathi A, Singh P, *et al*. 2021. Monocotyledonous plants graft at the embryonic root–shoot interface. Nature **602**, 280–286.

Rowhani A, Uyemoto JK, Golino DA, Daubert SD, Al Rwahnih M. 2017. Viruses involved in graft incompatibility and decline. In: Meng B, Martelli G, Golino D, Fuchs M, eds. Grapevine viruses: molecular biology, diagnostics and management. Cham: Springer, 289–302.

Rymen B, Kawamura A, Lambolez A, *et al*. 2019. Histone acetylation orchestrates wound-induced transcriptional activation and cellular reprogramming in Arabidopsis. Communications Biology **2**, 15.

Sachs T. 1968. On the determination of the pattern of vascular tissue in peas. Annals of Botany **32**, 781–790.

Sala K, Karcz J, Rypien A, Kurczynska EU. 2019. Unmethyl-esterified homogalacturonan and extensins seal Arabidopsis graft union. BMC Plant Biology **19**, 151.

Sauer M, Balla J, Luschnig C, Wisniewska J, Reinöhl V, Friml J, Benková E. 2006. Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. Genes and Development **20**, 2902–2911.

Savatin DV, Gramegna G, Modesti V, Cervone F. 2014. Wounding in the plant tissue: the defense of a dangerous passage. Frontiers in Plant Science **5**, 470.

Schoning U, Kollmann R. 1995. The function of phloem connections in regenerating in vitro-grafts. Botanica Acta **108**, 56–62.

Schoning U, Kollmann R. 1997. Phloem translocation in regenerating in vitro heterografts of different compatibility. Journal of Experimental Botany **48**, 289–295.

Simon SV. 1930. Transplantationsversuche zwischen *Solanum melongena* und *Iresine lindeni*. Jahrbucher für Wissenschaftliche Botanik **72**, 137–160.

Smetana O, Mäkilä R, Lyu M, *et al*. 2019. High levels of auxin signalling define the stem-cell organizer of the vascular cambium. Nature **565**, 485–489.

Solari LI, Johnson S, DeJong TM. 2006. Hydraulic conductance characteristics of peach (*Prunus persica*) trees on different rootstocks are related to biomass production and distribution. Tree Physiology **26**, 1343–1350.

Stegemann S, Bock R. 2009. Exchange of genetic material between cells in plant tissue grafts. Science **324**, 649–651.

Stegemann S, Keuthe M, Greiner S, Bock R. 2012. Horizontal transfer of chloroplast genomes between plant species. Proceedings of the National Academy of Sciences, USA **109**, 2434–2438.

Tedesco S, Fevereiro P, Kragler F, Pina A. 2022. Plant grafting and graft incompatibility: a review from the grapevine perspective. Scientia Horticulturae **299**, 111019.

Thomas H, Van den Broeck L, Spurney R, Sozzani R, Frank M. 2021. Gene regulatory networks for compatible versus incompatible grafts identify a role for SlWOX4 during junction formation. The Plant Cell **34**, 535–556.

Wulf KE, Reid JB, Foo E. 2020. What drives interspecies graft union success? Exploring the role of phylogenetic relatedness and stem anatomy. Physiologia Plantarum **170**, 132–147.

Xie LL, Dong CJ, Shang QM. 2019. Gene co-expression network analysis reveals pathways associated with graft healing by asymmetric profiling in tomato. BMC Plant Biology **19**, 373.

Xie L, Tian J, Peng L, Cui Q, Liu Y, Liu J, Li F, Zhang S, Gao J. 2022. Conserved regulatory pathways for stock–scion healing revealed by comparative analysis of Arabidopsis and tomato grafting transcriptomes. Frontiers in Plant Science **12**, 810465.

Yin H, Yan B, Sun J, Jia P, Zhang Z, Yan X, Chai J, Ren Z, Zheng G, Liu H. 2012. Graft-union development: a delicate process that involves cell–cell communication between scion and stock for local auxin accumulation. Journal of Experimental Botany **63**, 4219–4232.

Zhang A, Matsuoka K, Kareem A, Robert M, Roszak P, Blob B, Bisht A, De Veylder L, Voiniciuc C, Asahina M, Melnyk CW. 2022. Cell-wall damage activates DOF transcription factors to promote wound healing and tissue regeneration in Arabidopsis thaliana. Current Biology **32**, 1883–1894.