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Plant grafting: Molecular mechanisms and applications

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ABSTRACT

People have grafted plants since antiquity for propagation, to increase yields, and to improve stress tolerance. This cutting and joining of tissues activates an incredible regenerative ability as different plants fuse and grow as one. For over a hundred years, people have studied the scientific basis for how plants graft. Today, new techniques and a deepening knowledge of the molecular basis for graft formation have allowed a range of previously ungraftable combinations to emerge. Here, we review recent developments in our understanding of graft formation, including the attachment and vascular formation steps. We analyze why plants graft and how biotic and abiotic factors influence successful grafting. We also discuss the ability and inability of plants to graft, and how grafting has transformed both horticulture and fundamental plant science. As our knowledge about plant grafting improves, new combinations and techniques will emerge to allow an expanded use of grafting for horticultural applications and to address fundamental research questions.

Key words: plant grafting, regeneration, mobile molecules, tissue adhesion, vascular differentiation, stress tolerance

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INTRODUCTION AND THE HISTORY OF GRAFTING

For millennia, people have cut and joined different plants together through a process known as grafting. Shoots from one plant, known as the scion, are cut and joined to the stem or roots from another plant, known as the stock or rootstock (Figure 1). A rapid healing process ensues whereby tissues attach, cells divide, cells expand, and cells differentiate to form functional vascular connections between scion and rootstock. Botanical relatedness is important for grafting success, as is the age and types of tissues used (Garner and Bradley, 2013). How people discovered grafting remains unknown, though they were likely inspired by natural tissue fusions seen when branches attach or when parasitic plants grow on their host plants. By the fourth century BCE, grafting was practiced in the Mediterranean region and likely in China and the Middle East (Mudge et al., 2009). Some of the earliest species grafted included grapes, citrus, and apples. Many of these plants, such as citrus and apples, were not true breeding and could not be easily rooted (Garner and Bradley, 2013). To multiply desirable varieties, scions of the best trees were grafted to wild rootstocks. For other species such as grapes, the purpose of ancient grafting is less clear since grapes can be propagated from cuttings. With these, desirable scions were grafted to rootstocks that might have been better suited to the soil, perhaps one of the earliest examples of rootstock-specific benefits. By the Middle Ages, grafting was very much practiced and one notable example from 1472 first mentions the dwarf apple variety 'Paradise' (Mudge et al., 2009). It had poor fruit quality but rooted easily from cuttings and when used as a rootstock, gave strong dwarfing effects to the scion. Thus, 'Paradise' was likely one of the first clonal rootstocks used to improve yields through dwarfing, and today forms the basis for several popular apple dwarfing rootstocks. Another notable historical milestone was the widespread introduction of grape vine grafting in the late 1800s (Mudge et al., 2009). In response to the arrival of the insect pest phylloxera to Europe from North America, European grape vines died as they had no natural resistance. A clever solution was found whereby grafting was used to replace a sensitive European root with a disease-resistant North American one (Mudge et al., 2009). Thus, the practice of grafting American rootstocks to European scions was born and today is used where phylloxera is present, which is nearly all the wine growing regions globally. A third important development has been the use of vegetable grafting. Although

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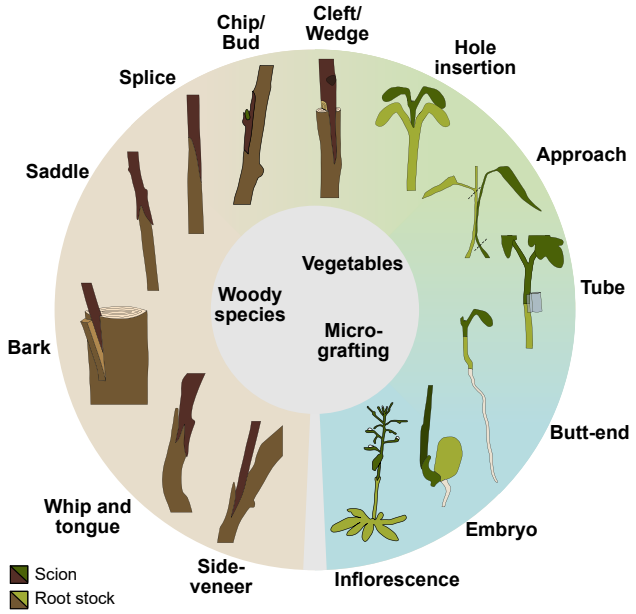

Figure 1. A spectrum of grafting techniques.
Several grafting techniques exist and are used depending on the species, time of year, and desired outcome. Woody species are often grafted with side-veneer, whip and tongue, bark, saddle, splice, chip/bud, cleft/wedge, or approach grafting. For vegetable grafting, cleft/wedge, hole insertion, approach or tube grafting are most commonly used. Several micrografting techniques including butt-end hypocotyl grafting, embryo grafting, and inflorescence grafting are mainly used for research purposes.

already mentioned in the first century BCE with bottle gourd grafting in China (Mudge et al., 2009), modern vegetable grafting was first proposed in the 1920s but did not gain popularity until the 1950s and 1960s (Lee et al., 2010). During this time, techniques emerged that made grafting economically viable and disease-resistant rootstocks became available (Lee et al., 2010). Today, over 1 billion vegetables, mainly tomatoes, peppers, cucumbers, and melons (Lee et al., 2010), are grafted for increased disease resistance, far outnumbering the number of woody species grafted per year. Finally, the recent use of grafting with small, young tissues, a process known as micrografting, has gained popularity (Figure 1). Micrografting has allowed an increase in the range of species that can be grafted and also facilitated the scientific study of grafting by allowing a large number of grafts in model plant species to be rapidly made and take little space.

The process of graft formation has interested people since ancient times, including graft failure, graft success, and the ability of the conjoined plants to maintain distinct phenotypes (Mudge et al., 2009). With modern tools and techniques such as micrografting, our scientific understanding of graft junction formation between scion and rootstock and the physiology of this process has rapidly progressed. In particular, grafting in *Arabidopsis* and tomato has provided mechanistic details of graft junction formation. Grafting with different species and genotypes has also been transformative for understanding the long-distance movement of molecules in plants. Given the recent advances made in grafting biology, we present here an updated view of the molecular mechanisms of graft junction formation. We discuss the early steps of tissue attachment through to the vascular connection process and why grafts fail or succeed. We also discuss the environmental regulation of graft junction formation and the current scientific and horticultural applications. Altogether, this rapidly expanding understanding of graft formation will facilitate horticultural applications and scientific progress.

THE ORIGINS OF GRAFTING

Grafting is a human-initiated process since it involves the cutting and joining of different plant varieties or species together (Figure 2). Such phenomena, whereby different plants are wounded and placed in contact at the sites of wounding, must be unusual in natural settings, begging the question of what natural process allows such a human-driven technique to succeed. Here, we propose three phenomena that occur in nature that could explain in part the ability of plants to efficiently graft.

First, many plants connect and fuse tissues and vasculature even in the absence of wounding. The carpels in the gynoecium attach during floral development in angiosperms to form fused carpels (Reyes-Olalde et al., 2013). During leaf vein development and axillary bud activation, vasculature strands are initially unconnected, but as leaves develop or buds activate, the vasculature differentiates and connects (Nelson and Dengler, 1997; Leyser, 2009). Second, many plant species have strong regenerative abilities. They readily form a proliferative mass of cells known as callus at cut sites, and heal wounds including deep incisions in the stems. The transcriptional responses to such wounds are similar to those observed during graft formation (Melnyk et al., 2018; Zhang et al., 2022), and conceptually, a deep cut through a stem that severs the vascular tissue might be similar to a self-grafted plant. For instance, both stem cutting and grafting activated *NAC DOMAIN-CONTAINING PROTEIN071* (*ANAC071*) and *ANAC096* expression, and mutations in these genes inhibit both cutting and graft healing (Matsuoka et al., 2021; Zhang et al., 2022). Both *WUSCHEL-LIKE HOMEOBOX13* (*WOX13*) and *ETHYLENE RESPONSE FACTOR115* (*ERF115*) transcription factors are also involved in wound healing, regeneration, and graft formation (Heyman et al., 2016; Ikeuchi et al., 2022; Zhang et al., 2022). In *Physcomitrium* and *Marchantia*, *WOX13-like* and an *ERF115*-like homolog, *PpWOX13L* and *MpERF15*, are upregulated in response to wounding, and knocking out these factors inhibits the ability to heal or respond to wounding (Sakakibara et al., 2014; Liang et al., 2022). Notably, the potential for grafting and wound healing varies greatly among tissue types. For instance, mature tissues from monocots have poor regenerative abilities and cannot form callus or graft (Muzik and La Rue, 1952; Ikeuchi et al., 2016; Hu et al., 2017). However, using embryonic tissues in monocots provides high regeneration competency and an ability to successfully graft (Ikeuchi et al., 2016; Reeves et al., 2022). Thus, aspects of wound healing and regeneration appear conserved between species and are likely the same as those used by plants to heal grafts.

A third possible explanation for the ability for plants to graft is their ability to form natural tissue fusions between different plants in the absence of human intervention. Plant parasitism, a natural example of plant-plant tissue attachments, has evolved at least

Figure 2. Examples of grafting and tissue fusion.
(A) A modern apple (*Malus domestica*) orchard of 'Red Fuji' apple scions grafted on M26 apple rootstocks. The orchard is located in Shaanxi Province, China.
(B) A bay laurel (*Laurus nobilis*) showing an example of tissue fusion after the process of tree shaping. The tree is located in Norrvikens trädgårdar, Sweden.
(C) Two cherry trees (*Prunus* sp.) grafted together demonstrating long-term differences in growth rates as the rootstock diameter outgrows the scion. The tree is located in Reagent's Park, London, UK.
(D) Grafting a chlorophyll-deficient cactus species (red; *Gymnocalycium mihanovichii*) onto another species capable of photosynthesis to create a visually appealing graft combination.
(E) A grafted tree rose. Shrub roses (floribunda variety 'Beijinghong') are grafted onto an elongated main stem of *Rosa canina*. The tree is located in Yunnan Province, China.
(F) An example of natural grafting in Persian Ironwood (*Parrotia persica*). The two stems are fused at different sites. The tree is located in Cambridge Botanic Gardens, UK.

12 independent times, suggesting that the ability for distantly related plants to fuse together has developed multiple times and is a recurrent developmental strategy (Westwood et al., 2010). The parasitic plant *Phtheirospermum japonicum* infects the host *Arabidopsis* through the formation of a haustoria that penetrates tissues and forms xylem connections between parasite and host (Kokla and Melnyk, 2018). This process activates genes including those related to xylem (*VASCULAR-RELATED NAC-DOMAIN 7*, *VND7*), cellulase-like genes (*GLYCOSYL HYDROLASE 9B3*, *GH9B3*), auxin transport (*PIN-FORMED 1*, *PIN1*), and cell division (*CYCLIN B1;2*), which are the same genes activated when *Phtheirospermum japonicum* is grafted to *Arabidopsis* (Kurotani et al., 2020), suggesting a degree of overlap between grafting and parasitism. In addition, the process of natural fusions or natural "grafting" is both widespread and common. There, when stems or roots are brought into contact with close neighbors, tissues can fuse and grow together (Mudge et al., 2009; Garner and Bradley, 2013). This process of natural "grafting" is known as inosculation and most commonly occurs within a species but can also form between species (Figure 2). In temperate climates, tissue fusion occasionally occurs in branches but is much more common in roots. In Norway spruce, 33–75% of trees after 10–20 years showed root fusions, while 36% of balsam fir trees fused roots to one another (Külla and Lõhmus, 1999; Quer et al., 2022). Root fusions between different species is less common but still occurs, particularly when species are taxonomically related (Garner and Bradley, 2013). Why roots and branches fuse between trees is unknown. It may be due to wounding from nematodes or abrasion, or a process related to pressure and growth allowing fusion. There may be advantages for nutrient exchange and for securing trees from wind damage and toppling over. However, root grafting is also an efficient means for pathogens such as fungi to move from tree to tree, and in forestry is undesirable since it contributes to pathogen spread (Külla and Lõhmus, 1999). Thus, there may be adaptive or evolutionary reasons plants choose to fuse limbs that involve healing, fusion, and patterning mechanisms discussed above.

TISSUE ADHESION AND PLASMODESMATA FORMATION

Successful grafting begins with cutting and the correct alignment of tissues by the grafter (Figure 1). The plant then begins a tissue adhesion process involving cell wall modifications, cell expansion, and cell division (Figure 3). Tissue adhesion is rapid and the strength of the graft increases first exponentially then linearly. After several weeks, the grafted tissue has similar strength to non-grafted tissues (Lindsay et al., 1974; Melnyk et al., 2018). Methods to measure graft attachment include using weights or manual bending to determine breaking strength and more recently, using an extensometer (Lindsay et al., 1974; Melnyk et al., 2018; Kawakatsu et al., 2020; Thomas et al., 2021). During the early stages of attachment, structural projections in the cell wall appear and cell wall components such as pectins, extensins, cellulases, hemicellulose, and arabinogalactan proteins are secreted to the extracellular region (Miller and Barnett, 1993; Sala et al., 2019; Notaguchi et al., 2020; Frey et al., 2022, 2023b). Low methyl-esterified homogalacturonans are the main pectin deposited on cut tissues in *Arabidopsis* and tomato (Sala et al., 2019; Frey et al., 2023b). Secretion of a specific cellulase called β-1,4-glucanases into the extracellular region is important for *Nicotiana* inter-family grafting (Notaguchi et al., 2020) and external application of cellulase or auxin enhances tissue adhesion in *Nicotiana* (Kawakatsu et al., 2020). It is likely that auxin application promotes adhesion through changes in cell wall composition at the graft junction, as it is known that auxin can influence cell wall properties (Nishitani and Masuda, 1981;

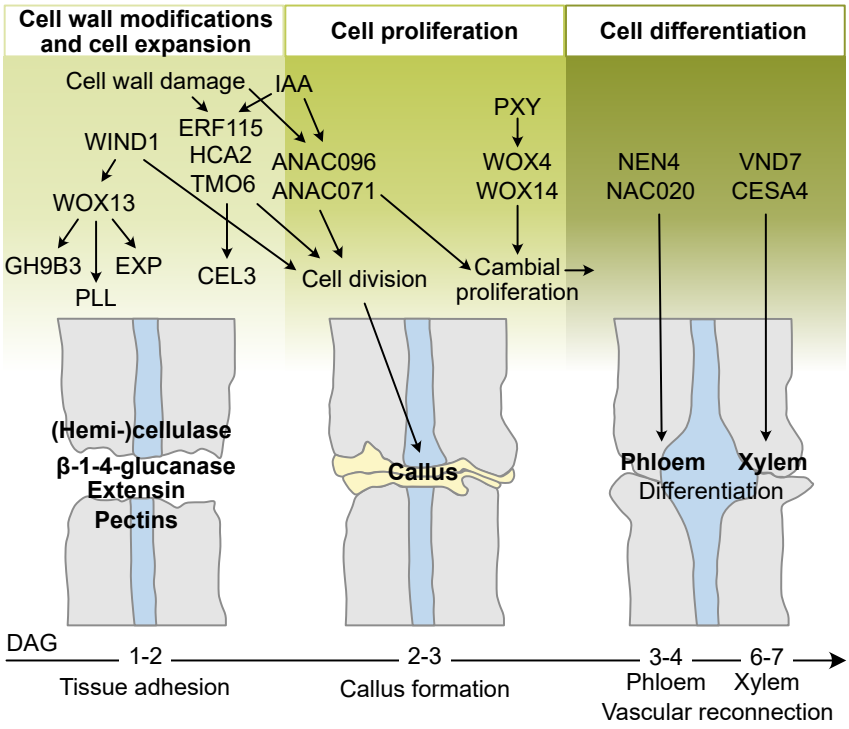

Figure 3. Molecular players involved in graft formation in *Arabidopsis thaliana*.
Grafting follows sequential events of tissue adhesion (1–2 days after grafting [DAG]), callus formation (2–3 DAG) and vascular reconnection with phloem reconnection (3–4 DAG) and xylem reconnection (6–7 DAG) (Melnyk et al., 2015). During the tissue adhesion phase, WOX13 induces GH9B3, PLL, and EXP (Ikeuchi et al., 2022). Additionally, cell wall damage and auxin (indole-3-acetic acid [IAA]) induce transcription factors including HCA2, TMO6, ANACs, and ERF115 (Zhang et al., 2022). These act upstream of CEL3 and contribute to cell divisions and vascular cambium activation leading to callus formation to fill the gap between scion and rootstock. During this phase, PXY together with WOX4 and WOX14 induce cambial proliferation. In the last phase, these cells are differentiated to phloem by factors such as NEN4 and NAC020 and to xylem by factors including VND7 and CESA4 (Melnyk et al., 2018).

Cosgrove, 2005). Accordingly, cellulase and auxin treatment have an additive effect on tissue adhesion (Kawakatsu et al., 2020). However, how cell wall modifications facilitate tissue adhesion is an outstanding question. Low methyl-esterified homogalacturonan might provide "sticky" surfaces enabling tissue adhesion due to its ability to form adhesive pectate gels by crosslinking with calcium ions (Goldberg et al., 1996; Sala et al., 2019). Similarly, extensins exhibit adhesive characteristics that may contribute to tissue adhesion (Miller and Fry, 1993). Thus, the adhesion between scion and rootstock is accomplished most likely by the deposition and subsequent polymerization of extracellular materials at the graft junction. Cell wall modifications may also play an important role to assist with cellular expansion since epidermal and cortex cells at the *Arabidopsis* graft rapidly expand where tissues have been cut and cells damaged (Melnyk et al., 2015; Matsuoka et al., 2016). Such expansions help fill the gaps and are likely mediated by changes in and remodeling of the cell wall. At the cellular level, cell wall modifications likely help with both cell wall loosening to allow for cellular expansion and cell wall crosslinking to join new cells together. What determines the process of loosening versus attachment is unknown but may rely on sensors that can detect the presence or absence of surrounding cells that could signify loosening (no neighbor) or attachment (a neighbor is present). Thus, the accumulating data support the notion that cell wall modifications facilitate tissue adhesion by both providing a molecular glue to hold cells together, but also by allowing cell wall loosening to facilitate cell expansion.

A second important step during early stages of graft formation is the activation of cell division and cell differentiation. Wound-induced callus forms at the cut sites and proliferates to help fill the gap between tissues (Melnyk et al., 2015; Ikeuchi et al., 2022). The origins of these cells are not well known, but may be derived from vascular and pericycle cells after wounding (Ikeuchi et al., 2017). In particular, the vascular cambium appears important for forming wound-induced callus (Serivichyaswat et al., 2023). Blocking auxin signaling in the cambium inhibits attachment, callus formation, and vascular reconnection, suggesting a link between these three processes (Serivichyaswat et al., 2023). Similarly, mutants with reduced callus formation have a lower efficiency of graft attachment (Ikeuchi et al., 2022). In *Arabidopsis*, *WOUND INDUCED DEDIFFERENTIATION1* (*WIND1*) mutants reduce wound-induced callus formation and inhibit leaf grafting, and a correlation between callus formation and grafting success is prevalent in the grafting literature (Garner and Bradley, 2013; Ikeuchi et al., 2022). However, many *Arabidopsis* mutants compromised in callus formation, including *WIND1* mutants, do not affect hypocotyl grafting (Melnyk et al., 2015) and thus more studies are needed to comprehend the absolute requirements of callus in successful graft formation. When tissues are tightly connected by a skilled grafter, callus formation may not be as critical for attachment but instead plays an important role for strengthening the attachment or filling gaps from imprecise alignment.

Soon after wounding, a rapid transcriptional response occurs including the upregulation of multiple genes related to cell wall biogenesis (Cookson et al., 2013; Melnyk et al., 2018; Notaguchi et al., 2020; Xie et al., 2021). In particular, several DNA binding with one finger (DOFs), ANAC, and ERF transcription factors are induced within 6 h of *Arabidopsis* grafting and mutations in these genes fail to activate cell wall-related genes including *EXPANSINs* (*EXP*), *XYLOGLUCAN ENDOTRANSGLUCOSYLASE 20* (*XTH20*), and *CELLULASE3* (*CEL3*) (Zhang et al., 2022) (Figure 3). The DOF transcription factor TARGET OF MONOPTEROS6 (TMO6) binds the promoter of *CEL3*, a homolog of *NbGH9B3* (Zhang et al., 2022). This gene encodes a β-1,4-glucanase upregulated during *Nicotiana* grafting (Notaguchi et al., 2020), suggesting that DOF activation can

directly activate cellulases important for tissue attachment. *WOX13* is also upregulated during wounding and regulates cell wall-modifying enzymes genes such as *EXP*, *PECTATE LYASE LIKE*s (*PLL*), and *GH9B3* after wounding in *Arabidopsis* (Ikeuchi et al., 2022). Accordingly, *wox13* mutant petioles are defective in tissue adhesion during grafting. Callus formation requires cell division and markers associated with cell division are typically activated within 24–48 h of graft formation in *Arabidopsis* (Melnyk et al., 2015, 2018). This activation occurs later than the upregulation of many cell wall-related genes and also later than the earliest stages of tissue attachment and cell expansion in *Arabidopsis*, suggesting cell division may be occurring after the earliest stages of attachment.

After tissue adhesion, a new shared cell wall is formed with plasmodesmata between the cells of the scion and rootstock (Jeffree and Yeoman, 1983; Kollmann and Glockmann, 1991; Kurotani and Notaguchi, 2021). A proper plasmodesmatal connection at the graft junction may represent successful graft formation, as limited plasmodesmatal connections are found in grafts that show late graft rejection and failure (Pina et al., 2012). Plasmodesmata are typically observed using transmission electron microscopes, which makes distinguishing the graft junction and which cells originated from which tissue challenging. To overcome this obstacle, grafts between species with different cellular morphology were performed that allowed the observation of plasmodesmata across cells at the junction between *Helianthus* and *Vicia*, an only partially successful graft combination (Kollmann et al., 1985; Kollmann and Glockmann, 1985). Using a correlative light electron microscopy approach with fluorescent markers has distinguished the graft junction in *Arabidopsis* with excellent resolution (Chambaud et al., 2022). There, four classes of plasmodesmata emerged by 3 days after grafting including many that spanned the junction and some that did not (Chambaud et al., 2022). Whether such intercellular connections contribute to grafting success is unknown, but there is speculation that such channels or even pores in the cell wall might contribute to organelle transfer across the graft junction (Hertle et al., 2021). Certainly highly modified plasmodesmata known as sieve plates are important for phloem function and play an important role in vascular formation during grafting (Melnyk et al., 2015).

VASCULAR FORMATION

The plant vascular system including phloem and xylem facilitates communication and transports organic compounds, water, and nutrients between shoot and root. Vascular reconnection is thus vital during grafting and failed reconnections lead to the long-term failure of most grafts (Garner and Bradley, 2013; Thomas et al., 2021). Hence, there is a pressing need to understand how the vasculature reconnects and to monitor connectivity success. Staining longitudinal sections of a wound site or the graft junction with dyes such as toluidine blue or basic fuchsin enabled the measurement of xylem reconnection with their distinctive cellular structure (Jacobs, 1952; Hardham and McCully, 1982; Moore, 1984b). However, non-destructive methods are technically easier, though rely on the systemic transport of visible molecules as an indirect measure of connectivity. Carboxyfluorescein diacetate (CFDA), esculin, acid fuchsin, and green fluorescent protein movement between shoot and scion have all been used to monitor grafting success (Yin et al., 2012; Melnyk, 2017a; Xu et al., 2022). Transducer systems can observe hydraulic connections, whereas infrared thermography and quantum yields can measure heat dissipation or photosynthetic activity, respectively, processes that can signify grafting success (Turquois and Malone, 1996; Frey et al., 2023a). However, costs and equipment availability for such systems remain barriers. Using a combination of fluorescent dye movement, gene expression analyses, and cell morphology in *Arabidopsis* hypocotyls revealed that phloem connects 3–4 days after grafting and xylem connects 6–7 days after grafting (Yin et al., 2012; Melnyk et al., 2015) (Figure 3). *Arabidopsis* inflorescent stems have a well-developed vascular union by 15 days after grafting (Flaishman et al., 2008), suggesting young tissues graft quicker. Rice grafting of embryonic hypocotyls show a similar sequence of events with phloem connecting at 5–7 days and xylem at 6–10 days (Reeves et al., 2022). In grafted tomato, xylem connected 4 and 5 days after grafting, whereas phloem connected between 5 and 6 days (Cui et al., 2021). In young conifer grafts, phloem and xylem connect during similar time frames (Feng et al., 2024). Thus, it seems there is no clear conservation for whether phloem or xylem differentiates first but instead the need for both to differentiate soon after grafting. These findings in gymnosperms, eudicots, and monocots also revealed similar dynamics of graft junction formation and a common activation of genes related to procambium, phloem, and xylem, suggesting a high degree of conservation regarding how plants graft (Melnyk et al., 2018; Reeves et al., 2022; Feng et al., 2024). Intriguingly, some graft combinations between different species appear viable or have high survival rates yet lack functional vascular connections such as *Arabidopsis* grafted to tomato (Flaishman et al., 2008) or eggplant grafted to pepper (Thomas et al., 2023). It could be that technical limitations prevented the accurate measurement of vascular connectivity, or that nonvascular symplastic and apoplastic transport is sufficient for vegetable grafts to survive and grow. For woody species, vascular connectivity is likely critical for long-term success (Garner and Bradley, 2013).

Early during graft formation, markers associated with provascular formation and wounding are rapidly upregulated in *Arabidopsis* including *ERF114*, *ERF115*, DOFs, *ANAC071*, and *ANAC096* transcription factors (Zhang et al., 2022). Overexpressing the DOF gene *HIGH CAMBIAL ACTIVITY2* (*HCA2*) or related DOF genes *DOF6*, *DOF2.1*, and *TMO6* promotes phloem reconnection (Melnyk et al., 2018; Zhang et al., 2022). A quadruple mutant of *dofQ* (*hca2*, *tmo6*, *dof2.1*, *dof6*) impairs phloem and xylem reconnection, and fails to upregulate multiple genes associated with vascular formation, while *anac071anac096* double mutants and *erf114erf115* double mutants decreased phloem reconnection (Zhang et al., 2022). Soon after cambial gene activation, cell cycle markers are induced after which cell differentiate begins when phloem and then xylem markers activate (Melnyk et al., 2018). Cambial markers such as *PHLOEM INTERCALATED WITH XYLEM* (*PXY*) and *WOX4*, phloem marker genes such as *NAC45/86-DEPENDENT EXONUCLEASE-DOMAIN PROTEIN 4* (*NEN4*) and *NAC020*, and xylem markers such as *VND7* and *CELLULOSE SYNTHASE A4* (*CESA4*) activate sequentially (Melnyk et al., 2018; Cui et al., 2021). *PXY* regulates cambium proliferation by promoting *WOX4* and *WOX14* (Etchells et al.,

2013). *WOX4* is also important for grafting since *Slwox4* mutants fail to form proper xylem connections in tomato (Thomas et al., 2021).

Plant hormones, in particular auxin and cytokinin, play a vital role in promoting the differentiation and regeneration of both phloem and xylem tissues (Wetmore and Rier, 1963; Sachs, 1981; Aloni, 1995). Auxin and cytokinin levels peaked around the graft junction 12 h after grafting in tomato (Cui et al., 2021) and there is a high overlap between graft activated genes and auxin responsive genes in both *Arabidopsis* and Norway spruce (Melnyk et al., 2018; Feng et al., 2024). In *Arabidopsis*, activation of auxin response at the graft junction appears similar above and below the junction by 6 to 24 h after grafting. In tomato, auxin initially accumulates above the junction but levels are similar above and below the junction by 72 h after grafting (Melnyk et al., 2018; Cui et al., 2021). Auxin transporters including *PIN1* are upregulated in the *Arabidopsis* scion, perhaps to help transport auxin across the graft junction (Melnyk et al., 2018). The role of auxin is particularly notable since mutations in auxin response, such as *auxin resistant 1* (*axr1*), *aberrant lateral root formation 4* (*alf4*), and *bodenlos* (*bdl*) all reduce grafting efficiency (Melnyk et al., 2015; Serivichyaswat et al., 2022). Treatment with inhibitors of auxin transport or auxin biosynthesis also prevents successful graft formation (Matsuoka et al., 2016; Reeves et al., 2022; Serivichyaswat et al., 2022, 2023). The effects of several mutations are stronger when in the rootstock, suggesting this tissue might be more sensitive to perturbations in auxin. Grafting using a rootstock overexpressing *iaaM*, which boosts auxin levels (Sitbon et al., 1992), raised graft success rates (Zhai et al., 2021). A role for cytokinin is less clear since several cytokinin mutants in *Arabidopsis* did not affect vascular connectivity (Melnyk et al., 2015). However, exogenous auxin or cytokinin application can increase the success rate of grafting in *Carya*, tobacco, tomato, and rice (Saravana Kumar et al., 2018; Cui et al., 2021; Zhai et al., 2021; Reeves et al., 2022), and auxin-like compounds are often included in the waxes used for grape vine grafting. Other plant hormones are also implicated in the establishment of vascular connections during graft formation. Ethylene (1-aminocyclopropane-1-carboxylate, ACC) accelerates graft union formation, while its biosynthesis inhibitor delays healing (Zhai et al., 2021). The gibberellin biosynthesis inhibitor paclobutrazol (PBZ) decreased monocotyledon grafting success, while combining gibberellin and auxin increased rice grafting success from 53% to 78% (Reeves et al., 2022). Gibberellin plays a role in promoting cell division in cut hypocotyls (Asahina et al., 2002) and cortex cell expansion at the graft junction (Matsuoka et al., 2016) though there is no clear upregulation of GA-related genes during grafting (Melnyk et al., 2018). Sugars are also mobile growth factors and during grafting, promote junction development in cucumber and pumpkin heterografts (Miao et al., 2021). In addition, the application of sugar promotes vascular formation in callus (Wetmore and Rier, 1963; Aloni, 1980). However, sugar treatment inhibited phloem reconnection in *Arabidopsis* (Melnyk et al., 2018). This could suggest sugar is important for growth and vascular differentiation, but depending on the sugar levels and location at the junction, may inhibit the vascular reconnection process. Likely, too, other hormones play important roles and by looking at phenotypes in more detail or using species with less robust graft healing will help reveal their roles (Nanda and Melnyk, 2018).

GRAFT COMPATIBILITY AND INCOMPATIBILITY

The use of grafting in horticulture relies on successful combinations of different species or genotypes. However, even with appropriate grafting methods and suitably sized tissues, many plants cannot be successfully grafted with each other (Rasool et al., 2020) (Figure 4). This phenomenon is known as graft incompatibility (Melnyk, 2017b) and has been described in many horticulturally important species including grapevine, pear, quince, lychee, apricot, and cherries (Loupit and Cookson, 2020). With increasing genetic distance, grafting success gets less likely and grafting between individuals of different families is usually not successful (Melnyk, 2017b; Rasool et al., 2020). There are two main types of graft incompatibility. In short-term incompatibility, the graft often does not survive more than a couple of weeks or months (Melnyk, 2017b) (Figure 4). In long-term incompatibility, the scion grows well initially but after several months or even years, grafts begin to fail (Errea et al., 1994; Pina and Errea, 2005; Melnyk, 2017b; Rasool et al., 2020). Rootstock and scion incompatibility varies and phylogenetic relationships do not always inform whether two partners are compatible (Melnyk, 2017b; Rasool et al., 2020). Both compatible and incompatible grafts may form callus and even plasmodesmata between them (Kollmann and Glockmann, 1985; Errea et al., 1994; Pina et al., 2012). However, short-term incompatible grafts are often characterized by a low attachment between the grafted partners and they form limited or no vascular connections. Limited vascular connections often lead to stunted shoot and root growth or the formation of suckers or adventitious roots (Garner and Bradley, 2013), illustrating that the formation of a vascular connection is an important indicator of graft compatibility. Long-term incompatible grafts typically show early signs of success but with months or years they have problems like graft junction breaking or stunted scion growth. In long-term incompatible apricot grafts, a portion of the callus evolves into a parenchymatous tissue instead of vascular tissue that coexists with the differentiated vascular tissue. This may cause graft breaking during later stages (Errea et al., 1994). In incompatible pear-quince heterografts, a decrease in programmed cell death processes caused delayed and limited vascular differentiation (Espen et al., 2005). Bulging at the graft junction or formation of a necrotic layer is also a common sign of long-term incompatibility that can impact the quality of the graft even several years after grafting (Pina and Errea, 2005).

Independent of the cause of graft incompatibility, it is important to identify incompatibility early to avoid economic losses (Rasool et al., 2020) (Table 1). One factor used to estimate graft compatibility is the quality and quantity of phenolics (Pina and Errea, 2008; Pina et al., 2012; Babar et al., 2023). Graft combinations that are less compatible show high concentrations of phenolic compounds, but in general the identification of robust metabolitic markers has been challenging (Loupit and Cookson, 2020; Loupit et al., 2022, 2023). Recent studies using a tissue-specific approach in grapevine indicated that α-viniferin accumulates at graft junctions with low grafting success rates, while resveratrol accumulates at heterograft junctions with high success rates (Loupit and Cookson, 2020; Loupit et al., 2022, 2023). Incompatible grafts also tend to

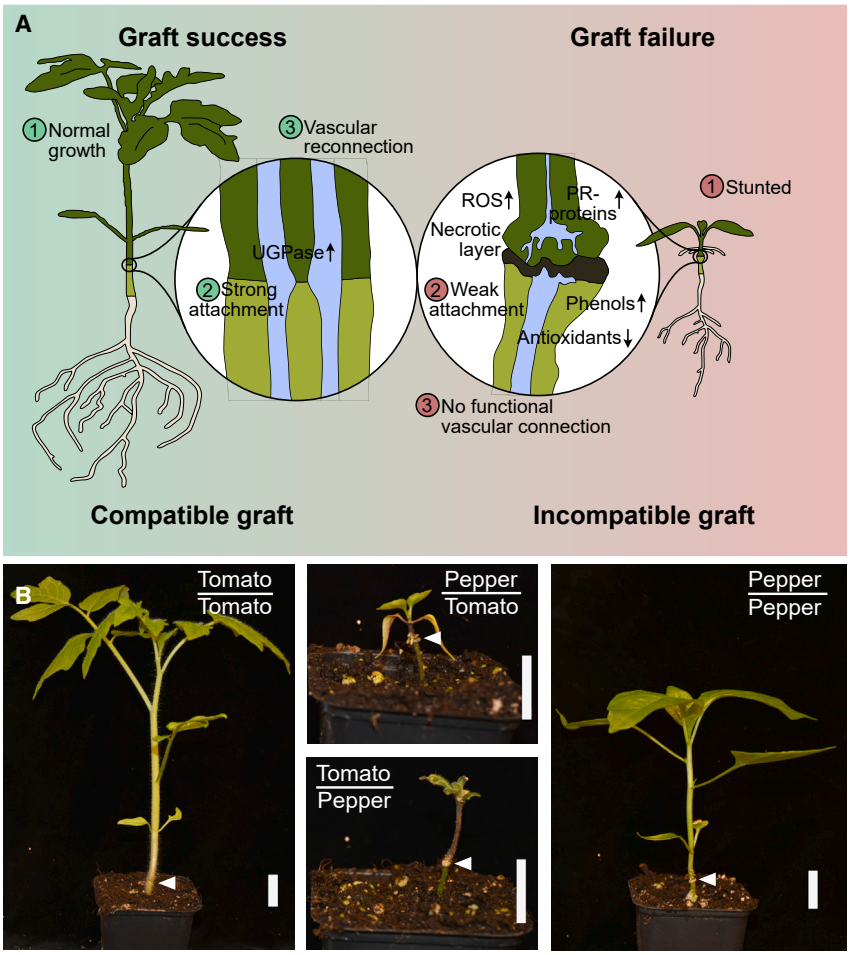

Figure 4. Graft success and failure.

(A) Cartoon depicting symptoms of compatible grafts leading to graft success and incompatible grafts leading to graft failure. On the left, a compatible graft showing (1) normal growth, (2) strong attachment, and (3) vascular reconnection. UDP-glucose pyrophosphorylases (UGPases) are upregulated at the graft junction. On the right, an incompatible graft exhibiting (1) stunted growth, (2) low attachment, and (3) no functional vascular connection. ROS, defense related proteins (PR-proteins) and phenols are upregulated, while antioxidants levels are decreased. A necrotic layer at the graft junction has formed.

(B) Tomato and pepper homo- and heterografts 28 days after grafting (DAG). Plants were grafted 7 days after germination on agar plates and transferred to soil 14 DAG. While homografts grow well, the heterografts exhibit signs of graft incompatibility (see A). White triangles indicate the graft junction, scale bars, 2 cm.

produce more stress response compounds including the reactive oxygen species hydrogen peroxide and in some cases produce lower levels of antioxidants (Aloni et al., 2008; Irisarri et al., 2015; Loupit and Cookson, 2020; Babar et al., 2023). Furthermore, measuring peroxidase activity can estimate graft compatibility (Babar et al., 2023) since peroxidase activity at the graft interface of incompatible grafts is often higher than that of compatible grafts (Loupit and Cookson, 2020). In grapevine, compatible grafts have an earlier and higher expression of genes involved in metabolic, developmental and hormonal pathways and at the same time, a reduced expression of phenolic metabolism genes and of the oxidative stress response (Assunção et al., 2019). In incompatible apricot-plum callus grafts, UDP-glucose pyrophosphorylase (UGPase) is expressed at lower levels compared with compatible grafts and could thus be used as a marker of graft compatibility (Pina and Errea, 2008). Another sign of graft compatibility is the chlorophyll concentration, which can be used to estimate stress levels (Tedesco et al., 2020). The identification of such molecular markers for grafting success would be an advantage for genetic research and rootstock selection programs (Loupit and Cookson, 2020). Recently, advances have been made to identify the genetic basis of graft incompatibility in apricots-plum grafts using crosses between compatible and incompatible apricot varieties. A genome-wide quantitative trait loci mapping of F1 apricots identified two genomic regions associated with incompatibility, necrosis and wood discontinuity (Irisarri et al., 2019; Pina et al., 2021). Refined mapping of these regions and identification of the causative genes would be very informative for understanding incompatibility in woody species and could represent useful breeding markers in the future.

Even though many signs of incompatibility have been identified, it is not clear whether these are a cause or a consequence of incompatibility. Incompatibility may be caused locally or on a systemic level (Melnyk, 2017b). One theory is that graft incompatibility is caused locally by cell recognition mechanisms and activation of defense and stress responses at the graft junction (Yeoman et al., 1978; Tedesco et al., 2022). This has been supported by stress response genes being locally upregulated at the graft junction of heterografts compared with homografts (Cookson et al., 2014). The failure of local cell-to-cell recognition important for tissue attachment and vascular formation could also cause graft incompatibility (Jeffree and Yeoman, 1983). Overcoming graft incompatibility using a third plant in the middle that is compatible with the scion and rootstock, a process known as intergrafting or double working, allows some incompatible graft combinations to succeed and also argues for a local mechanism contributing to at least some examples of graft incompatibility. On the other hand, systemic effects may exist that cause graft incompatibility. According to this other theory, incompatibility is caused by an imbalance of the mobile graft promoting morphogens, such as auxins, and graft inhibiting toxins, such as cyanides, at the graft junction (Moore, 1984a). In incompatible grafts, morphogens are overwritten by toxins (Moore, 1984a). This view is supported by grafting with certain melon and pumpkin combinations that fail but incompatibility can be overcome by changing auxin transport dynamics suggesting systemically transported auxin might affect graft incompatibility (Aloni et al., 2008). Another example is pear and

Compatible/Graft success	Incompatible/Graft failure
Adhesion and callus (wound response) Strong attachment Vascular (phloem and xylem) reconnection Expression of grafting specific genes Both nonvascular and vascular connections	Adhesion and callus (wound response) Lower attachment Limited vascular reconnection Necrotic layer at the graft junction More ROS Less antioxidants More phenolic compounds Expression of pathogen related proteins Stunted shoot and root growth The formation of suckers or adventitious roots Large, bulging graft junctions

Table 1. Graft failure and graft success

quince grafts where cyanogenic glycosides from the quince rootstock, such as prunasin produced in response to temperature stress, move into the pear scion where they are broken down to toxic cyanides leading to necrosis at the junction and graft failure (Gur et al., 1968; Moore, 1984c). Using an intergraft, however, can allow a successful pear-quince graft to form (Garner and Bradley, 2013). Thus, it appears that both local and systemic factors are relevant for grafting success.

Recently, advances have been made in overcoming graft incompatibility. Monocots show very low rates of grafting success and are considered incompatible, potentially due to a lack of a vascular cambium and their scattered arrangement of vascular bundles (Melnyk and Meyerowitz, 2015). However, grafting with the embryonic hypocotyl tissue (mesocotyl in grasses) of monocots overcame grafting incompatibility both within a species and between different genera of the monocots (Reeves et al., 2022). Using this technique, crops such as durum wheat, rice, pearl millet, pineapple, banana, onion, tequila agave, oil palm, and date palm could self-graft. Hexaploid wheat even formed inter-species grafts with durum wheat and inter-generic grafts with rye and inter-tribal grafts with oat (Reeves et al., 2022). Such inter-genus and inter-tribal grafts are uncommon in eudicots suggesting monocots have fewer inter-species barriers to grafting, or possibility, that very young meristematic tissues allow such wide grafts to form. Recent findings in gymnosperms found a similar trend whereby using young tissue allowed distantly related grafts to form. Inter-species grafts could form with *Picea* and *Pinus*, while inter-genus grafts with *Pinus* and *Larix* scions could form with *Picea abies* rootstocks (Feng et al., 2024). Notably, grafts were successful even 2.5 years after grafting, suggesting longer term compatibility. Such inter-genus grafts are normally not possible with conventional grafting (Jayawickrama et al., 1991), suggesting very young *Picea abies* rootstocks were more accepting of divergent scions. *Picea abies* scions were not inter-genus compatible demonstrating an example of graft polarity (Feng et al., 2024), when combinations in one orientation are compatible but not in the other. Several members of the Solanacea family including *Nicotiana benthamiana* and *Petunia hybrida* exhibit diverse intra- and inter-family graft compatibilities (Notaguchi et al., 2020; Kurotani et al., 2022). *N. benthamiana* grafts to a range of angiosperms (Notaguchi et al., 2020), although it is debatable whether some show signs of delayed incompatibility during later stages of growth. Important for *Nicotiana* grafting success is the upregulation of the β-1,4-glucanase *GH9B3* that is secreted into the extracellular space and facilitates cell wall reconstruction at the graft junction (Notaguchi et al., 2020). Upregulation of β-1,4-glucanases has been seen in inter-family grafts in *N. benthamiana*, *Petunia hybrida*, and also *Phtheirospermum japonicum* grafted to *Arabidopsis* (Kurotani et al., 2020; Kurotani et al., 2022; Notaguchi et al., 2020). Within the Solanacea family, graft compatibility varies widely. Tomato scions can be grafted on potato root stocks creating the commercially available TomTato® or Ketchup 'n' Fries™ plant and eggplant scions on potato rootstocks can be purchased as Egg & Chips® plants (Melnyk, 2017b). Other combinations such as tomato and pepper or tomato and physalis are not successful (Thomas et al., 2023) (Figure 4). Transcriptome analyses of compatible and incompatible Solanacea members revealed the upregulation of *SlWOX4* during successful grafting, and *slwox4* mutant fail to form xylem connections across the junction (Thomas et al., 2021).

Given the strong regenerative ability of many plants, perhaps it should come as no surprise that self-grafted plants can heal grafts after severing when tissues are well aligned and attached. However, the ability for different plant species of substantial taxonomic distance to join after wounding is surprising. Such an ability for plants to join to different or even distantly related plants is hard to reconcile from an evolutionary point of view. It may be due to the lack of a system that can not efficiently distinguish self from non-self or instead a tolerance to differences and a desire to overcome or adapt to it. While much work has been done to identify processes related to graft incompatibility in different plant combinations, more work is needed to understand whether these are symptoms of the graft failure or the cause of incompatibility. Good model systems are also needed for the study of long-term incompatibility (Bartusch and Melnyk, 2020). Although the cause of graft incompatibility has not been completely deciphered, it is reasonable to assume that several mechanisms are involved including both cell-to-cell and systemic signaling, and that different combinations will have different reasons for failure, making understanding and overcoming incompatibility more challenging.

ENVIRONMENTAL REGULATION OF GRAFTING

The role of biotic and abiotic factors, including the environment, can dramatically influence the success or failure of graft formation. One important factor is temperature, which influences the growth

rate of the plant and also the rate of wound healing and regeneration (Lee and Seo, 2017; Lambolez et al., 2022). For many woody plants, grafting is performed during dormancy or at the end of their dormancy, for instance, grape vines, conifers, apples, and cherries (Larson, 2006; Garner and Bradley, 2013). In grapevine grafting, scion and rootstock cuttings are collected during dormancy. After cold storage, the dormant scion and rootstock are grafted together. The grafts are then incubated at an appropriate temperature to promote healing, followed by rooting the grafts in soil (Waite et al., 2015). Other species are grafted during their growth periods, such as olives (Fabbri et al., 2004), and for vegetable crops, they can be grafted once plants are large enough. Depending on the time of year, grafting methods can vary to improve success of woody species. Whip and tongue grafting is effective at the end of dormancy for apples, but chip or t-budding is more appropriate during active growth of apples (Garner and Bradley, 2013) (Figure 1). A good knowledge of when to graft woody plants and what technique to use helps ensure success. Temperature is also relevant during the graft healing period. Elevated, but not stress inducing, temperatures seem particularly helpful for vegetable crops. Increased temperatures accelerate graft healing in watermelons, eggplants, and tomatoes (Shibuya et al., 2007; Yang et al., 2016; Serivichyaswat et al., 2022). In *Arabidopsis*, raising the healing temperature from 22°C to 27°C speeds up grafting and vascular reconnection by approximately 25% (Turnbull et al., 2002; Serivichyaswat et al., 2022), likely due to increased growth and regeneration. High temperatures promote auxin biosynthesis in the cotyledons and this auxin is thought to move the graft junction where it promotes healing and vascular formation (Serivichyaswat et al., 2022). In some woody species, localized heating of the graft junction has proven extremely effective in promoting healing. Walnut graft success increases from 6% to 73% with localized heating (Avanzato and Tamponi, 1988). However, elevated temperatures can also have negative effects such as promoting pear-quince graft failure discussed earlier. High healing temperatures in grape vines can also promote weaker junctions, and if temperatures are elevated for too long, vascular formation is inhibited (Waite et al., 2015). In addition, light quality and humidity are also important during graft recovery to help reduce stress on the scion while the vasculature reconnects. High humidity for several days after grafting promotes tomato graft healing but extended high humidity is detrimental as it can enhance disease (Vu et al., 2013). Tomato grafts heal poorly in darkness and instead, heal best under natural light conditions (Vu et al., 2014) or a 70:30 ratio of red and blue LEDs (Yousef et al., 2021).

In addition to the graft healing environment, biotic factors can also play a role. A common problem is the presence of viruses and pathogens in plant material that can be efficiently transmitted across the graft junction or natural graft. Viruses can weaken the rootstock, scion, or both tissues and lead to less vigorous plants and reduced yields. Since many scions and rootstocks are clonally propagated, efforts are made to reduce viruses in them. Heat treatment combined with micrografting or shoot tip culture removes viruses in many woody species (Wang et al., 2018). The EMLA apple rootstocks are heat treated to remove viruses from high-value dwarfing apple rootstocks (Garner and Bradley, 2013). Viruses can also cause graft failure when different species are combined. For instance, the Grapevine Leafroll-associated virus 2 (GLRaV-2) in grapevines can move from the European scion to the American rootstock where this genotype is more sensitive leading to graft failure (Rowhani et al., 2017; Habili et al., 2023). The citrus tristeza virus (CTV) is found in many citrus trees but causes particular problems for sour orange (*Citrus aurantium*) rootstocks commonly used in *Citrus* grafting. The sour orange rootstock is highly sensitive to CTV and over 100 million grafted trees were lost from CTV infections in rootstocks (Moreno et al., 2008). Thus, care must be taken to use virus-free grafting material and to monitor rootstock and scion health. Taken together, factors such as graft timing and healing environment can dramatically alter our ability to successfully graft. Optimizing these in recalcitrant grafting species should be a priority, as well as combining these effects with recent techniques such as grafting with young tissues.

GRAFTING IN RESEARCH

Plant grafting serves as a powerful tool in scientific research across various fields of study (Figure 5). Some of the first scientific uses of grafting were to study the transmission of acquired characteristics. Early studies used plants that were induced to flower and grafted these with plants not induced and found that a mobile substance, known as florigen, could move across the junction and induce flowering (Chailakhyan, 1937). Grafting later helped identify that the mobile protein FLOWERING LOCUS T (FT), which acts as a strong inducer of flowering, was synthesized in the leaves and transported to the plant's meristems (Corbesier et al., 2007). The idea behind using grafting to demonstrate mobility is that a plant lacking a substance is grafted to a plant with that substance. If the substance is detected in the deficient plant after grafting, then it is consistent with mobility of the substance. A nice illustration of this technique is the use of grafting to assay RNA mobility. Phloem sap contains a number of RNA molecules including small RNAs and mRNAs (Yoo et al., 2004; Buhtz et al., 2008). Grafting rootstocks lacking small interfering RNAs (siRNAs) to scions containing siRNAs showed a substantial restoration of siRNAs in the rootstocks (Molnar et al., 2010). When siRNA production was blocked in the scion, fewer siRNAs were present in the rootstock consistent with siRNA movement from scion to rootstock (Molnar et al., 2010). An application of this RNA mobility is grafting wild-type scions to transgenic rootstocks silencing viral sequences. In cherry trees, this can help confer virus resistance to the scion through the mobility of transgenic siRNAs from the rootstock targeting the virus (Zhao and Song, 2014). miRNAs are also mobile. Grafting experiments demonstrated that miRNA399 mobility is important for phosphate starvation responses (Pant et al., 2008) and miRNA156 acts as a potential graft-transmissible signal influencing both plant architecture and tuber development in potatoes (Bhogale et al., 2013). The transport of mRNA has also been demonstrated by grafting. *GIBBERELLIC ACID INSENSITIVE* (*GAI*) mRNA, negatively regulating gibberellin response, is transported in both directions between scion and rootstock (Haywood et al., 2005; Xu et al., 2010). Thousands of mRNAs are mobile in *Arabidopsis*, and there, the presence of a t-RNA like signature appears important for mobility (Thieme et al., 2015; Zhang et al., 2016). By fusing this t-RNA like mobility signal to non-mobile RNAs, mobility is achieved and can even be applied to Cas9 and guide RNA transcripts. By grafting with such Cas9-guides with mobility motifs, genome editing can be performed in the recipient tissues without the need for transgenic DNA (Yang

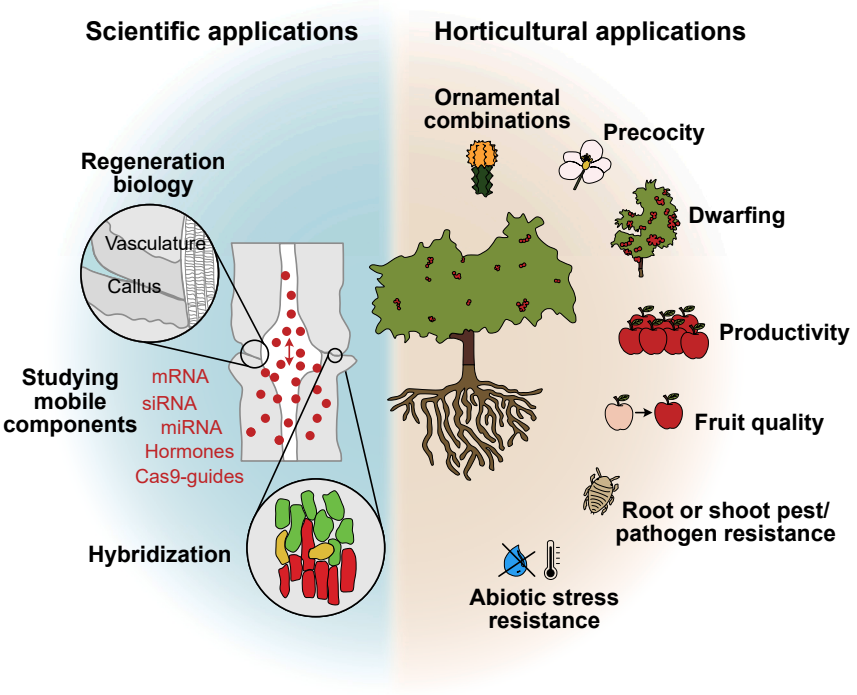

Figure 5. Grafting applications.
Grafting is widely used both for scientific and horticultural reasons. Scientific applications, on the left, include studying regeneration at the graft junction, studying mobile substances that are transported between scion and rootstock, and studying hybridization events leading to the formation of cells at the graft junction. Horticultural application, on the right, include producing new ornamental combinations, inducing precocity or dwarfing for easier cultivation, enhancing productivity or fruit quality, and enhancing resistance against pathogens and abiotic stress.

et al., 2023). Grafting experiments have also revealed that phytohormones are mobile. Prior to the identification of strigolactones, grafting with branching mutants revealed the presence of a graft-transmissible branch-inhibiting hormone, later revealed to be strigolactone (Waldie et al., 2014). Similarly, grafting experiments have demonstrated the mobility of GA12, a gibberellic acid precursor (Regnault et al., 2015).

A second important use of grafting is to study regeneration and wound healing. Plants have a remarkable ability to regenerate new tissue after wounding including tissue repair, de novo organogenesis, and meristem reconstruction (Sena et al., 2009; Ikeuchi et al., 2016). Plant grafting involves a regeneration process including wounding-induced callus formation and tissue fusion followed by vascular healing (Lindsay et al., 1974; Yeoman and Brown, 1976). Enhancing our comprehension of grafting allows us to uncover essential pathways of regeneration and identify the genes involved in tissue regeneration. For instance, graft transcriptomic studies identified four DOF transcription factors rapidly induced and these were found to also have defects in callus formation and stem incision healing (Zhang et al., 2022). Likewise, grafting transcriptomes identified *ENHANCER OF VISUAL AND GRAFTING 1* (*EVG1*) and mutations in this gene affect graft formation, xylem differentiation, and callus formation perhaps through an interaction with *RECEPTOR LIKE PROTEIN 44* (*RLP44*) (Mazumdar et al., 2023). Grafting studies have also been informative regarding the influence of temperature upon regeneration (Serivichyaswat et al., 2022), what cells and tissues contribute to tissue adhesion (Serivichyaswat et al., 2023) and the role of auxin movement during xylem differentiation (Sachs, 1968; 1981).

A third use of grafting is the study of hybridization. Occasionally, cells or tissues can emerge from the junction that combine the characteristics of both parent plants, which are graft chimeras or graft hybrids (Frank and Chitwood, 2016). Chimeras have cells from both graft partners but these cells remain distinct and typically form different layers or regions (Frank and Chitwood, 2016). Such chimeras can have horticultural interest and include chimeras between two distinct species. Examples include between *Laburnum anagyroides* and *Cytisus purpureus*, and between *Camellia sasanqua* and *Camellia japonica* (Neilson-Jones, 1969; Stewart et al., 1972). The 'Bizzaria' orange,between *Citrus medica* and *Citrus aurantium*, has existed since at least the 1600s and graft chimeras have been scientifically studied and regenerated since the early 20th century (Frank and Chitwood, 2016). The graft hybrid concept was proposed by Darwin (Darwin, 1868) and a modern interpretation is that chromosomes or DNA from both graft partners combine in a cell to form a new species. Only recently has this phenomenon been observed when different genotypes or species of transgenic *Nicotiana* were grafted. After healing, the graft junction was excised and cultured on media containing antibiotics that would select for cells with resistance markers from both scion and rootstock genotypes. The transfer of substantial DNA portions, complete plastid genomes, or nuclear genomes between scion and rootstock could occur (Stegemann and Bock, 2009; Stegemann et al., 2012; Gurdon et al., 2016), resulting in the creation of novel, fertile, and stable allopolyploid species (Fuentes et al., 2014). Such hybrids might be fairly common at the junction, occurring in 1/20 to 1/40 of contacting cells (Hertle et al., 2021), yet identifying and regenerating these events without transgenes is currently challenging. Recently, using *in vitro* callus grafts, it appeared that plastids became highly mobile and might move through pores in the cell wall at the graft junction (Hertle et al., 2021). How nuclear genomes could migrate is unknown, and the possibility of cell fusion at the junction leading to hybrids has not been ruled out. Thus, horizontal genome transfer at the graft junction provides an attractive tool to asexually generate hybrid plants or new species.

GRAFTING IN HORTICULTURE

Grafting plays a crucial role in horticulture (Figures 2 and 5) and an increasing number of plants are grafted each year. Over 70

woody perennial species are grafted and 80% of the most produced fruit and nut trees are regularly grafted (Warschefsky et al., 2016). In the 1950s vegetable grafting became more common, and today is the most commonly done grafting with over 1 billion plants grafted per year, typically tomato, cucumber, melons, peppers, and watermelons (Lee et al., 2010). Grafting is one of the more labor-intensive forms of plant propagation and thus there needs to be a good economic incentive for it (Rysin and Louws, 2015). For instance, grafted tomato plants cost four times more than non-grafted tomato plants but the costs are justified under high nematode pressure (Barrett et al., 2012). Various methods have been developed for successful grafting, including whip and tongue, chip grafts, approach grafts, and butt-end grafts (Figure 1). The grafting method and tissue used is important for success and varies depending on the species and timing of grafting (Larson, 2006; Garner and Bradley, 2013). Despite these considerations, grafting remains popular and the benefits often outweigh the costs.

Grafting was thought to be developed for clonal propagation of plants that were difficult to root from cuttings and that were not true breeding (Mudge et al., 2009). Today, grafting is still used for such woody plant propagation but this use has decreased due to improved tissue culture techniques, micropropagation, and the use of rooting hormones (Larson, 2006). However, grafting is still used to clonally propagate woody plants such as oak, maple, dogwood, witch-hazel, and pine (Larson, 2006). One of the most important features of grafting is to change the physiological and morphological features of the scion. Since the 1400s, apple rootstocks with dwarfing capabilities have been used and today, there are a range of dwarfing rootstocks available (Mudge et al., 2009). For instance, apple trees grafted onto M9 rootstocks that dwarf trees by 60% of full size are used for garden and orchards. Previously, MM111 rootstock that dwarf trees by 20% were used in commercial orchards (Mudge et al., 2009), but today, there is a trend to use smaller trees (Wang et al., 2019). With M26 or M9 highly dwarfing rootstocks, tree planting density increases, typically in rows, which improves yields, tree management, and fruit harvesting (Figure 2). The reason why rootstocks cause dwarfing remains unknown. M9 rootstocks are less efficient than MM111 rootstocks in the absorption of macronutrients, perhaps contributing to a reduction in scion growth (Amiri et al., 2014). Dwarfing rootstocks might also restrict water supply to the scion, affect long-distance hormone transport, or be a result of mild incompatibility that affects junction formation or growth (Webster, 2004). Two loci contributing to dwarfing in apples, *Dw1* and *Dw2*, have been identified (Foster et al., 2015). However, the specific genes have not been discovered and it is not clear how they cause dwarfing. Grafting can also improve the fruit yield and quality in both woody species and vegetables (Davis et al., 2008; Garner and Bradley, 2013). In watermelon grafts, *Lagenaria* rootstocks produced 27–106% higher yields than the ungrafted plants (Yetisir and Sari, 2003). Furthermore, grafting can alter the growth habits of both scions and rootstocks. Grafting aubergine onto a woody Solanum rootstock yields aubergine fruits continuously for 3 years and increases yields (The grand challenge of breeding by design, 2022). Grafting can also make visually captivating combinations in ornamental plants, exemplified by tree roses, where shrub roses are grafted onto an elongated main stem (Figure 2) thus modifying the form and growth of the plant.

Graft propagation can also lead to a significant reduction in juvenility (Zimmerman, 1972; Hackett, 1985). For instance, apple seedlings grafted onto M9 rootstocks exhibited a 43% flowering rate after 6 years, compared with only 3% for ungrafted plants (Tydeman, 1961), a substantial acceleration.

Another important reason for grafting is to improve biotic and abiotic stress tolerance (Figure 5). Perhaps the most famous example was during the mid-1800s when vineyards employed grafting European scions to American rootstocks to confer resistance against phylloxera (Mudge et al., 2009). Vegetables, the mostly commonly grafted plants, are typically grafted for disease resistance. For instance, bacterial wilt (*Ralstonia solanacearum*) is a deadly disease with a wide host range, including tomatoes. Using *Ralstonia*-resistant rootstocks for grafting significantly reduced bacterial wilt in sensitive tomato varieties (Rivard et al., 2012). Root-knot nematodes are also important pathogens that inhibit the growth of infected plants by parasitizing their roots. Employing a tomato rootstock (Brigeor F1) for grafting with an eggplant scion (Bonica F1) provided nearly complete protection against root-knot nematodes (Ioannou, 2001). Plants grafted onto rootstocks from the wild watermelon line RKVL 318 exhibited notably reduced root galling from knot nematodes in comparison with non-grafted 'Fiesta' watermelon plants (Thies et al., 2010). In addition to biotic stress, grafting is also used to enhance tolerance to salt (Estañ et al., 2004), low or high temperatures (Rivero et al., 2003; Venema et al., 2008), drought and flooding (Nilsen et al., 2014; Bahadur et al., 2015), and heavy metal stresses (Savvas et al., 2010). For example, grafting with cold-tolerant hybrid squash rootstocks improved cucumber yields by 1.8–18.2 times compared with non-grafted cucumbers when grown in cool temperatures (Guan et al., 2020). Grafting can also increase nutrient uptake and utilization efficiency of rootstocks (Savvas et al., 2010). Thus, there are substantial benefits to grafting, particularly when rootstocks can confer tolerance or resistance to multiple stresses.

Given the importance of grafting in horticulture, robot-aided grafting has been used to improve success rates and productivity (Lee et al., 2010; Xie et al., 2020). Using Solanaceae family members, one robotic system operated by two people could graft up to 2250 plants an hour with success rates approaching 100% (Xie et al., 2020). Comparing to a skilled vegetable grafter who can graft up to 500 plants per hour (Xie et al., 2020), this is an improvement, although further work is needed to justify the costs of robots and currently, manual grafting remains the most popular method (Lee et al., 2010).

OUTSTANDING QUESTIONS AND PERSPECTIVES

Grafting provides a flexible toolkit that horticulturists can use to modify and enhance plant traits, improve disease resistance, and produce better crop yields. Numerous successful grafting combinations exist in horticulture including multiple inter-species and inter-genus grafts. This range is increasing, though our fundamental understanding of why some grafts succeed and others fail is still lacking. Research using examples of incompatibility within the Solanaceae could prove useful given the striking examples of

compatibility and incompatibility within the family (Thomas et al., 2023), as could better understanding of why species such as monocots and cacti have a strong ability to successfully form intra-family grafts. Further work is also needed in woody species to better understand long-term incompatibility. Efforts have characterized a range of symptoms and chemical markers associated with failure but work is needed to distinguish between the cause and consequence of incompatibility. By focusing on early stages of graft formation and using chemical, technical, or genetic means to overcome incompatibility could help identify causes. One important development has been the use of juvenile tissues to overcome incompatibility. In monocots, this has been transformative and may lie with the ability for these tissues to have a procambium or stem cell-like nature (Reeves et al., 2022). In gymnosperms, the advantages of juvenile tissues may lie in part with a group of transcription factors including *PHYTOCHROME A SIGNAL TRANSDUCTION 1* (*PAT1*) expressed during grafting in young tissues (Feng et al., 2024). Whether grafting with extremely juvenile tissues in eudicots similarly improves compatibility needs investigating. In addition, findings with *Nicotiana* and *Petunia* inter-family grafts (Notaguchi et al., 2020; Kurotani et al., 2022) is promising and further work is needed to translate these findings in other species to broaden inter-family graft compatibility. By using such developments to both understand how distant grafts form, and to also develop new model systems for incompatibly would be hugely informative.

Grafting is growing in popularity, particularly with vegetable crops, and new opportunities are available but challenges remain. Rootstock breeding should take a priority for many of the commonly grafted species. Examples with viruses attacking orange and grape rootstocks are of concern and demonstrate the vulnerability of combining different species together. Given the high costs and manual labor associated with grafting, there is resistance to deploy this technology more widely (Rysin and Louws, 2015). Lowering the costs of grafting through automation, providing better information to growers, and making available high-quality rootstocks would be hugely beneficial. Certain industries with long-lived species could also benefit from grafting, for instance, forestry. There, rootstocks that improve nutrient acquisition or stress tolerance could be combined with high-yielding scions to produce superior trees. The finding that graft hybrids form at the junction is also interesting and such hybrids could be selected and grown as a means to asexually hybridize species. Given the broadening range of grafted species, this could be a feasible way to hybridize species and have advantages over other techniques such as protoplast fusions that have challenges regenerating protoplasts from different species (Reed and Bargmann, 2021). Graft chimeras can also emerge from the junction and in some instances, might have horticultural or agricultural relevance. Such chimeras could also be an elegant tool to investigate the cell-to-cell movement of substances.

The future of grafting research remains strong with potential for new combinations, new applications, and a deeper understanding of the mechanism. Recent developments with mobile CRISPR-Cas9 editing across the junction (Yang et al., 2023) also provide novel means to deploy grafting more widely to genetically engineer plants. Combined with further research, our ability to understand and deploy grafting will continue to grow and bridge a gap between science and horticulture.

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