Review

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Review

Hunting for the Basis of Graft Compatibility: Insights from Diverse Plant-Plant Interactions

Running Title: Graft Compatibility and Plant-Plant Interactions

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Abstract

As sessile organisms, plants have developed intricate strategies to interact with their environment, including a variety of plant-plant interactions that range from mutualistic to antagonistic. Among these interactions, plant grafting stands out as a significant horticultural technique for enhancing productivity, disease resistance, and stress tolerance. Despite its widespread application, the mechanisms underlying graft compatibility remain poorly understood. This review explores the diverse field of plant-plant interactions, focusing on parallel mechanisms from other systems that may explain how "non-self" is determined during graft incompatibility. We first discuss the role of inter-plant signaling and the possibility of exudate-regulated compatibility. Next, we identify similarities between the parasitic plant haustoria and graft junctions, offering valuable insights into overcoming immunologic and physiologic barriers during vascular reconnection. We then delve into the potential roles of wound signaling and damage-associated molecular patterns (DAMPs) in grafting. Lastly, we provide an overview of pollen self-incompatibility as a case study for the detection of non-self throughout the plant kingdom. Overall, this review underscores the need for interdisciplinary approaches to unravel the complexities of graft compatibility, suggesting that future research should integrate knowledge from various fields of plant-plant interactions to improve the utilization of grafting and expand graft compatibility.

Keywords: grafting; graft compatibility; plant-plant interactions; parasitic plants

Introduction

Plants must employ a wide range of strategies to respond to their constantly changing environment. Much like animal systems, plants encounter a diverse array of friends and foes, and it is critical that complex surveillance systems are in place to identify foreign organisms as "non-self" (Janeway, 1992). Furthermore, it is key that appropriate responses are elicited to defend against or promote these interactions.

One of the most well-studied areas of plant biology revolves around the response of plants to pathogens. Significant progress has been made in understanding the plant immune system, whether bacteria-, fungi-, or insect-mediated. Overlapping with research on insect pathogens, the field of plant wound response has also shed significant light on the role of cellular damage as a mobile signal. In addition to pathogens, plants respond to other plants during plant-plant interactions. During infestation by parasitic plants that leach water and nutrients from their hosts, hosts must fend off

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enemies within the same kingdom, thereby allowing the evolution of plant-inclusive immune surveillance machinery. In addition to plant parasitism, plants interact with one another for various reasons, such as protecting against self-pollination, allocating resources, and signaling.

One additional type of plant-plant interaction is plant grafting. The origins of plant grafting are ancient, thought to have originated in oak (*Quercus* spp.), where pressure over time can force two neighboring branches together (Mudge *et al.*, 2009). Similarly, natural grafting of tree roots occurs in oak and yellow birch (*Betula alleghaniensis*), where density forces nearby roots to press against one another, exposing their vascular tissues to one another (Mudge *et al.*, 2009). While both instances are natural, evolved processes driven by the necessity of wound healing, humans have co-opted these processes to generate grafted plants for commercial use. The apical portion of a graft is referred to as the scion, and the root system as the rootstock. Grafting is an effective strategy used across a wide range of species, including trees and herbaceous crops, to enhance productivity and resilience (Goldschmidt, 2014). Grafting can be used for various purposes, such as inducing dwarfism to improve planting and harvest practices. For example, the "M9" rootstock is one of the most commonly used dwarfing varieties in apple (*Malus domestica*), where auxin signaling underlies the reduced stature of the grafted scion (Li *et al.*, 2024). Grafting can be a strategy to improve fruit production, such as through graft-induced vigor. In *Arabidopsis thaliana* and Tomato (*Solanum lycopersicum*), *msh1* mutant rootstocks have been shown to induce vigor via root-to-shoot-mobile siRNA, which regulates methylation of phytohormone genes in the scion and subsequent progeny (Kundariya *et al.*, 2020). Grafting is also used to improve disease resistance. Among many examples, the well-known use of the North American grape (*Vitis labrusca*) rootstocks prevented the complete destruction of European wine cultivars (*Vitis vinifera*) by the invasive phylloxera insect pathogen (Mudge *et al.*, 2009). Lastly, grafting is a key technique to promote abiotic stress tolerance. Interspecies grafting of *S. peruvianum* or *S. habrochaite*s rootstocks with *S. lycopersicum* scions can be used to promote temperature-related stress tolerance (Venema *et al.*, 2008, Lee *et al.*, 2024). While grafting requires combining genetically distinct plants to achieve these processes, the mechanism that enables the joining of plant varieties, species, and sometimes even families remains poorly understood.

For two plants to be successfully grafted, substantial vascular connections must form between the scion and the rootstock (Thomas *et al.*, 2022). These successful grafts are known as compatible. In contrast, incompatible grafts may fail to regenerate or regenerate only non-vascular tissue (Thomas *et al.*, 2023). Historically, understanding graft incompatibility was limited by the lack of an herbaceous model system. Recent work in *Arabidopsis* has identified many novel genes involved in grafting (Melnyk *et al.*, 2018), and the development of tomato and pepper as a model for graft incompatibility has dramatically expanded the field (Thomas *et al.*, 2022; Thomas *et al.*, 2023). Despite this, much remains unknown about how plants detect graft partners and determine compatibility status.

Drawing from various fields of plant-plant studies, new information has recently been uncovered that may be relevant for a better understanding of plant grafting. This review aims to describe the phenomenon involved in plant-plant interactions, the processes that mediate "non-self" determination, and reveal parallel mechanisms that may regulate graft healing and compatibility.

Plant-plant interactions allow plants to perceive non-self counterparts and initiate appropriate responses. A notable instance of such interactions is grafting, where distinct plant varieties are cut and joined, then allowed to heal. However, not all combinations succeed, a phenomenon referred to as graft compatibility. The criteria for determining compatibility remain elusive; nonetheless, plant-plant interactions may elucidate the mechanisms underlying non-self detection and response during grafting. Communication between plants can involve root exudates or airborne signals. Similar to plant-pathogen interactions and responses to injury, plants have developed sophisticated surveillance and signaling systems to defend against threats. Parasitic plants represent one of the most obvious cases of negative plant-plant interactions, whereby hosts attempt to elicit immune responses to repel parasites, while parasites endeavor to circumvent these defenses and establish vascular connections. In this capacity, parasitic plants demonstrate a high degree of compatibility, forming vascular connections with diverse host species. Furthermore, different plant species employ

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varied mechanisms to recognize self-pollen through self-incompatibility. These examples collectively illustrate the myriad ways in which plants can detect non-self entities, suggesting that analogous mechanisms may underpin the determination of graft compatibility.

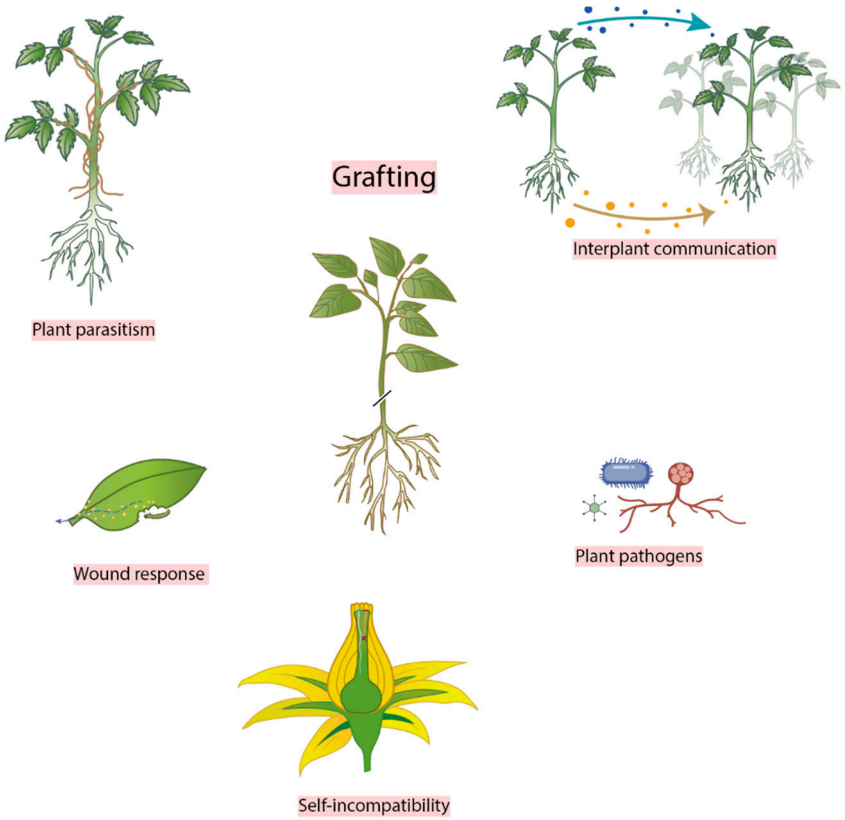

Figure 1. Diverse plant-plant interactions highlight mechanisms of non-self detection in the Plant Kingdom.

Inter-Plant Communication

The Rhizosphere and Root Exudates

Plants interact with other plants through a wide array of secreted compounds. Some of these exudates are released from the roots and have been shown to regulate the rhizosphere by affecting the chemical ecology of the surrounding soil. For example, well-studied beneficial microbial associations, such as those involving rhizobia, are promoted by plant-secreted flavonoids (Peters *et al.*, 1986). Plants can also secrete compounds, such as coumarins (Steinauer *et al.*, 2016), that promote beneficial microbes while inhibiting pathogens (Huang *et al.*, 2019, Hu *et al.*, 2018, Stringlis *et al.*, 2018). The rhizome can also protect aerial plant parts from disease, a process known as induced systemic resistance (ISR) (Pieterse *et al.*, 2014). The presence of certain root-associated mutualists, such as *Trichoderma spp.*, can prime the plant immune system for subsequent pathogen infection, further underscoring the important role of the root system in plant health and protection (Walters *et al.*, 2013).

Root exudates can also directly affect neighboring plants (Sorty *et al.*, 2025). Beneficial associations include intercropping-induced mutualistic resource allocation (Si *et al.*, 2025) or floral timing cues from neighboring plants (Stirnemann & Sasse, 2025). However, root exudates can also act as plant inhibitors by repressing seed germination or root growth (Sorty *et al.*, 2025). Chemically mediated negative plant-plant interactions are known as allelopathy. These instances of inhibition can prevent parasitic plant invasion (Cimmino *et al.*, 2015), protect limited soil resources (Gattullo *et al.*, 2018), or help a plant to dominate the canopy for light (Kegge *et al.*, 2015). For example, benzoxazinoids are secreted by many grasses to inhibit the growth of neighboring plant species (Schandry & Becker, 2020). Two such compounds are 4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one

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(DIBOA) and 4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA). Analysis of various wheat grasses (*Triticeae* spp.) found that exudates from *T. durum* resulted in a 60.3% inhibition of *Sinapis alba* root growth, with 69.4% of that effect estimated to be due to DIBOA and DIMBOA, validating the strong phytotoxic effect of root exudates (Belz & Hurle, 2005).

While root systems regulate the rhizosphere of ungrafted plants, the same principle applies to grafted plants, where beneficial phenotypes arise from root-specific interactions with microorganisms. In grafted plants, interactions between the rootstock and the scion can be seen as an intimate form of plant-plant interaction. For example, the rootstock's ability to regulate the root microbiome is hypothesized to contribute to graft-induced vigor of the scion (Williams *et al.*, 2021). Indeed, during tomato grafting, the use of the F1 hybrid "Maxifort" (*S. lycopersicum* × *S. habrochaites*) rootstock was found to increase rhizosphere microbial biodiversity compared with self-grafted or ungrafted tomato (*S. lycopersicum*). Similarly, in grafted grape and apple, different rootstocks were found to affect soil microbial communities. In apple, vigorous rootstocks were found to promote a more diverse bacterial microbiome than the dwarfing M9 (Liu et al., 2018), while grape rootstocks contained bacteria associated with growth-promoting traits (Marasco *et al.*, 2018). This phenomenon has been hypothesized to result from the evolution of holobionts, in which rootstock and microbes may have co-evolved. Through grafting, these beneficial relationships can regulate the scion of other varieties and cultivars. In contrast, grafted partners may utilize exudates to repress healing. The presence of graft-limiting metabolites has been hypothesized for decades (Mudge *et al.*, 2009), yet only a single instance of metabolic graft incompatibility has been clearly studied: quince-pear grafts. Quince (*Cydonia oblonga*) is commonly used as a dwarfing rootstock to pear (*Pyrus communis)* (Browning & Watkins, 1991); however, several economically valuable pear varieties, such as "Williams", are graft-incompatible with quince (Tomaz *et al.*, 2009; Herrero, 1951). This was attributed to high levels of the cyanogenic glucoside prunasin in quince rootstocks (Sánchez-Pérez *et al.*, 2012). Because prunasin is phloem-mobile, it accumulates at the pear graft interface, where it is degraded by β-glucosidase, prunasin hydrolase, leaving behind the toxic byproduct, hydrogen cyanide (HCN) (Sánchez-Pérez et al., 2012), which leads to cell death and vascular degradation at the graft junction. Quince-pear incompatibility was successfully overcome using interstock grafting with the pear variety, "Old Home", which contains the enzyme inhibitor capable of halting prunasin breakdown (Hudina *et al.*, 2014).

Much like the ability of certain rootstocks to positively regulate scion growth through graft-induced vigor, it is possible that chemical exudates, metabolites, or other effects by the rootstock can dysregulate the scion either through response to a secreted compound, such as allelopathy, or by altering the rhizosphere such that necessary nutrients or microbe-interactions are limited. The communication between rootstock and scion has not been well studied and may be involved in compatibility determination. Furthermore, it is well established that root exudates can inhibit the growth of neighboring plants, suggesting that other root- or scion-derived compounds might also impair graft healing. In the study of allelopathy, root exudates from plant cultures are screened for phytotoxic effects (Bais *et al.*, 2006). When exudates containing putative allelopathic compounds are identified, the components of the exudate mixtures are analytically separated, and the inhibitory effect is individually validated. Perhaps a similar protocol could be used to screen incompatible graft combinations for toxic metabolites. An attempt to profile metabolic inhibition of callus formation in tomato and pepper was conducted using culture media contaminated with cross-species exudates, but no effect was observed (Thomas *et al.*, 2024). However, the role of exudates on graft healing remains poorly explored.

Volatile Organic Compounds

Plants also release volatile organic compounds (VOCs), which allow inter-plant communication. One of the most well-studied VOCs is methyl jasmonate, a volatile gaseous derivative of jasmonic acid (JA). Jasmonates are released by shoots and roots (Kulkarni *et al.*, 2024), especially after wounding, and are key regulators of defense against herbivory (Howe & Jander, 2008; Glauser *et al.*,

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2008). JA has been shown to be present in wheat root exudate and sensed by up to 100 plant species, making it a broadly applicable plant-plant communication signal (Kong *et al.*, 2018). Jasmonates also move within tissues from shoot to root along the vasculature and cell-to-cell in nonvascular tissue, sending distal wound signals that induce *de novo* biosynthesis in unwounded tissue (Gasperini *et al.*, 2015).

Additionally, jasmonates are involved in localized tissue healing. In *Arabidopsis*, partial wounding to the floral hypocotyl led to high auxin/low JA above the wound, and low auxin/high JA below (Asahina *et al.*, 2011). Low auxin and high JA induced the expression of *Related to APETALA2.6-like* (*RAP2.6L)*, which is necessary for wound-induced cell proliferation (Matsuoka *et al.*, 2018). While JA is critical for healing and is indeed upregulated during grafting in *Arabidopsis*, the JA-biosynthesis mutant, *allene oxide synthase (aos)*, was still capable of grafting, suggesting the exact role of JA during graft healing is more complex than wounding alone (Matsuoka *et al.*, 2018, Kong *et al.*, 2018). Regardless, it has been shown that JA is graft-mobile (Kong *et al.*, 2018) and present in the cambium (Sehr *et al.*, 2010), making it a potential plant-plant communication signal relevant to graft compatibility. And indeed, during Solanaceous grafting, incompatible tomato-pepper (*Capsicum annuum*) grafts had an upregulated JA response, including the production of JA-dependent defensive metabolites, steroidal glycoalkaloids (SGA) in the scion (Bai *et al.*, 2025, Thomas *et al.*, 2024).

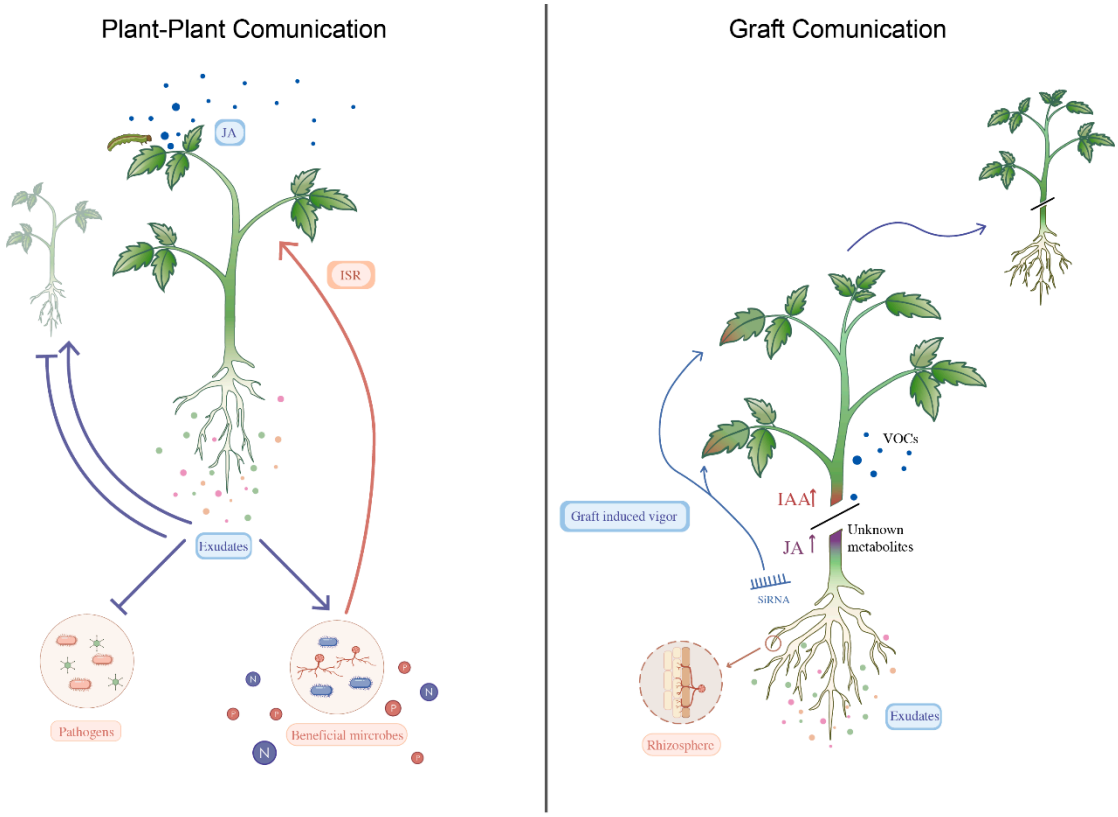

Figure 2. Plants promote and inhibit neighboring plants through various signals.

Plants communicate with adjacent plants through numerous compounds (left). Root systems may secrete substances that promote beneficial microbes while inhibiting pathogens. These beneficial microbes not only supply nutrients to plants but can also prime the aerial shoot via induced systemic resistance (ISR). These exudates can further promote or inhibit the germination or growth of neighboring plants. Wounds, such as those caused by insect herbivory, produce mobile signals such as jasmonic acid (JA), which can travel through vascular tissue, cell-to-cell, as soil exudates, or even through the air. Furthermore, grafted rootstocks interact with their scions in similar manners (right). Rootstocks can modulate the rhizosphere and enhance growth by improving nutrient uptake. Rootstocks also send graft mobile signals, such as siRNA, into the scion, where gene regulation

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influences growth and development. The accumulation of hormones during grafting regulates the healing process, while unidentified metabolites may suppress regeneration. As grafting is a wound-healing process, volatile organic compounds (VOCs) such as jasmonic acid (JA) might also regulate nearby plants.

The Immune System and Graft Compatibility

Drawing on hundreds of years of plant-pathogen interactions, recent findings have identified novel instances of plant-plant immune response. A fundamental aspect of this response is the ability of plants to distinguish 'self' from 'non-self' (Sanabria *et al.*, 2010). In animals, a complex immune system identifies foreign organisms through mobile, rapidly responding innate and adaptive immune cells (McComb *et al.*, 2026). Adaptive immune cells exhibit a delayed response and immunological memory from prior encounters with specific antigens, enabling extreme precision in response (McComb *et al.*, 2026). Whereas plants employ a more ancestral immune system based fully on innate immunity. This system relies largely on broad, non-specific responses, which have helped plants to adapt to biotic stressors, such as bacteria, fungi, insects, and herbivores (Miller *et al.*, 2017, Abdul Malik *et al.*, 2020). In plants, non-self recognition is mediated by two systems: pathogen-associated molecular pattern-triggered immunity (PTI) and effector-triggered immunity (ETI).

The first layer of defense is PTI, which comprises extracellular receptors known as pattern recognition receptors (PRRs) that detect microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs), herbivore-associated molecular patterns (HAMPs), and damage-associated molecular patterns (DAMPs). When these molecules are bound by PRRs, a cascade of processes is activated, such as reactive oxygen species (ROS) burst, Ca^{2+} influx, the activation of mitogen-activated protein kinases (MAPK) cascades, and callose induction, to name a few (Bigeard *et al.*, 2015, Yu *et al.*, 2024). Plant PRRs are molecule-specific, cell surface-localized receptor kinases that often co-bind with more broadly employed receptors, such as BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) (DeFalco & Zipfel, 2021).

The second layer of defense is ETI. ETI is an intracellular response in which pathogenic proteins, known as effectors, are sensed by intracellular nucleotide-binding and leucine-rich repeat receptors (NLRs), sometimes known as R genes. Effectors aim to evade the plant immune system and instead deactivate certain components of the immune response, making the plant susceptible to infection. Each R gene has evolved to bind to one or a few corresponding effectors, leading to a stressor-specific response (Dodds & Rathjen, 2010, Sanabria *et al.*, 2010). Once perceived, effectors can activate hypersensitive response (HR)-related programmed cell death (PCD) in local tissue or trigger systemic acquired resistance (SAR)-mediated salicylic acid (SA) production systemically to prevent infection throughout the plant (Sanabria *et al.*, 2010).

Pathogens

During PTI, PAMPs signal the plant immune system of potential infection. Several PAMPs have been extensively studied, such as bacterial flagellin (flg22) (Felix *et al.*, 1999), lipopolysaccharides (LPS) (Newman *et al.*, 1995), peptidoglycan (PGN) (Gust *et al.*, 2007), exopolysaccharide (EPS), and elongation factor Tu (EF-Tu) (Kunze *et al.*, 2004) as well as fungal chitin (Felix *et al.*, 1993) and β-glucan (Umemoto *et al.*, 1997). Each PAMP has a main PRR that triggers downstream processes, such as flg22-FLAGELLIN SENSING2 (FLS2) (Chinchilla *et al.*, 2006) or chitin-CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) (Miya *et al.*, 2007). PTI manifests as an activated immune system. For example, pretreatment with flg22 in *Arabidopsis* leads to an increased resistance to *Pseudomonas syringae* pv. tomato DC3000 (Pst) due to the defensive production of extracellular ROS, and activated defense genes (Zeidler *et al.*, 2010, Wang *et al.*, 2023), such as WRKYs (Logemann *et al.*, 2013), and JA/SA biosynthesis (Zhang *et al.*, 2010, Feys *et al.*, 1994). A mutation in any of these PRRs leaves plants susceptible to infection by disrupting their ability to mount a rapid immune response.

Most work on PTI is based on local signaling, but recent studies have shown that chitin perception in soil induces PTI in leaves, such as Ca^{2+} influx and ROS induction (Makechemu *et al.*,

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2025), highlighting a long-distance component. Although the systemic signal was not identified, they showed that this effect was graft-mobile in *Arabidopsis*, suggesting that inducing PTI in rootstocks may be a novel strategy for protecting susceptible scions.

While PTI tends to elicit local responses, ETI is known to elicit greater-intensity, larger-scale, long-lasting responses. When NLRs bind to pathogenic effectors, they form large protein complexes known as resistosomes (Makechemu *et al.*, 2025). These complexes then form and activate diverse processes, but most utilize an influx of intracellular Ca^{2+} to trigger transcriptional changes or even HR. One of the critical outputs of ETI is SAR, in which the perception of a pathogen in one location confers disease resistance in distant, non-infected tissues. SA is a key component of this response. In 1985, experiments with grafted cucumbers (*Cucumis sativus*) showed that infection of rootstock leaves with the fungus *Colletotrichum lagenarium* could confer anthracnose resistance in the scion via a mobile signaling element (Dean & Kuc, 1986). Since then, the mode of transport for this signal has been mostly clarified to be the phloem (Kiefer & Slusarenko, 2003), with several signals posited as the mobile element. Originally thought to be SA itself (Malamy *et al.*, 1990, Metraux *et al.*, 1990), it is now suggested that N-hydroxy-pipecolic acid may be the true cue, due to its necessity in SAR (Chen *et al.*, 2018) and presence in the phloem (Schnake *et al.*, 2020). Throughout this research timeline, grafting has played an integral part in our understanding of SAR. Indeed, graft experimenters were able to not only show that SAR moves from the site of infection to the distal leaves (Guedes *et al.*, 1980), that the original infected leaf is the source of the mobile signal (Metraux *et al.*, 1990), but that SA was not the mobile signal due to the ability of SA-deficient *nahG* tobacco (*Nicotiana tabacum*) rootstocks to still confer SAR in grafted scions (Dean & Kuc, 1986). Despite its early involvement in the field of SAR, only a few studies (Makechemu *et al.*, 2025, Jensen *et al.*, 2012) have investigated how graft mobile immune signaling might be used to confer graft-induced disease resistance. While grafting remains a key technique for conferring localized disease resistance in the soil (Louws *et al.*, 2010), the presence of non-autonomous graft-mobile immune signals is a poorly investigated area with potential for future advancements.

Parasitic Plants

Recently, progress in the study of how plants detect and defend against parasitic plants has highlighted that immune processes originally identified in disease also operate during plant parasitism. Parasitic plants have evolved a structure known as a haustorium, capable of penetrating into the plant vascular system and extracting not only water and nutrients, but also transferring signaling molecules between the parasite and host through the haustoria (Kim & Westwood, 2015, Shen *et al.*, 2023). Parasitic plants, including the well-studied *Striga* spp., *Orobanche* spp., and *Cuscuta* spp., are considered serious threats to crops. Most host plants fail to detect these invading parasites, making numerous crops susceptible to infestation. Because parasitic plants can impact yield, research has focused on identifying R genes in resistant species.

Cuscuta spp. are incredibly destructive plants that draw water and nutrients from their host's stem. Unlike most hosts, domesticated tomato is resistant to *Cuscuta reflexa* (Kaiser *et al.*, 2015). During infestation, tomato elicits an immune response that induces the production of SA and JA (Runyon *et al.*, 2010) and triggers HR in the cells where haustoria have penetrated (Albert *et al.*, 2004, Ihl. *et al.*, 1988). It was later found that extracts from *C. reflexa* contained a glycine-rich cell wall peptide, coined Crip21 (Hegenauer *et al.*, 2020), which could trigger typical PTI responses, such as a ROS burst, via a cell surface PRR, CUSCUTA RECEPTOR 1 (CuRe1) and the co-receptor SlSOBIR1 (Hegenauer *et al.*, 2016).

In the *Orobanche cumana-Helianthus annuus* (sunflower) interaction, in which *O. cumana* penetrates the sunflower root to enter the vascular system, recent work has shown that resistance to *O. cumana* can be conferred by a member of the sunflower *Or* gene family, *HaOr7* (Duriez *et al.*, 2019). Similar to PRRs, which perceive pathogens, HaOr7 is a LEUCINE-RICH REPEAT RECEPTOR-LIKE KINASE (LRR-RLK) and a predicted homolog of Xa21 in rice (Song *et al.*, 1995). In rice, Xa21 is key for resistance to *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*) by recognizing the bacterial peptide, Ax21 (Park

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& Ronald, 2012). Due to the sequence homology, HaOr7 is predicted to act as a PRR by binding to a hypothetical *O. cumana* peptide deemed *Avr (Avirulence)-Or7*.

These two examples demonstrated the presence of plant PAMPs, suggesting that plants have likely evolved the ability to detect non-self plant interactions through cell-surface receptors, much as they detect microbes and insects. Similarly, in the same way that pathogens deliver pathogenic effector proteins capable of disarming the plant immune system, a process that plants counteract through the evolution of NLRs, parasitic plants also behave in a similar way.

Evidence for parasitic plant effectors was first identified in *Striga*, an aggressive root parasite of the forage crop cowpea (*Vigna unguiculata* L. Walp.), where the resistant cultivar B301 (Timko. *et al.*, 2007) suddenly became susceptible to *Striga gesnerioides race 4* (*SG4*) (Huang *et al.*, 2012). This phenomenon exemplified the gene-for-gene model underlying R gene evolution (Flor, 1971). Furthermore, it was later shown that B301 resistance to *S. gesnerioides race 3 (SG3*) was conferred by the NLR RSG3-301, although the effector remains unidentified (Li & Timko, 2009). Similarly, an *SG4* effector protein, Suppressor of Host Resistance 4z (SG4z), was identified as highly expressed in the haustorium and capable of attenuating the HR required for resistance (Su *et al.*, 2020). Here, the effector was found to bind the cowpea ubiquitin E3 ligase POB1, VuPOB1, a regulator of HR (Su *et al.*, 2020).

In contrast to pathogen-derived molecules, it has also been shown that parasitic plants can perceive host-derived molecular patterns. The quinone, 2,6-dimethoxy-1,4-benzoquinone (DMBQ), is perceived by the LRR-RLK CANNOT RESPOND TO DMBQ 1 (CARD1) (Laohavisit *et al.*, 2020). Unlike PTI, which induces anti-pathogenic processes, DMBQ perception by CARD1 in *Phtheirospermum japonicum* induced Ca^{2+}-mediated haustorium induction. Interestingly, in non-parasitic *Arabidopsis*, DMBQ could also be perceived, leading to Ca^{2+} influx and MAPK induction, but not ROS, suggesting that quinone sensing is also involved in plant immunity, but does not overlap with PTI. Previous work identified quinones as stomatal regulators (Toh *et al.*, 2018), and indeed, *card1* mutants were perturbed in stomatal immunity and more susceptible to *Pseudomonas syringae* pv. *tomato* DC3000 infection than WT (Laohavisit *et al.*, 2020).

While PTI and ETI were originally considered pathogen-specific processes, it is now clear that plant PAMPs and effectors also exist. This opens the intriguing possibility that graft compatibility has an immunity-controlled basis. Further supporting this, incompatible tomato-pepper grafts exhibit graft-localized cell death and upregulation of hundreds of rootstock NLRs, suggesting that this pairing reflects genetic incompatibility that induces NLR-mediated cell death (Thomas *et al.*, 2024). The signal that induces this process remains unknown, but species-specific proteins may trigger immune responses leading to immunologically mediated cell death.

An alternative hypothesis for tomato-pepper incompatibility focuses on the significant similarities between this genetic graft incompatibility and hybrid necrosis (Hollingshead, 1930), which occurs when hybridized species have incompatible immune components, usually involving at least one NLR (Bomblies *et al.*, 2007). Although observed in many plants, including *Crepis spp.* (Hollingshead, 1930), rice (*Oryza sativa*) (Yamamoto *et al.*, 2010), *Nicotiana* (Yamada & Marubashi, 2003), wheat (*Triticum aestivum* L.) (Chu *et al.*, 2006), and *Capsella spp.* (Sicard *et al.*, 2015), hybrid necrosis was most clearly described in *Arabidopsis*, where crosses between Cdm-0 and other accessions, such as TueScha-9, led to severe necrosis in the cotyledon stage (Barragan *et al.*, 2021). This instance of hybrid necrosis was found to be due to the presence of a truncated singleton NLR, DANGEROUS MIX 10 (DM10), in Cdm-0, which, when crossed with accessions containing DM11, triggered SA and JA production, upregulated hundreds of NLRs, and eventually led to cell death (Barragan *et al.*, 2021). Interestingly, tomato-pepper incompatible grafts were also found to have upregulated SA and JA signaling, upregulated NLRs, and graft junction-specific cell death (Thomas *et al.*, 2024). During grafting, tissues from two individuals are in such close proximity that there is a high chance of genetic exchange at the graft interface, and indeed, RNA (Thieme *et al.*, 2015), DNA (Stegemann & Bock, 2009), extracellular circular DNA (Zhang *et al.*, 2024), organelles (Hertle *et al.*, 2021), and even entire nuclear genomes (Fuentes *et al.*, 2014) can be horizontally transferred between

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cells at the graft interface. Therefore, similar mechanisms that control hybrid necrosis may also influence interspecies graft compatibility, in which components of separate immune systems, when mixed within a single cell, lead to cell death.

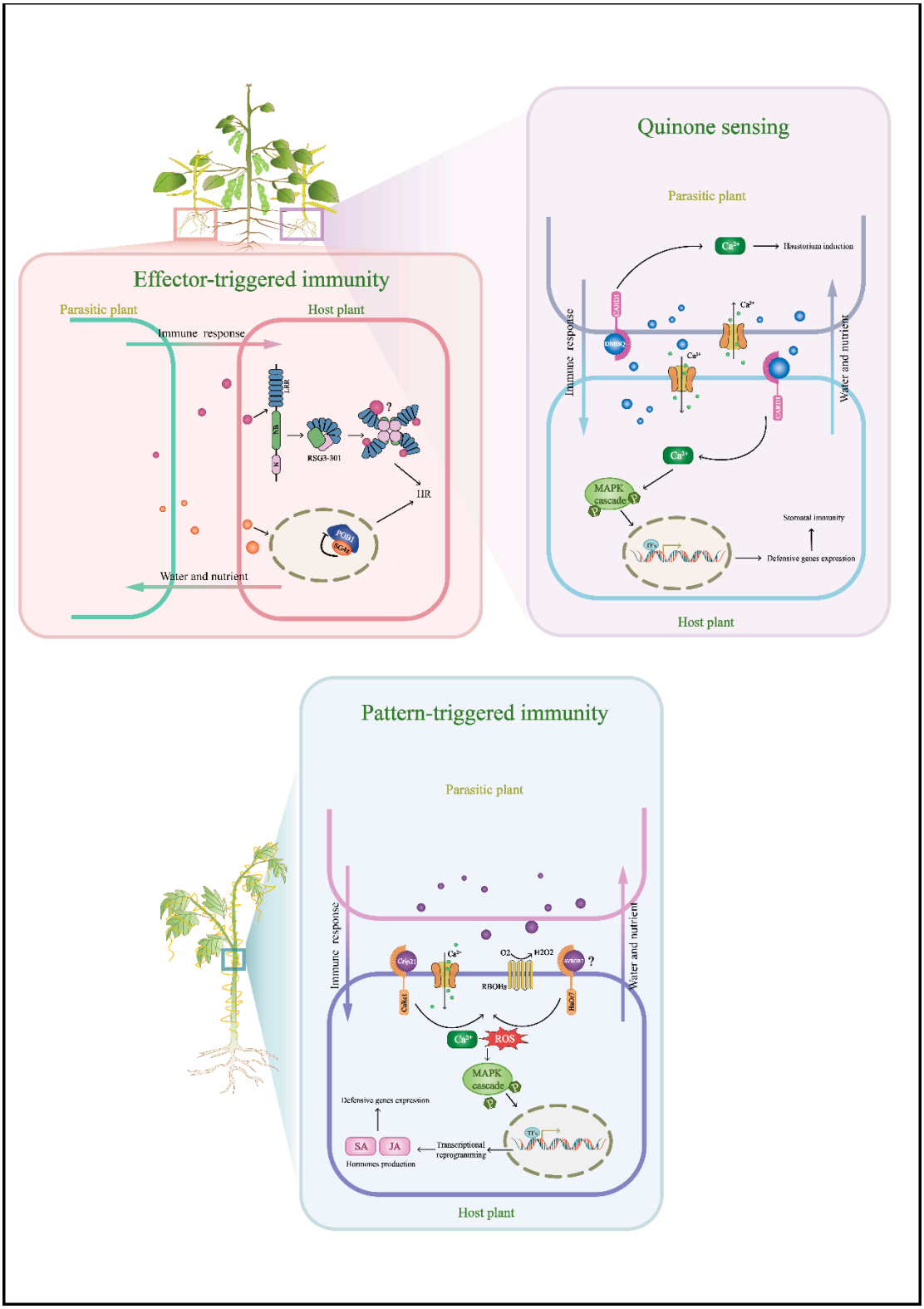

Figure 3. The plant immune system can detect non-self plants during parasitism

(Top left) Detection of non-self plants can occur through effector-triggered immunity (ETI). The *Striga* plants parasitize the roots of cowpea. *S. gesnerioides race 4* evades the plant immune system by secreting an effector protein, SG4z, in the haustorium that targets the host gene, *POB1*, which is required for hypersensitive response

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(HR). In contrast, resistance to *S. gesnerioides* race 3 is attributed to a host NLR, RSG3-301, which induces HR to prevent parasitic colonization; however, the specific effector protein of Striga remains unidentified. (Bottom) Additionally, detection of non-self plants also involves PAMP-triggered immunity (PTI). PAMPs from parasitic plants, such as Crip21 from *C. reflexa*, are recognized through tomato PRR, CUSCUTA RECEPTOR 1 (CuRe1). Similarly, an unknown PAMP from *O. cumana,* designated AvrOr7, activates PTI via the sunflower receptor HaOr7. The induction of PTI encompasses Ca^{2+} influx, apoplastic reactive oxygen species (ROS) production, MAP kinase (MAPK) activation, and transcriptional regulation including the synthesis of defensive hormones. (To right) Furthermore, plants also perceive non-self stimuli through quinone sensing. 2,6-dimethoxy-1,4-benzoquinone (DMBQ) is detected by the host LRR-RLK CANNOT RESPOND TO DMBQ 1 (CARD1), which initiates Ca^{2+} influx and MAPK activation. Transcriptional responses to DMBQ result in stomatal closure, thereby safeguarding *Arabidopsis* against bacterial invasion. Conversely, host-derived DMBQ also functions as a cue for haustorium induction in *P. japonicum.*

Small RNAs, Gene Silencing, and Graft Mobile Technology

Although distinct from PTI/ETI, another mechanism that parasitic plants and pathogens use to evade detection involves small RNAs (sRNAs). sRNAs regulate gene silencing by targeting mRNA for cleavage or epigenetic regulation. It was shown in tomato and *Arabidopsis* that infection with the fungus *Botrytis cinerea* led to the production of pathogenic sRNAs that could "hijack" the host AGONAUT1 (AGO1) protein and degrade host immune genes via RNA-interference (RNAi) (Weiberg *et al.*, 2013). Similarly, *Cuscuta campestris* was found to produce 22 nt microRNAs capable of targeting *Arabidopsis* mRNA, resulting in mRNA cleavage of genes involved in growth and defense, such as *BOTRYTIS-INDUCED KINASE 1 (BIK1)* (Lu *et al.*, 2010). In contrast, host-induced gene silencing (HIGS) occurs when transgenic small interfering RNA (siRNA) is introduced into the host, targeting and degrading pathogen mRNA (Baulcombe, 2015). All these examples suggest that sRNAs are excellent targets for plant-plant genetic interactions. This is bolstered by the knowledge that numerous sRNAs are graft mobile (Li *et al.*, 2021, Palauqui *et al.*, 1997). In fact, grafting was key to demonstrating that 24 nt sRNA moved through the vasculature to mediate epigenetic silencing (Molnar *et al.*, 2010). Thus, it also stands to reason that sRNA movement, either locally at the graft junction or systemically via vascular transport, may affect graft healing, compatibility, or trait inheritance.

Furthermore, efforts have been made to use grafting to introduce siRNA from the rootstock into the floral meristem (Zhang *et al.*, 2014). This technique allows grafting to serve as a molecular "vaccine," delivering siRNA to existing plants via the phloem at the graft junction (Zhang *et al.*, 2014). This same concept has been famously utilized in 2023 to generate heritable transgene-free CRISPR Cas9-edited offspring via a graft-mobile editing system in *Arabidopsis* and *Brassica rapa* (Yang *et al.*, 2023).

Plant Regeneration: Grafting Versus Haustorium

One of the most critical facets of plant-plant interactions is the ability of distinct plants to physically interact and form chimeric tissues. Parasitic plants use a haustorium, an invasive organ, to attach to and penetrate the host stem, forming vascular connections between the parasite and its host (Kaiser *et al.*, 2015, Kirschner *et al.*, 2023, Shen *et al.*, 2023). The establishment of vascular connections universally occurs during stem wounding, parasitic plant infection, and grafting (Nishitani *et al.*, 2002, Melnyk, 2017, Jhu & Sinha, 2022, Thomas *et al.*, 2022). A more complete understanding of how plants heal in these parallel processes will further elucidate the mechanisms underlying graft incompatibility.

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Auxin

The vascular system transports water, nutrients, and signaling molecules throughout the plant. Wound healing, parasitism and grafting all require the induction of wound sealing, cell differentiation, and vascular reconstructions (Nishitani *et al.*, 2002, Kirschner *et al.*, 2023, Thomas *et al.*, 2024). One of the most important regulators of plant regeneration is auxin (Asahina *et al.*, 2011, Wang *et al.*, 2014). Blocking auxin signaling using the *bdl* (*Atiaa12*) mutant significantly reduced graft success in *Arabidopsis* (Serivichyaswat *et al.*, 2024, Melnyk *et al.*, 2015, Matsuoka *et al.*, 2016). Numerous studies have shown that removal of polar auxin transport is sufficient to interrupt both wound healing and graft reconnection (Asahina *et al.*, 2011, Feng *et al.*, 2024, Serivichyaswat *et al.*, 2024). For example, apical auxin is required for the induction of *DNA BINDING WITH ONE FINGER* (*DOF*) transcription factors *HIGH CAMBIAL ACTIVITY2* (*HCA2*), *TARGET OF MONOPTEROS6* (*TMO6*), *DNA BINDING WITH ONE FINGER* (*DOF2.1*), and *DOF6* (Melnyk *et al.*, 2018). DOFs are highly induced during *Arabidopsis* wounding and grafting and are required for vascular and non-vascular healing. It was shown that DOF induction required auxin and cell wall damage, adding a second layer of input to the necessary expression (Melnyk *et al.*, 2018, Zhang *et al.*, 2022). The authors went on to show that TMO6 could induce *CELLULASE3* (*CEL3*)/*GLYCOSYL HYDROLASE9B3* (*GH9B3*), a β-1,4-glucanases shown to be key in interfamily graft compatibility (Notaguchi *et al.*, 2020). Similarly, parasitic plants require auxin to induce haustorium formation. In *P. japonicum*, the perception of haustorium-inducing factors in the soil (such as plant exudates) leads to the induction of the auxin biosynthesis gene, *YUCCA3 (YUC3)* (Ishida *et al.*, 2016), a process that could be blocked in *Triphysaria versicolor* using the auxin transport inhibitor, triiodobenzoic acid (TIBA) (Tomilov *et al.*, 2005).

Cell Wall Remodeling

Many of the first genes identified as critical for regeneration in wounded inflorescence hypocotyls of *Arabidopsis* were involved in non-vascular healing and cell wall remodeling. For example, wounded hypocotyls exhibited a unique spatial segregation, with *ANAC071/096* highly expressed above the wound and *RAP2.6L* below (Asahina *et al.*, 2011, Matsuoka *et al.*, 2021). This was later found to be due to spatial accumulation of phytohormones, in which high auxin and ethylene promote *ANAC071*, and low auxin and high JA induce *RAP2.6L*. While *RAP2.6L* and JA were induced during grafting, they were later shown not to be required for healing (Matsuoka *et al.*, 2018). In contrast, *ANAC071/096* were found to play a critical role not only in wound healing but also in grafting. Here, the authors showed that auxin-induced *ANAC071* and *ANAC096* positively regulated vascular proliferation during grafting, and that *anac071anac096* double mutants healed poorly after grafting (Matsuoka *et al.*, 2016, Zhang *et al.*, 2022, Matsuoka *et al.*, 2021). ANAC071 was also shown to regulate the expression of *ENDOTRANSGLUCOSYLASE/HYDROLASE* (*XTH19*) and *XTH20*, required for non-vascular cell differentiation in wounded stems (Pitaksaringkarn *et al.*, 2014). The *XTH* gene family has been validated as broadly upregulated during melon (*Cucumis melo*) grafting, especially during compatible grafting; A *CmXTH9* knockdown and an *Arabidopsis xth4xth7* double mutant both showed reduced graft healing and decreased callus proliferation (Xiong *et al.*, 2026). In tomato, gene regulatory networks based on the first week of compatible healing identified several *XTH* genes downstream of central hubs. *XTH16* was predicted to be regulated by *TOMATO HOMEOBOX GENE 1* (*THOM1*), and *SlXTH6* was predicted to be co-regulated by *JASMONATE-RESPONSIVE ERF 4* (*JRE4*) and *ETHYLENE RESPONSE FACTOR* (*ERF4*) (Thomas *et al.*, 2022). In pepper grafts, the hub genes *LATERAL ORGAN BOUNDARIES DOMAIN 4* (*LBD4*), *MYB86*, *NGATHALIKE 1* (*NGAL1-like*), and two *ERFs* were all predicted to co-regulate *XTH22* and *XTH38*. *XTHs* have also been implicated in *Cuscuta* infection, with *CrXTH1* and *CrXTH2* upregulated in the developing haustoria (Olsen *et al.*, 2016, Johnsen *et al.*, 2015, Hozumi *et al.*, 2017). Haustorium penetration is often associated with cell wall-related genes, so the involvement of *XTHs* is logical (Ranjan *et al.*, 2014, Yang *et al.*, 2015, Johnsen *et al.*, 2015, Hozumi *et al.*, 2017). Whether *CrXTH1* and *CrXTH2* are more critical for remodeling of the host or parasite cell wall remains unclear.

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Cell wall remodeling has been shown to be key not just to graft healing but also for compatibility. Interfamily grafting was demonstrated in tobacco (Notaguchi *et al.*, 2020) and petunia (*Petunia hybrida*; Kurotani *et al.*, 2022) due to the highly secreted β-1,4-glucanase, *GH9B3*, which is thought to degrade cellulose in the apoplast. Similarly, in *P. japonicum*, *PjGH9B3* was highly expressed during infection of *Arabidopsis* and during *P. japonicum-Arabidopsis* interspecies grafting (Kurotani *et al.*, 2020). RNAi of *PjGH9B3* significantly reduced haustoria penetration, demonstrating that cell wall remodeling is critical for distant plant-plant regeneration. Since adhesion is the initial step in regeneration, it stands to reason that genes that optimize early graft healing are key targets for plant breeding to expand graft compatibility.

LBD25 was also identified as highly expressed in C. campestris haustorium (Jhu *et al.*, 2021). In Arabidopsis, LBD25 functions in auxin signaling during lateral root formation (Mangeon *et al.*, 2011). Using HIGS in tomato, they showed that CcLBD25 is critical for haustorium initiation, potentially through modification of the host cell wall (Jhu *et al.*, 2021). In alignment with this, LBD25 was among the most highly upregulated genes in the Thesium chinense haustorium, suggesting that this gene is activated in diverse species during infection (Ichihashi *et al.*, 2018). The role of *LBD25* in reconnection is further supported by the identification of *SlLBD25* as a predicted hub gene during early tomato graft formation (Thomas *et al.*, 2022).

In addition to auxin, ethylene has been linked to tissue regeneration. Ethylene is induced and required for wound response (Li *et al.*, 2018), but ethylene mutants in *Arabidopsis* (ethylene insensitive 2; *ein2*, ethylene response 1; *etr1*) appear to graft normally (Melnyk *et al.*, 2015). This is contradicted in tobacco, where the application of an ethylene precursor (ACC) could enhance graft formation, whereas the ethylene biosynthesis inhibitor (AVG) delayed healing (Zhai *et al.*, 2021). However, in *Arabidopsis*, the *ctr1* mutant, which over-accumulates ethylene, showed reduced phloem connectivity (Melnyk *et al.*, 2015). Unlike the somewhat ambiguous effect of ethylene on graft healing, ethylene appears to positively regulate haustorium formation. In P. japonicum infection, mutations to EIN2 and ETR1, as well as the ethylene signaling inhibitor, $AgNO_3$, all resulted in severely compromised infection rates (Cui *et al.*, 2020).

Vascular Regulators

In addition to nonvascular healing and cell wall remodeling, wound response requires a competent cambium to induce *de novo* differentiation of vascular tissues, such as xylem and phloem. The need for cambial contact has long been noted in horticultural handbooks (Hartmann *et al.*, 2002), but until recently, the genetic regulators of cambial differentiation during grafting were ambiguous. Cambial genes such as *PHLOEM INTERCALATED WITH XYLEM* (*PXY*), *ARABIDOPSIS THALIANA HOMEOBOX FACTOR8* (*ATHB8*), *SUPPRESSOR OF MAX2 1-LIKE PROTEIN 5* (*SMXL5*), and *WUSCHEL RELATED HOMEOBOX4* (*WOX4*) have been identified as key for graft healing in *Arabidopsis* and tomato (Thomas *et al.*, 2022, Serivichyaswat *et al.*, 2024). This is a process shared between grafted and parasitized plants. By inducing haustorium production in *C. campestris*, transcriptional regulators of xylem formation in the haustorium were identified using RNA-seq (Kaga *et al.*, 2020). Similar to grafting, the cambium was necessary for the formation of a vascular bridge between the two joined plants, with genes such as *CcWOX4*, *CcPXY-like*, *CcMP*, and *CcTMO5* induced during the transition of search hyphae into xylem hyphae. *WOX4* expression was also observed in *P. japonicum* haustorium using fluorescent markers (Wakatake *et al.*, 2018).

During grafting, phloem reconnection occurs before the xylem (Melnyk *et al.*, 2015, Melnyk *et al.*, 2018). In *Arabidopsis*, *ABERRANT LATERAL ROOT FORMATION 4* (ALF4), *AUXIN RESISTANT 1* (AXR1), and HCA2 were first identified as key phloem regulators in the stock (Melnyk *et al.*, 2015, Melnyk *et al.*, 2018). However, *Arabidopsis* lines lacking auxin signaling in the promoter region of *ALTERED PHLOEM DEVELOPMENT* (*APL*), a phloem companion cell marker, showed a reduced, but non-significant reduction in phloem connectivity (Serivichyaswat *et al.*, 2024). While not all parasitic plants form both xylem and phloem connections, Orobanche and *Cuscuta spp.* can (Dorr & Kollmann, 1995, Haupt *et al.*, 2001, Aly *et al.*, 2011). *C. japonica* expressed *CjAPL* and *CjSEOR1*, a sieve

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element marker gene, in the haustorium during infection, showing that similar genetic regulators control the haustorium phloem development (Shimizu *et al.*, 2018). Despite this, the genetic regulation of phloem formation, grafting, and parasitism remains less well understood than other processes, such as xylem formation.

In contrast to phloem, the *VASCULAR-RELATED NAC*-DOMAIN (*VND*) family of proteins is a key regulator of xylem formation. As xylem connections formed between the *C. campestris* haustorium and the *Arabidopsis* host, the key xylem differentiation factor *VND7* (Kubo *et al.*, 2005), along with known downstream genes *MYB46*, *MYB86*, *IRREGULAR XYLEM* (*IRX3*), and *IRX5* were upregulated (Kaga *et al.*, 2020). *PjIRX3* was visualized in the haustorium using fluorescent markers, clearly building strong genetic parallels between the two parasitic plants (Wakatake *et al.*, 2018). While *VNDs* are integral for xylem formation, single mutants fail to show phenotypes due to redundancy in the family (Gushino *et al.*, 2024, Tan *et al.*, 2018). Similarly, in tobacco, *Nbvnd7* mutants showed no effect on grafting, whereas inducible overexpression lines increased xylem formation in *Arabidopsis*-tobacco interspecies grafts (Huang *et al.*, 2025).

An interesting finding in the *C. japonica* haustorium was the expression of the Tracheary element differentiation inhibitory factor (TDIF), *CLAVATA3/EMBRYO SURROUNDING REGIONRELATED 41* (*CLE41*), *GLYCOGEN SYNTHASE KINASE* (*GSK3*) and *BRI1-EMS-SUPPRESSOR (BES1)* (Shimizu *et al.*, 2018). In *Arabidopsis*, *CLE41* and *CLE44*-derived CLE41/44 peptides are expressed in the phloem and transported to the procambium, where they bind PXY, activating cambium maintenance via WOX4 and repressing xylem formation via GSK3/BES1 (Hunziker & Greb, 2024). Identification of this cambium-xylem pathway in the haustorium suggests that all vascular formations may share a core set of genetic regulators involved in vascular regeneration, including mobile CLE peptides.

In 1969, Dean & Kuijt referred to the haustorium as a "perfect graft" (Dean & Kuijt, 1969). Indeed, there are significant parallels between grafting and plant parasitism, including the need for cell wall remodeling, the importance of auxin, and vascular regeneration. It is worth noting that while grafts face compatibility limitations, parasitic plants are largely capable of infecting and thus fusing their vascular systems, with almost all species. In this way, most parasitic plants can be considered not just perfect grafts but examples of ultra-compatibility. Understanding how parasitic plants evade the host immune system, overcome physiological barriers, and coordinate the molecular signaling required for healing may hold the key to overcoming graft incompatibility.

Overlap between various genes has identified potential regulators of graft compatibility. Hypercompatibility can be seen in inter-family grafts as well as parasitic plants. These traits have been linked to enhanced cell wall remodeling and adhesion via GLYCOSYL HYDROLASE9B3 (GH9B3) and ENDOTRANSGLUCOSYLASE/HYDROLASE (XTH). Furthermore, repression of the host immune system can be accomplished via parasitic plant effectors such as SH4z. Both parasitic plants and compatible grafts require auxin signaling (YUCCAs (YUCs) and LATERAL ORGAN BOUNDARIES DOMAIN 4 (LBD4)), cambium maintenance (TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTORS (TDIF)-PHLOEM INTERCALATED WITH XYLEM (PXY)-WUSCHEL RELATED HOMEOBOX4 (WOX4)), phloem (ALTERED PHLOEM DEVELOPMENT (APL)), and xylem (VASCULAR-RELATED NAC-DOMAIN 7 (VND7)). Graft incompatibility has been associated with vascular repression (ENHANCED XYLEM AND GRAFTING 1 (EXG1)) and the upregulation of NLRs associated with immune-mediated cell death. By targeting these known processes, it may be possible to genetically overcome graft incompatibility.

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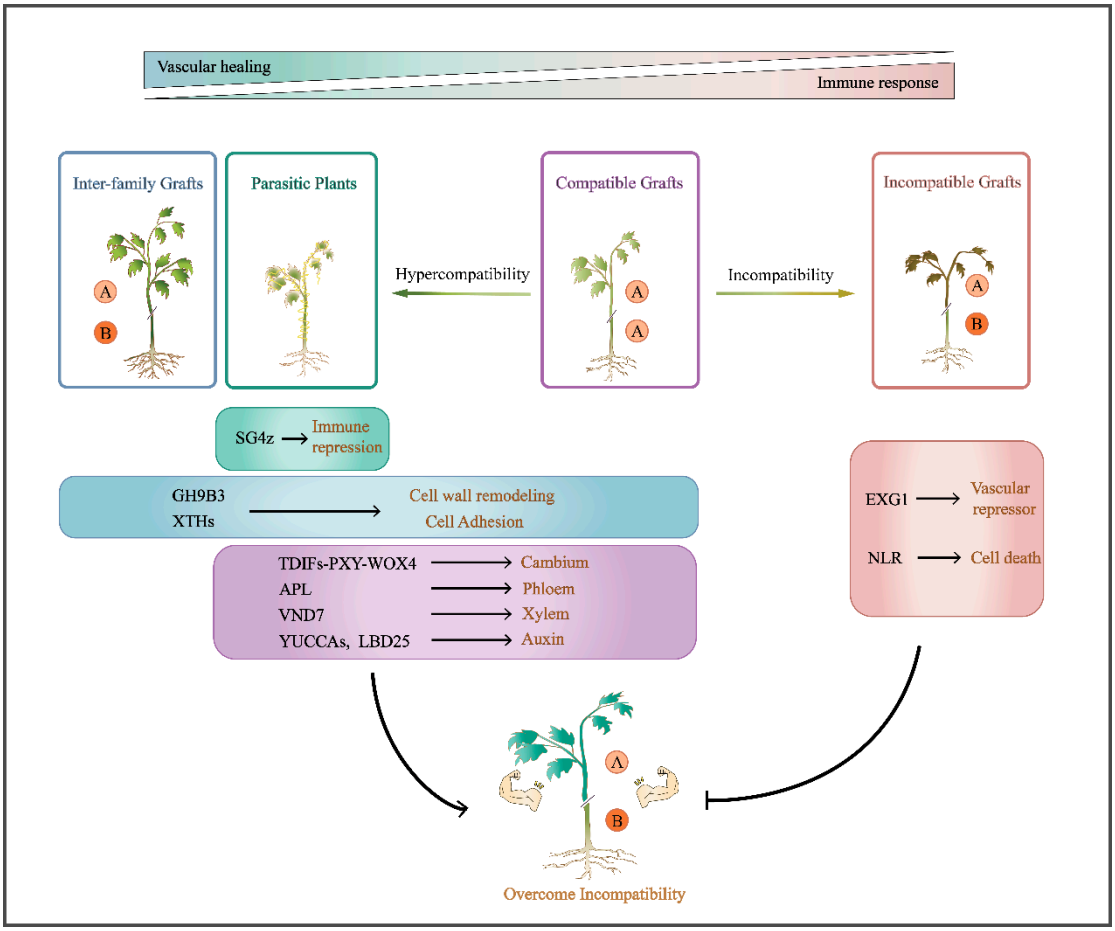

Figure 4. Grafted and parasitic plants span a continuum of compatibility.

Wound Signals

Several genes have been proposed as damage- or wound-activated during grafting in recent years. *ENHANCED XYLEM AND GRAFTING1* (*EXG1*), a negative regulator of vascular tissue regeneration, is rapidly upregulated after nematode infection, grafting, and infection with *Agrobacterium* (Mazumdar *et al.*, 2025). Although the authors did not identify the elicitors for this, they hypothesized that hormones, ROS, changes to the cell wall, or turgor pressure may activate this gene. Similarly, *ERF114* and *ERF115* have been shown to be induced during both wounding and graft healing of *Arabidopsis*, spruce, tomato, and pepper (Feng *et al.*, 2024, Thomas *et al.*, 2024, Zhang *et al.*, 2022, Canher *et al.*, 2022). Treating *Arabidopsis* with cell wall-modifying enzymes, such as cellulase and pectinase, was sufficient to activate *ERF115* expression in the stem, but, when combined with auxin, the effect was amplified (Zhang *et al.*, 2022). Similarly, treatment with macerozyme, a pectinase, induced *ERF114* in the root (Canher *et al.*, 2022). DNA damage to the root tip in the JA mutant, *coronatine insensitive1* (*coi1*), was able to show that wound-mediated *ERF115* expression did not require JA signaling, and while auxin was not required for *ERF115* induction following wounding, it was necessary for wound recovery (Canher *et al.*, 2020). The authors then suggested *ERF114* and *115* could be induced by mechanical stress. Using the mechanosensory mutant, *feronia* (*fer*), which showed increased mechanical strain, in cooperation with auxin, was likely responsible for the induction of ERF114 and 115 (Canher *et al.*, 2022).

Plants release a plethora of wound signals, and while none have been clearly linked to graft incompatibility, they all have potential activity. Because grafting is, at its core, an instance of wounding, signals involved in the wound response are worth exploring. To be considered potential regulators of compatibility, the signal must be released during grafting, be mobile over short or long distances, and induce incompatibility-associated symptoms, such as cell death.

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Constitutive DAMPs

Damage-associated molecular patterns (DAMPs) are molecules that are either induced during wounding or constitutively present in cells but released into the apoplast during damage (Tanaka & Heil, 2021). Like PAMPs, these signals are perceived by receptors and trigger additional immune responses. DAMPs are extremely diverse, spanning sugars, nucleotides, peptides, and more, many of which have unknown receptors.

Most carbohydrates that act as DAMPs are components of the plant cell wall. These molecules are released into the apoplast during cellular degradation by pathogenic enzymes. Perhaps more relevant to grafting is the breakdown of homogalacturonan pectin into oligogalacturonides (OGs) by endogenous polygalacturonases, which occurs following wounding, as demonstrated in tomato leaves (Orozco-Cardenas & Ryan, 1999). OGs are one of the most extensively studied cell wall DAMPs (Bishop *et al.*, 1981, Davidsson *et al.*, 2017). Treatment with OGs activates PTI processes such as MAPK activation, ROS burst, and resistance against *Botrytis* infection (Denoux *et al.*, 2008, Galletti *et al.*, 2011, Galletti *et al.*, 2008, Kohorn *et al.*, 2009). Evidence first identified WALL-ASSOCIATED KINASE 1 and 2 (WAK1 and 2) as the proposed receptors due to their ability to bind OGs and trigger downstream responses (Brutus *et al.*, 2010, Decreux *et al.*, 2006, Kohorn *et al.*, 2009), but recent work involving an *Arabidopsis* mutant lacking all five *WAK* genes was found to still execute immune responses when treated with exogenous OGs (Herold *et al.*, 2024), demonstrating WAKs are not required for OG-induced PTI. Furthermore, studies have shown that WAKs function in coordination with other receptors, such as FERONIA (FER) (Dünser et al., 2019), which act as mechanical sensors of cell wall strain to elicit defense responses during wounding. Currently, the main OG receptor remains unclear.

During cell rupture, cytoplasmic contents spill into the extracellular matrix, where they can act as DAMPs, triggering PTI responses. Extracellular ATP (eATP) is present at high concentrations within the cell, making it an excellent marker of compromised cellular integrity. Unlike OGs, which lack a clear functional receptor, P2 Receptor Kinase 1 and 2 (P2K1 and 2) have been validated as active receptors that trigger immune processes in response to high eATP (Choi *et al.*, 2014, Pham *et al.*, 2020, Tanaka *et al.*, 2014, Jeter *et al.*, 2004). Other cellular components, such as the amino acid glutamate, are released during wounding (Bellandi *et al.*, 2022). Glutamate (glu) moves throughout the xylem and extracellular space, where it binds to and triggers the GLUTAMATE-LIKE RECEPTORS 3.3 (GLR) and GLR3.5 in *Arabidopsis*, which induce the intracellular influx of calcium (Toyota *et al.*, 2018, Mousavi *et al.*, 2013). The movement of glu in the plant was shown to trigger both the propagation of electrical signals via calcium and ROS waves. While *Arabidopsis* and tomato mutant rootstocks lacking long-distance electrical (*Atglr3.3glr3.6 or Slglr3.3glr3.5*) and ROS (*Atrbohdrhohf* or *Slrbho1*) signals could still be successfully grafted onto WT scions (Zhan *et al.*, 2025, Wang *et al.*, 2019), it is interesting to consider the role that these long-distance immune-mediating signals might play prior to or during compatibility determination.

Another nucleotide-based DAMP is extracellular DNA (eDNA), which can occur in the apoplast during necrotrophic infection and wounding. In pea (*Pisum sativum*), *Fusarium solani* produces a secreted DNase, which degrades host DNA (Klosterman *et al.*, 2001). Fragmented DNA (less than 700 bp) can trigger PTI responses, including Ca^{2+} influx, ROS, MAPK activation, and resistance to *Pseudomonas syringae* (Duran-Flores & Heil, 2018, Barbero *et al.*, 2016). Interestingly, while all fragmented host DNA could elicit host immune responses, treatment with fragmented DNA from other species elicited a significantly attenuated response, suggesting that, however eDNA is surveilled, plants are capable of distinguishing self from non-self. Interestingly, the symptoms associated with DAMP-induced PTI align with many of the graft incompatibility symptoms described in the tomato-pepper grafts. It is worth noting that in a related process, *BREAST CANCER SUSCEPTIBILITY GENE 1* (*BRCA1*) and *BRCA1 ASSOCIATED RING DOMAIN PROTEIN* 1 (*BARD1*) homologs were identified as upregulated in incompatibly grafted tomato (Thomas *et al.*, 2024). These genes are involved in DNA repair after genotoxic damage, suggesting that incompatibility may trigger DNA breakdown in tomato. It is possible that a molecular signal released by the opposite

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species or propagated by the host during failed healing (cell wall components or fragmented DNA) triggers many of the symptoms observed in graft incompatibility (Thomas *et al.*, 2023). To date, no published work has explored the role of OGs, eATP, glutamate or other DAMPs during grafting, making this an important area for future studies.

DAMP Peptides

Plants also secrete peptides during wound response. Peptide-DAMPs that have identified receptors include RALFs, which bind to FER (Stegmann *et al.*, 2017, Pearce *et al.*, 2001); PLANT ELICITOR PEPTIDES (PEPs), which bind to PLANT ELICITOR PEPTIDE RECEPTOR 1 (PEPR1) and PEPR2 (Huffaker *et al.*, 2006, Yamaguchi *et al.*, 2010, Yamaguchi *et al.*, 2006); PLASMA MEMBRANE INTRINSIC PROTEINS1/2 (PIP1/2), which binds to RECEPTOR-LIKE KINASES 7 (RLK7) (Hou *et al.*, 2014); Systemin, which binds to SYSTEMIN RECEPTOR 1 (SYR1/2) (Pearce *et al.*, 1991, Wang *et al.*, 2018); SERINE-RICH ENDOGENOUS PEPTIDE 12 (SCOOP12), which binds to MALE DISCOVERER 1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2) (Gully *et al.*, 2019, Rhodes *et al.*, 2021); and phytosulfokines (PSKs) that bind to PHYTOSULFOKINE RECEPTOR 1/2 (PSKR1/2) (Matsubayashi & Sakagami, 1996). Aside from species-specific DAMPs, most of these peptides are presumed to be secreted during grafting.

Recent work has suggested that PEPs and PSKs could be important to graft compatibility (Lori *et al.*, 2015). PEPs are conserved species-specific DAMPs (Lori *et al.*, 2015) that elicit PTI responses (Bartels *et al.*, 2013). In contrast, PSKs, while first identified as wound-inducible, have since been shown to regulate growth and cell division (Kutschmar *et al.*, 2009, Yang *et al.*, 2000). It had previously been shown in tomato that PSK-regulated immunity to *Botrytis* was dependent on auxin signaling, further supporting PSK in the growth-defense nexus (Zhang *et al.*, 2018). In rice, wounding triggers expression of the OsPep3 precursor within 15 minutes. Wounding and Pep3 treatment induced rapid changes, including MAPK activation, JA signaling and defense genes such as *WRKY* TFs, as well as delayed expression of PSK precursors, especially OsPSK3 (Harshith *et al.*, 2024). They then showed that the receptor, OsPSKR, associates with SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE 1 (SERK1), a homolog of *A. thaliana.* BRI1-ASSOCIATED RECEPTOR KINASE 1 (AtBAK1). *OsPSKR*-overexpression (OE) lines exhibited reduced wound response, whereas the *pskr* mutants displayed exaggerated and prolonged cell death, indicating that PSKR represses the late immune response to PEPs. The "constitutive cell death" phenotype seen in *pskr* is reminiscent of *Atbak1* mutants, in which loss of repression of immune processes led to autoimmune-induced cell death and hyperactivation of NLRs (Wu *et al.*, 2020). Due to the striking similarity with tomato-pepper genetic incompatibility (Thomas *et al.*, 2024), it is critical that peptide DAMPs be carefully assayed for their role in compatibility.

The DAMP peptides, PLANT ELICITOR PEPTIDES (PEP) and PHYTOSULFOKINE (PSK), antagonistically balance early defense responses and the transition into growth and healing. PEPs are secreted within minutes of wounding, whereas PSKs are expressed hours later. It is possible that a similar mechanism is activated during compatible grafting, downregulating the wound response to allow the graft junction to heal. Failure to repress defense may lead to a non-self-induced immune response and a cell-death phenotype. Adapted from Harshith *et al.*, 2024.

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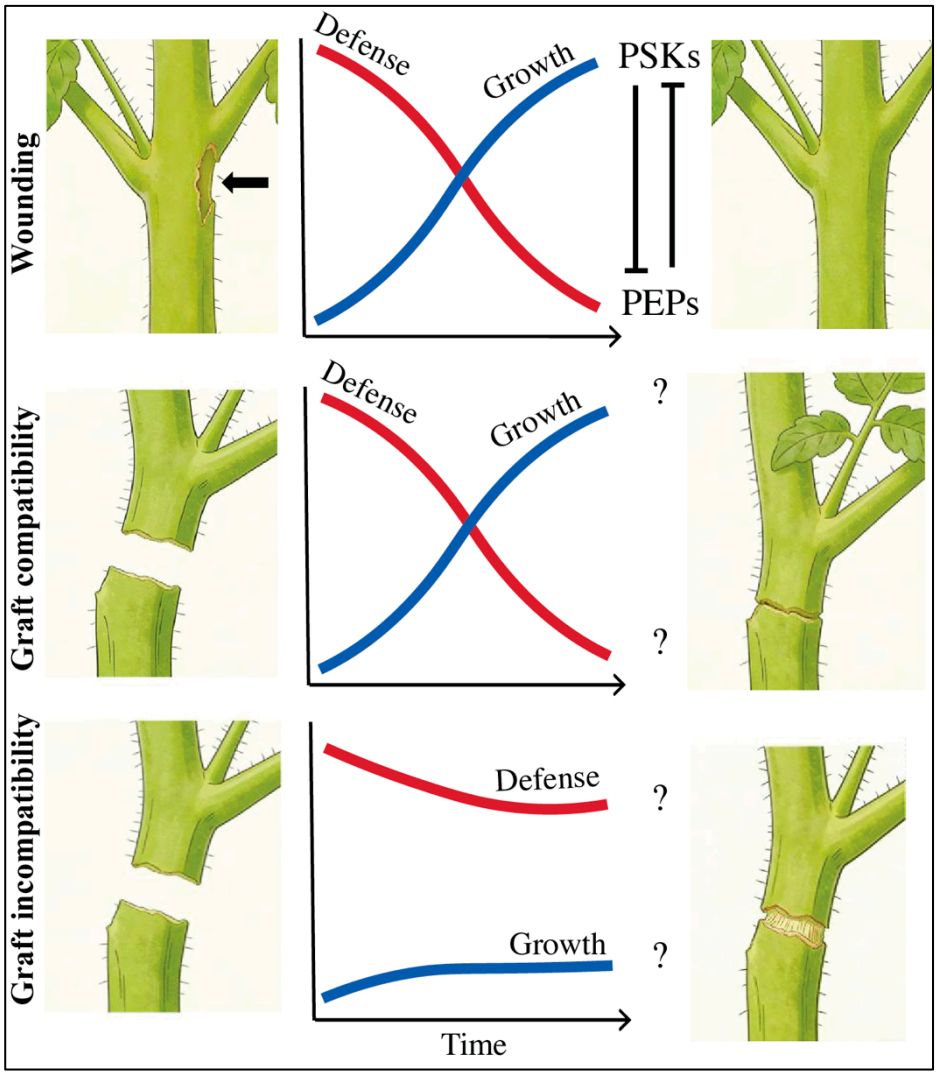

Figure 5. Graft compatibility may be regulated by DAMPs.

Incompatibility: From Pollen to Grafting

A remarkable adaptation observed in plants that facilitates outcrossing is self-incompatibility (SI), which allows plants to reject genetically similar pollen, preventing excessive inbreeding. SI has evolved independently in up to 40% of angiosperms, resulting in distinct types of SI (Igic *et al.*, 2008, Charlesworth, 2010, Allen. & Hiscock., 2008). Because the process of SI is so diverse, we aim only to draw parallels between graft compatibility and SI. For a more in-depth study, see (Takayama & Isogai, 2005).

SI is largely controlled by one polymorphic locus called the S-locus. Through distinct molecular mechanisms, the S gene encodes alleles that regulate compatibility determination during pollen-pistil interaction. For example, Type I (*Solanaceae*) SI is controlled by a single pistil-expressed S-RNase and multiple pollen-expressed cytosolic S-loci F boxes (SLFs) (Liu *et al.*, 2014, Luu *et al.*, 2000). S-RNAses are taken up into the pollen tube, where they bind to SLFs (Anderson *et al.*, 1986). Non-self-recognition leads to the ubiquitination and degradation of the RNases, allowing pollen tube growth, whereas SI leads to RNA degradation of the pollen tube via the pistil RNase (Entani *et al.*, 2014). Other types of SI involve ligand-receptor pairings that trigger molecular cascades that lead to pollen death, such as Type 3 SI (*Papaveraceae*), where self-recognition involves the pollen S-gene (*P. rhoeas pollen S : PrpS*), which encodes a transmembrane receptor, and the stigma S-gene (*P. rhoeas stigma S : PrsS*), which encodes a secreted protein. When SI occurs, it triggers Ca^{2+} influx and a cascade of responses, including increased ROS, MAPK9 activation, ATP depletion, and caspase-3-like activity, resulting in

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PCD (Wheeler *et al.*, 2009, Bosch & Franklin-Tong, 2008, Wheeler *et al.*, 2010, Franklin-Tong *et al.*, 2002, Chai *et al.*, 2017, Wang *et al.*, 2022). During Type 4 SI (*Brassica*), the stigma S-locus receptor kinase (SRK) binds the pollen S-locus cysteine-rich protein (SCR) (Takayama *et al.*, 2000, Takasaki *et al.*, 2000). SI leads to the breakdown of fertilization-required compatibility factors (exocyst complex subunit, EXO70A1; glyoxalase 1, GLO1; and phospholipase D alpha 1, PLDα1) and ROS production (Samuel *et al.*, 2009, Sankaranarayanan *et al.*, 2015, Scandola & Samuel, 2019). In *Arabidopsis*, this leads to autophagy of the pollen tube cell (Macgregor *et al.*, 2022).

Parallels between pollen SI and the plant immune system have been previously drawn (Allen & Hiscock, 2008), as many SI systems are based on ligand-receptor pairings reminiscent of PAMP-PRRs. Furthermore, SRKs are receptor-like kinases that belong to the same protein family as PRRs (Lee *et al.*, 2021). Additionally, similarities exist among PrsS/SCRs and plant defensins, which are antimicrobial proteins identified in numerous plant species, as all are cysteine-rich secreted proteins with significant structural similarities (Thomma *et al.*, 2002). While it is unlikely that grafting, a process largely propagated by humankind, has led to the evolution of an unknown ligand-receptor pairing, it is possible that wound responses have utilized existing signaling components to protect against non-self interactions. In this way, it is curious to consider whether graft incompatibility relies on the detection of non-self, as in Type I SI, or on the detection of self, as in Type 3/4 SI. It seems most likely that a mechanism similar to Type I may exist in which non-self is detected rather than self. Regardless, it remains key to carefully explore secreted peptides, highly expressed receptors, DNases, and RNases at graft interfaces to determine whether similar mechanisms regulate non-self detection during grafting as they do during pollination.

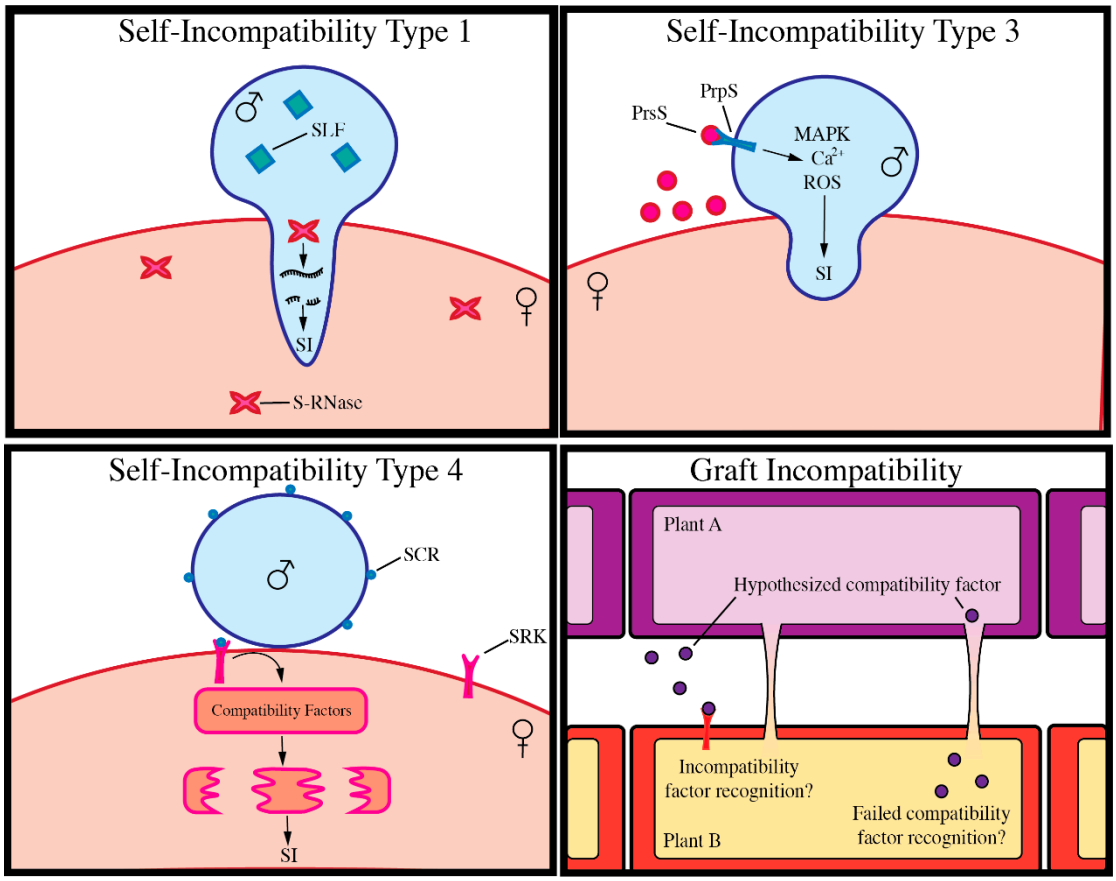

Figure 6. Plants utilize diverse mechanisms to detect and avoid self-interactions during pollination
During pollination, detection of self-pollen triggers pollen tube degradation through a process known as self-incompatibility (SI). Various mechanisms facilitate SI depending on the plant family. In Type I SI, failure for pollen SLFs to bind to stigma S-RNases leads to active RNA breakdown in the pollen tube. During Type 3 SI, secreted PrsS from the stigma will bind to the PrpS receptor on self-pollen, leading to pollen death. During Type

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4 SI, SCR peptides coating the pollen bind to stigma SRK self-receptors, thereby facilitating the breakdown of required compatibility factors. At the interface of a graft junction, two distinct plant varieties touch. The mechanism for the detection of non-self remains unknown. It is possible that, like with SI, vegetative tissue contains compatibility factors that are either required for compatibility or trigger incompatibility. These may be intracellularly transmitted via plasmodesmata or secreted and bound by unknown receptors.

Conclusion

Plant-plant interactions encompass a diverse range of processes, including secreted substances, airborne signals, and physical contact. Many of the fields studying these interactions have remained separate and are seldom considered when discussing the role of grafting. From the existing literature, it is evident that plants possess numerous mechanisms to promote and prevent contact with other plants. Furthermore, plants have evolved complex signaling pathways to alert the immune system to pathogens and wounding. Despite a limited understanding of how plants distinguish compatible and incompatible graft partners, substantial knowledge exists regarding how plants monitor non-self. Interdisciplinary collaboration is crucial to unlocking the secrets of graft compatibility, emphasizing the need to integrate expertise across fields to address this challenge. Future research should carefully consider existing data to inform hypotheses regarding the underlying mechanisms of graft incompatibility.

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References

Abdul Malik, N. A., Kumar, I. S. and Nadarajah, K. (2020) Elicitor and receptor molecules: Orchestrators of plant defense and immunity. *Int J Mol Sci,* **21**.

Albert, M., Werner, M., Proksch, P., Fry, S. C. and Kaldenhoff, R. (2004) The cell wall-modifying xyloglucan endotransglycosylase/hydrolase LeXTH1 is expressed during the defence reaction of tomato against the plant parasite *Cuscuta reflexa*. *Plant Biol,* **6,** 402–407.

Allen., A. M. and Hiscock., S. J. (2008) *Evolution and phylogeny of self-incompatibility systems in angiosperms*. Berlin, Heidelberg, GERMANY: Springer Berlin / Heidelberg.

Aly, R., Hamamouch, N., Abu-Nassar, J., Wolf, S., Joel, D. M., Eizenberg, H., *et al.* (2011) Movement of protein and macromolecules between host plants and the parasitic weed *Phelipanche aegyptiaca* Pers. *Plant Cell Rep,* **30,** 2233–2241.

Anderson, M. A., Cornish, E. C., Mau, S. L., Williams, E. G., Hoggart, R., Atkinson, A., *et al.* (1986) Cloning of cDNA for a stylar glycoproteinassociated with expression of self-incompatibility in *Nicotiana alata*. *Nature,* **321,** 38–44.

Asahina, M., Azuma, K., Pitaksaringkarn, W., Yamazaki, T., Mitsuda, N., Ohme-Takagi, M., *et al.* (2011) Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis*. *Proc Natl Acad Sci U S A,* **108,** 16128–16132.

Bai, F., Wu, M., Huang, W., Xu, W., Wang, Y., Zhang, Y., *et al.* (2025) Removal of toxic steroidal glycoalkaloids and bitterness in tomato is controlled by a complex epigenetic and genetic network. *Sci Adv,* **11,** eads9601.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S. and Vivanco, J. M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology,* **57,** 233–266.

 doi:10.20944/preprints202605.1538.v1

Barbero, F., Guglielmotto, M., Capuzzo, A. and Maffei, M. E. (2016) Extracellular Self-DNA (esDNA), but not heterologous plant or insect DNA (etDNA), induces plasma membrane depolarization and calcium signaling in Lima Bean (*Phaseolus lunatus*) and Maize (*Zea mays*). *Int J Mol Sci,* **17,** 1659.

Barragan, A. C., Collenberg, M., Wang, J., Lee, R. R. Q., Cher, W. Y., Rabanal, F. A., *et al.* (2021) A truncated singleton NLR causes hybrid necrosis in *Arabidopsis thaliana*. *Mol Biol Evol,* **38,** 557–574.

Bartels, S., Lori, M., Mbengue, M., van Verk, M., Klauser, D., Hander, T., *et al.* (2013) The family of Peps and their precursors in *Arabidopsis*: differential expression and localization but similar induction of pattern-triggered immune responses. *J Exp Bot,* **64,** 5309–5321.

Baulcombe, D. C. (2015) VIGS, HIGS and FIGS: small RNA silencing in the interactions of viruses or filamentous organisms with their plant hosts. *Curr Opin Plant Biol,* **26,** 141–146.

Bellandi, A., Papp, D., Breakspear, A., Joyce, J., Johnston, M. G., de Keijzer, J., *et al.* (2022) Diffusion and bulk flow of amino acids mediate calcium waves in plants. *Sci Adv,* **8,** eabo6693.

Belz, R. G. and Hurle, K. (2005) Differential exudation of two benzoxazinoids - One of the determining factors for seedling allelopathy of triticeae species. *J Agric Food Chem,* **53,** 250–261.

Bigeard, J., Colcombet, J. and Hirt, H. (2015) Signaling mechanisms in pattern-triggered immunity (PTI). *Mol Plant,* **8,** 521–539.

Bishop, P. D., Makus, D. J., Pearce, G. and Ryan, C. A. (1981) Proteinase inhibitor-inducing factor activity in tomato leaves resides in oligosaccharides enzymically released from cell walls. *Proc Natl Acad Sci U S A,* **78,** 3536–3540.

Bomblies, K., Lempe, J., Epple, P., Warthmann, N., Lanz, C., Dangl, J. L., *et al.* (2007) Autoimmune response as a mechanism for a dobzhansky-uuller-type incompatibility syndrome in plants. *PLOS Biol,* **5,** 1962–1972.

Bosch, M. and Franklin-Tong, V. E. (2008) Self-incompatibility in *Papaver*: signalling to trigger PCD in incompatible pollen. *J Exp Bot,* **59,** 481–490.

Browning, G. and Watkins, R. (1991) Preliminary evaluation of new quince (*Cydonia oblonga* Miller) hybrid rootstocks for pears. *J Hortic Sci,* **66,** 35–42.

Brutus, A., Sicilia, F., Macone, A., Cervone, F. and De Lorenzo, G. (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proc Natl Acad Sci U S A,* **107,** 9452–9457.

Canher, B., Heyman, J., Savina, M., Devendran, A., Eekhout, T., Vercauteren, I., *et al.* (2020) Rocks in the auxin stream: Wound-induced auxin accumulation and ERF115 expression synergistically drive stem cell regeneration. *Proc Natl Acad Sci U S A,* **117,** 16667–16677.

Canher, B., Lanssens, F., Zhang, A., Bisht, A., Mazumdar, S., Heyman, J., *et al.* (2022) The regeneration factors ERF114 and ERF115 regulate auxin-mediated lateral root development in response to mechanical cues. *Mol Plant,* **15,** 1543–1557.

Chai, L., Tudor, R. L., Poulter, N. S., Wilkins, K. A., Eaves, D. J., Franklin, F. C. H., *et al.* (2017) MAP Kinase PrMPK9-1 contributes to the self-incompatibility response. *Plant Physiol,* **174,** 1226–1237.

Charlesworth, D. (2010) Self-incompatibility. *F1000 Biol Rep,* **8,** 68.

Chen, Y. C., Holmes, E. C., Rajniak, J., Kim, J. G., Tang, S., Fischer, C. R., *et al.* (2018) N-hydroxy-pipecolic acid is a mobile metabolite that induces systemic disease resistance in *Arabidopsis*. *Proc Natl Acad Sci U S A,* **115,** E4920–E4929.

Chinchilla, D., Bauer, Z., Regenass, M., Boller, T. and Felix, G. (2006) The Arabidopsis receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. *Plant Cell,* **18,** 465–476.

Choi, J., Tanaka, K., Cao, Y., Qi, Y., Qiu, J., Liang, Y., *et al.* (2014) Identification of a plant receptor for extracellular ATP. *Science,* **343,** 290–294.

Chu, C. G., Faris, J. D., Friesen, T. L. and Xu, S. S. (2006) Molecular mapping of hybrid necrosis genes Ne1 and Ne2 in hexaploid wheat using microsatellite markers. *Theor Appl Genet,* **112,** 1374–1381.

Cimmino, A., Fernández-Aparicio, M., Avolio, F., Yoneyama, K., Rubiales, D. and Evidente, A. (2015) Ryecyanatines A and B and ryecarbonitrilines A and B, substituted cyanatophenol, cyanatobenzo[1,3]dioxole, and benzo[1,3]dioxolecarbonitriles from rye (*Secale cereale* L.) root exudates: Novel metabolites with allelopathic activity on *Orobanche* seed germination and radicle growth. *Phytochemistry,* **109,** 57–65.

 doi:10.20944/preprints202605.1538.v1

Cui, S., Kubota, T., Nishiyama, T., Ishida, J. K., Shigenobu, S., Shibata, T. F., *et al.* (2020) Ethylene signaling mediates host invasion by parasitic plants. *Sci Adv,* **6,** eabc2385.

Davidsson, P., Broberg, M., Kariola, T., Sipari, N., Pirhonen, M. and Palva, E. T. (2017) Short oligogalacturonides induce pathogen resistance-associated gene expression in *Arabidopsis thaliana*. *BMC Plant Biol,* **17,** 19.

Dean, H. L. and Kuijt, J. (1969) The Biology of Parasitic Flowering Plants. *Bio Science*.

Dean, R. A. and Kuc, J. (1986) Induced systemic protection in cucumbers - the source of the signal. *Physiol Mol Plant Pathol,* **28,** 227–233.

Decreux, A., Thomas, A., Spies, B., Brasseur, R., Van Cutsem, P. and Messiaen, J. (2006) In vitro characterization of the homogalacturonan-binding domain of the wall-associated kinase WAK1 using site-directed mutagenesis. *Phytochemistry,* **67,** 1068–1079.

DeFalco, T. A. and Zipfel, C. (2021) Molecular mechanisms of early plant pattern-triggered immune signaling. *Mol Cell,* **81,** 3449–3467.

Denoux, C., Galletti, R., Mammarella, N., Gopalan, S., Werck, D., De Lorenzo, G., *et al.* (2008) Activation of defense response pathways by OGs and Flg22 elicitors in *Arabidopsis* seedlings. *Mol Plant,* **1,** 423–445.

Dodds, P. N. and Rathjen, J. P. (2010) Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat Rev Genet,* **11,** 539–548.

Dorr, I. and Kollmann, R. (1995) Symplasmic sieve element continuity between *Orobanche* and its host. *Bot Acta,* **108,** 47–55.

Duran-Flores, D. and Heil, M. (2018) Extracellular self-DNA as a damage-associated molecular pattern (DAMP) that triggers self-specific immunity induction in plants. *Brain Behav Immun,* **72,** 78–88.

Duriez, P., Vautrin, S., Auriac, M. C., Bazerque, J., Boniface, M. C., Callot, C., *et al.* (2019) A receptor-like kinase enhances sunflower resistance to *Orobanche cumana*. *Nat Plants,* **5,** 1211–1215.

Entani, T., Kubo, K., Isogai, S., Fukao, Y., Shirakawa, M., Isogai, A., *et al.* (2014) Ubiquitin-proteasome-mediated degradation of S-RNase in a solanaceous cross-compatibility reaction. *Plant Journal,* **78,** 1014–1021.

Felix, G., Duran, J. D., Volko, S. and Boller, T. (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J,* **18,** 265–276.

Felix, G., Regenass, M. and Boller, T. (1993) Specific perception of subnanomolar concentrations of chitin fragments by tomato cells - induction of extracellular alkalinization, changes in protein-phosphorylation, and establishment of a refractory state. *Plant J,* **4,** 307–316.

Feng, M., Zhang, A., Nguyen, V., Bisht, A., Almqvist, C., De Veylder, L., *et al.* (2024) A conserved graft formation process in *Norway spruce* and Arabidopsis identifies the PAT gene family as central regulators of wound healing. *Nat Plants,* **10,** 53–65.

Feys, B. J. F., Benedetti, C. E., Penfold, C. N. and Turner, J. G. (1994) Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male-sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *Plant Cell,* **6,** 751–759.

Flor, H.H. (1971). Current status of gene-for-gene concept. *Annu. Rev. Phytopathol*, **9**, 275-296.

Franklin-Tong, V. E., Holdaway-Clarke, T. L., Straatman, K. R., Kunkel, J. G. and Hepler, P. K. (2002) Involvement of extracellular calcium influx in the self-incompatibility response of *Papaver rhoeas*. *Plant J,* **29,** 333–345.

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

Galletti, R., Denoux, C., Gambetta, S., Dewdney, J., Ausubel, F. M., De Lorenzo, G., *et al.* (2008) The AtrbohD-mediated oxidative burst elicited by oligogalacturonides in Arabidopsis is dispensable for the activation of defense responses effective against *Botrytis cinerea*. *Plant Physiol,* **148,** 1695–1706.

Galletti, R., Ferrari, S. and De Lorenzo, G. (2011) Arabidopsis MPK3 and MPK6 play different roles in basal and oligogalacturonide- or flagellin-induced resistance against *Botrytis cinerea*. *Plant Physiol,* **157,** 804–814.

Gasperini, D., Chauvin, A., Acosta, I. F., Kurenda, A., Stolz, S., Chételat, A., *et al.* (2015) Axial and radial oxylipin transport. *Plant Physiol,* **169,** 2244–2254.

Gattullo, C. E., Pii, Y., Allegretta, I., Medici, L., Cesco, S., Mimmo, T., *et al.* (2018) Iron mobilization and mineralogical alterations induced by iron-deficient cucumber Plants (*Cucumis sativus* L.) in a calcareous soil. *Pedosphere,* **28,** 59–69.

 doi:10.20944/preprints202605.1538.v1

Glauser, G., Grata, E., Dubugnon, L., Rudaz, S., Farmer, E. and Wolfender, J. (2008) Spatial and temporal dynamics of jasmonate synthesis and accumulation in *Arabidopsis* in response to wounding. *J Biol Chem,* **283,** 16400–16407.

Goldschmidt, E. E. (2014) Plant grafting: new mechanisms, evolutionary implications. *Front Plant Sci,* **5,** 727.

Guedes, M. E. M., Richmond, S. and Kuc, J. (1980) Induced systemic resistance to anthracnose in cucumber as influenced by the location of the inducer inoculation with colletotrichum-lagenarium and the onset of flowering and fruiting. *Physiol Mol Plant Pathol,* **17,** 229–233.

Gully, K., Pelletier, S., Guillou, M. C., Ferrand, M., Aligon, S., Pokotylo, I., *et al.* (2019) The SCOOP12 peptide regulates defense response and root elongation in *Arabidopsis thaliana*. *J Exp Bot,* **70,** 1349–1365.

Gushino, S., Tsai, A. Y., Otani, M., Demura, T. and Sawa, S. (2024) VND genes redundantly regulate cell wall thickening during parasitic nematode infection. *Plant Cell Physiol,* **65,** 1224–1230.

Gust, A. A., Biswas, R., Lenz, H. D., Rauhut, T., Ranf, S., Kemmerling, B., *et al.* (2007) Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in *Arabidopsis*. *J Biol Chem,* **282,** 32338–32348.

Harshith, C. Y., Pal, A., Chakraborty, M., Nair, A., Raju, S. and Shivaprasad, P. V. (2024) Wound-induced small-peptide-mediated signaling cascade, regulated by OsPSKR, dictates balance between growth and defense in rice. *Cell Rep,* **43,** 114515.

Hartmann, H. T., Kester, D. E., Davies, F. T. and Geneve, R. L. (2002) *Plant Propagation: Principles and Practices*. Upper Saddle River: Prentice Hall.

Haupt, S., Oparka, K. J., Sauer, N. and Neumann, S. (2001) Macromolecular trafficking between *Nicotiana tabacum* and the holoparasite *Cuscuta reflexa*. *J Exp Bot,* **52,** 173–177.

Hegenauer, V., Fürst, U., Kaiser, B., Smoker, M., Zipfel, C., Felix, G., *et al.* (2016) Detection of the plant parasite *Cuscuta* reflexa by a tomato cell surface receptor. *Science,* **353,** 478–481.

Hegenauer, V., Slaby, P., Körner, M., Bruckmüller, J. A., Burggraf, R., Albert, I., *et al.* (2020) The tomato receptor CuRe1 senses a cell wall protein to identify *Cuscuta* as a pathogen. *Nat Commun,* **11,** 5299.

Herold, L., Ordon, J., Hua, C., Kohorn, B. D., Nürnberger, T., DeFalco, T. A., *et al.* (2024) Arabidopsis WALL-ASSOCIATED KINASES are not required for oligogalacturonide-induced signaling and immunity. *Plant Cell,* **37,** koae317.

Herrero, J. (1951) Studies of compatible and incompatible graft combinations with special reference to hardy fruit trees. *J Hortic Sci,* **26,** 186–237.

Hertle, A. P., Haberl, B. and Bock, R. (2021) Horizontal genome transfer by cell-to-cell travel of whole organelles. *Sci Adv,* **7,** eabd8215.

Hollingshead, L. (1930) A lethal factor in crepis effective only in an interspecific hybrid. *Genetics,* **15,** 114–140.

Hou, S., Wang, X., Chen, D., Yang, X., Wang, M., Turrà, D., *et al.* (2014) The secreted peptide PIP1 amplifies immunity through receptor-like kinase 7. *PLoS Pathog,* **10,** e1004331.

Howe, G. A. and Jander, G. (2008) Plant immunity to insect herbivores. *Annu Rev Plant Biol,* **59,** 41–66.

Hozumi, A., Bera, S., Fujiwara, D., Obayashi, T., Yokoyama, R., Nishitani, K., *et al.* (2017) Arabinogalactan proteins accumulate in the cell walls of searching hyphae of the stem parasitic plants, *Cuscuta campestris* and *Cuscuta japonica*. *Plant Cell Physiol,* **58,** 1868–1877.

Hu, L. F., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B. B., *et al.* (2018) Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat Commun,* **9,** 2738.

Huang, A. C. C., Jiang, T., Liu, Y. X., Bai, Y. C., Reed, J., Qu, B. Y., *et al.* (2019) A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. *Science,* **364,** eaau6389.

Huang, C., Toyokura, K., Murakami, E. I., Ishiwata, A., Kurotani, K. I. and Notaguchi, M. (2025) Nicotiana benthamiana VASCULAR-RELATED NAC-DOMAIN7-2 (NbVND7-2) has a role in xylem formation during interfamily grafting. *J Exp Bot,* **76,** 2207–2221.

Huang, K., Mellor, K. E., Paul, S. N., Lawson, M. J., Mackey, A. J. and Timko, M. P. (2012) Global changes in gene expression during compatible and incompatible interactions of cowpea (*Vigna unguiculata* L.) with the root parasitic angiosperm *Striga gesnerioides*. *BMC Genomics,* **17,** 402.

Hudina, M., Orazem, P., Jakopic, J. and Stampar, F. (2014) The phenolic content and its involvement in the graft incompatibility process of various pear rootstocks (*Pyrus communis* L.). *J Exp Bot,* **171,** 76–84.

 doi:10.20944/preprints202605.1538.v1

Huffaker, A., Pearce, G. and Ryan, C. A. (2006) An endogenous peptide signal in *Arabidopsis* activates components of the innate immune response. *Proc Natl Acad Sci U S A,* **103,** 10098–10103.

Hunziker, P. and Greb, T. (2024) Stem cells and differentiation in vascular tissues. *Annu Rev Plant Biol,* **75,** 399–425.

Ichihashi, Y., Kusano, M., Kobayashi, M., Suetsugu, K., Yoshida, S., Wakatake, T., *et al.* (2018) Transcriptomic and metabolomic reprogramming from roots to haustoria in the parasitic plant, *Thesium chinense*. *Plant Cell Physiol,* **59,** 724–733.

Igic, B., Lande, R. and Kohn, J. (2008) Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences,* **169,** 93–104.

Ihl., B., Tutakhil., N., Hagen., A. and Jacob., F. (1988) Studien an *Cuscuta reflexa* Roxb: VII. Zum Abwehrmechanismus von Lycopersicon esculentum Mill. *Flora,* **181,** 383–393.

Ishida, J. K., Wakatake, T., Yoshida, S., Takebayashi, Y., Kasahara, H., Wafula, E., *et al.* (2016) Local auxin biosynthesis mediated by a YUCCA flavin monooxygenase regulates haustorium development in the parasitic plant *Phtheirospermum japonicum*. *Plant Cell,* **28,** 1795–1814.

Janeway, C. A., Jr. (1992) The immune system evolved to discriminate infectious nonself from noninfectious self. *Immunol Today,* **13,** 11–16.

Jensen, P. J., Halbrendt, N., Fazio, G., Makalowska, I., Altman, N., Praul, C., *et al.* (2012) Rootstock-regulated gene expression patterns associated with fire blight resistance in apple. *BMC Genomics,* **13**.

Jeter, C. R., Tang, W., Henaff, E., Butterfield, T. and Roux, S. J. (2004) Evidence of a novel cell signaling role for extracellular adenosine triphosphates and diphosphates in *Arabidopsis*. *Plant Cell,* **16,** 2652–2664.

Jhu, M. Y., Ichihashi, Y., Farhi, M., Wong, C. and Sinha, N. R. (2021) LATERAL ORGAN BOUNDARIES DOMAIN 25 functions as a key regulator of haustorium development in dodders. *Plant Physiol,* **186,** 2093–2110.

Jhu, M. Y. and Sinha, N. R. (2022) Parasitic plants: An overview of mechanisms by which plants perceive and respond to parasites. *Annu Rev Plant Biol,* **73,** 433–455.

Johnsen, H. R., Striberny, B., Olsen, S., Vidal-Melgosa, S., Fangel, J. U., Willats, W. G., *et al.* (2015) Cell wall composition profiling of parasitic giant dodder (*Cuscuta reflexa*) and its hosts: a priori differences and induced changes. *New Phytol,* **207,** 805–816.

Kaga, Y., Yokoyama, R., Sano, R., Ohtani, M., Demura, T., Kuroha, T., *et al.* (2020) Interspecific signaling between the parasitic plant and the host plants regulate xylem vessel cell differentiation in haustoria of *Cuscuta campestris*. *Front Plant Sci,* **11,** 193.

Kaiser, B., Vogg, G., Fürst, U. B. and Albert, M. (2015) Parasitic plants of the genus *Cuscuta* and their interaction with susceptible and resistant host plants. *Front Plant Sci,* **6**.

Kegge, W., Ninkovic, V., Glinwood, R., Welschen, R. A. M., Voesenek, L. and Pierik, R. (2015) Red: far-red light conditions affect the emission of volatile organic compounds from barley (*Hordeum vulgare*), leading to altered biomass allocation in neighbouring plants. *Ann Bot,* **115,** 961–970.

Kiefer, I. W. and Slusarenko, A. J. (2003) The pattern of systemic acquired resistance induction within the *Arabidopsis* rosette in relation to the pattern of translocation. *Plant Physiol,* **132,** 840–847.

Kim, G. and Westwood, J. H. (2015) Macromolecule exchange in *Cuscuta*–host plant interactions. *Curr Opin Plant Biol,* **26,** 20–25.

Kirschner, G. K., Xiao, T. T., Jamil, M., Al-Babili, S., Lube, V., Blilou, I., *et al.* (2023) A roadmap of haustorium morphogenesis in parasitic plants. *J Exp Bot,* **74,** 7034–7044.

Klosterman, S. J., Chen, J., Choi, J. J., Chinn, E. E. and Hadwiger, L. A. (2001) Characterization of a 20 kDa DNase elicitor from *Fusarium solani* f. sp. *phaseoli* and its expression at the onset of induced resistance in *Pisum sativum*. *Mol Plant Pathol,* **2,** 147–158.

Kohorn, B. D., Johansen, S., Shishido, A., Todorova, T., Martinez, R., Defeo, E., *et al.* (2009) Pectin activation of MAP kinase and gene expression is WAK2 dependent. *Plant J,* **60,** 974–982.

Kong, C. H., Zhang, S. Z., Li, Y. H., Xia, Z. C., Yang, X. F., Meiners, S. J., *et al.* (2018) Plant neighbor detection and allelochemical response are driven by root-secreted signaling chemicals. *Nat Commun,* **9,** 3867.

Kubo, M., Udagawa, M., Nishikubo, N., Horiguchi, G., Yamaguchi, M., Ito, J., *et al.* (2005) Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev,* **19,** 1855–1860.

 doi:10.20944/preprints202605.1538.v1

Kulkarni, O. S., Mazumder, M., Kini, S., Hill, E. D., Aow, J. S. B., Phua, S. M. L., *et al.* (2024) Volatile methyl jasmonate from roots triggers host-beneficial soil microbiome biofilms. *Nat Chem Biol,* **20,** 473–483.

Kundariya, H., Yang, X., Morton, K., Sanchez, R., Axtell, M. J., Hutton, S. F., *et al.* (2020) MSH1-induced heritable enhanced growth vigor through grafting is associated with the RdDM pathway in plants. *Nat Commun,* **11,** 5343.

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T. and Felix, G. (2004) The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. *Plant Cell,* **16,** 3496–3507.

Kurotani, K., Huang, C., Okayasu, K., Suzuki, T., Ichihashi, Y., Shirasu, K., *et al.* (2022) Discovery of the interfamily grafting capacity of Petunia, a floricultural species. *Hortic Res,* **9,** uhab056.

Kurotani, K. I., Wakatake, T., Ichihashi, Y., Okayasu, K., Sawai, Y., Ogawa, S., *et al.* (2020) Host-parasite tissue adhesion by a secreted type of β-1,4-glucanase in the parasitic plant *Phtheirospermum japonicum*. *Commun Biol,* **3,** 407.

Kutschmar, A., Rzewuski, G., Stührwohldt, N., Beemster, G. T. S., Inzé, D. and Sauter, M. (2009) PSK-α promotes root growth in Arabidopsis. *New Phytol,* **181,** 820–831.

Laohavisit, A., Wakatake, T., Ishihama, N., Mulvey, H., Takizawa, K., Suzuki, T., *et al.* (2020) Quinone perception in plants via leucine-rich-repeat receptor-like kinases. *Nature,* **587,** 92–93.

Lee, C., Harvey, J. T., Qin, K., Joshi, V. and Leskovar, D. I. (2024) Exploring the potential of *Solanum pennellii* and *Solanum peruvianum* as rootstocks for enhancing thermotolerance of tomato plants. *Environ Exp Bot,* **221,** 105741.

Lee, D. H., Lee, H. S. and Belkhadir, Y. (2021) Coding of plant immune signals by surface receptors. *Curr Opin Plant Biol,* **62,** 102044.

Li, J. X. and Timko, M. P. (2009) Gene-for-gene resistance in *Striga*-cowpea associations. *Science,* **325,** 1094–1094.

Li, S., Han, X., Yang, L., Deng, X., Wu, H., Zhang, M., *et al.* (2018) Mitogen-activated protein kinases and calcium-dependent protein kinases are involved in wounding-induced ethylene biosynthesis in *Arabidopsis thaliana*. *Plant Cell Environ,* **41,** 134–147.

Li, S., Wang, X. T., Xu, W. Y., Liu, T., Cai, C. M., Chen, L. Y., *et al.* (2021) Unidirectional movement of small RNAs from shoots to roots in interspecific heterografts. *Nat Plants,* **7,** 50–59.

Li, W., Chu, C., Li, H., Zhang, H., Sun, H., Wang, S., *et al.* (2024) Near-gapless and haplotype-resolved apple genomes provide insights into the genetic basis of rootstock-induced dwarfing. *Nat Genet,* **56,** 505–516.

Liu, W., Fan, J., Li, J., Song, Y., Li, Q., Zhang, Y., *et al.* (2014) SCF(SLF)-mediated cytosolic degradation of S-RNase is required for cross-pollen compatibility in S-RNase-based self-incompatibility in *Petunia hybrida*. *Front Genet,* **5,** 228.

Logemann, E., Birkenbihl, R. P., Rawat, V., Schneeberger, K., Schmelzer, E. and Somssich, I. E. (2013) Functional dissection of the PROPEP2 and PROPEP3 promoters reveals the importance of WRKY factors in mediating microbe-associated molecular pattern-induced expression. *New Phytol,* **198,** 1165–1177.

Lori, M., van Verk, M. C., Hander, T., Schatowitz, H., Klauser, D., Flury, P., *et al.* (2015) Evolutionary divergence of the plant elicitor peptides (Peps) and their receptors: interfamily incompatibility of perception but compatibility of downstream signalling. *J Exp Bot,* **66,** 5315–5325.

Louws, F. J., Rivard, C. L. and Kubota, C. (2010) Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Sci Hortic,* **127,** 127–146.

Lu, D. P., Wu, S. J., Gao, X. Q., Zhang, Y. L., Shan, L. B. and He, P. (2010) A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc Natl Acad Sci U S A,* **107,** 496–501.

Luu, D. T., Qin, X., Morse, D. and Cappadocia, M. (2000) S-RNase uptake by compatible pollen tubes in gametophytic self-incompatibility. *Nature,* **407,** 649–651.

Macgregor, S. R., Lee, H. K., Nelles, H., Johnson, D. C., Zhang, T., Ma, C., *et al.* (2022) Autophagy is required for self-incompatible pollen rejection in two transgenic *Arabidopsis thaliana* accessions. *Plant Physiol,* **188,** 2073–2084.

Makechemu, M., Goto, Y., Schmid, M. W., Zbinden, H., Widrig, V., Kaufmann, M., *et al.* (2025) Chitin soil amendment triggers systemic plant disease resistance through enhanced pattern-triggered immunity. *Plant Biotechnol J,* **23,** 5032–5044.

 doi:10.20944/preprints202605.1538.v1

Malamy, J., Carr, J. P., Klessig, D. F. and Raskin, I. (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science,* **250,** 1002–1004.

Mangeon, A., Bell, E. M., Lin, W. C., Jablonska, B. and Springer, P. S. (2011) Misregulation of the LOB domain gene DDA1 suggests possible functions in auxin signalling and photomorphogenesis. *J Exp Bot,* **62,** 221–233.

Matsubayashi, Y. and Sakagami, Y. (1996) Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. *Proc Natl Acad Sci U S A,* **93,** 7623–7627.

Matsuoka, K., Sato, R., Matsukura, Y., Kawajiri, Y., Iino, H., Nozawa, N., *et al.* (2021) Wound-inducible ANAC071 and ANAC096 transcription factors promote cambial cell formation in incised Arabidopsis flowering stems. *Commun Biol,* **4,** 369.

Matsuoka, K., Sugawara, E., Aoki, R., Takuma, K., Terao-Morita, M., Satoh, S., *et al.* (2016) Differential cellular control by cotyledon-derived phytohormones involved in graft reunion of *Arabidopsis* hypocotyls. *Plant Cell Physiol,* **57,** 2620–2631.

Matsuoka, K., Yanagi, R., Yumoto, E., Yokota, T., Yamane, H., Satoh, S., *et al.* (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 Mol Biol,* **96,** 531–542.

Mazumdar, S., Augstein, F., Zhang, A., Musseau, C., Anjam, M. S., Marhavy, P., *et al.* (2025) Damage activates EXG1 and RLP44 to suppress vascular differentiation during regeneration in *Arabidopsis*. *Plant Commun,* **6,** 101256.

McComb, S., Thiriot, A., Akache, B., Krishnan, L. and Stark, F. C. (2026) Introduction to the immune system. *Methods Mol Biol,* **2980,** 1–31.

Melnyk, C. W. (2017) Connecting the plant vasculature to friend or foe. *New Phytol,* **213,** 1611–1617.

Melnyk, C. W., Gabel, A., Hardcastle, T. J., Robinson, S., Miyashima, S., Grosse, I., *et al.* (2018) Transcriptome dynamics at *Arabidopsis* graft junctions reveal an intertissue recognition mechanism that activates vascular regeneration. *Proc Natl Acad Sci U S A,* **115,** E2447–e2456.

Melnyk, C. W., Schuster, C., Leyser, O. and Meyerowitz, E. M. (2015) A developmental framework for graft formation and vascular reconnection in *Arabidopsis thaliana*. *Curr Biol,* **25,** 1306–1318.

Metraux, J. P., Signer, H., Ryals, J., Ward, E., Wyssbenz, M., Gaudin, J., *et al.* (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science,* **250,** 1004–1006.

Miller, R. N., Costa Alves, G. S. and Van Sluys, M. A. (2017) Plant immunity: unravelling the complexity of plant responses to biotic stresses. *Ann Bot,* **119,** 681–687.

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., *et al.* (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proc Natl Acad Sci U S A,* **104,** 19613–19618.

Molnar, A., Melnyk, C. W., Bassett, A., Hardcastle, T. J., Dunn, R. and Baulcombe, D. C. (2010) Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science,* **328,** 872–875.

Mousavi, S. A., Chauvin, A., Pascaud, F., Kellenberger, S. and Farmer, E. E. (2013) GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling. *Nature,* **500,** 422–426.

Mudge, K., Janick, J., Scofield, S. and Goldschmidt, E. E. (2009) A history of grafting. In: *Hortic Rev.* pp. 437–493.

Newman, M. A., Daniels, M. J. and Dow, J. M. (1995) Lipopolysaccharide from *Xanthomonas campestris* induces defense-related gene expression in *Brassica campestris*. *Mol Plant Microbe Interact,* **8,** 778–780.

Nishitani, C., Demura, T. and Fukuda, H. (2002) Analysis of early processes in wound-induced vascular regeneration using *TED3* and *ZeHB3* as molecular markers. *Plant Cell Physiol,* **43,** 79–90.

Notaguchi, M., Kurotani, K. I., Sato, Y., Tabata, R., Kawakatsu, Y., Okayasu, K., *et al.* (2020) Cell-cell adhesion in plant grafting is facilitated by β-1,4-glucanases. *Science,* **369,** 698–702.

Olsen, S., Striberny, B., Hollmann, J., Schwacke, R., Popper, Z. and Krause, K. (2016) Getting ready for host invasion: elevated expression and action of xyloglucan endotransglucosylases/hydrolases in developing haustoria of the holoparasitic angiosperm *Cuscuta*. *J Exp Bot,* **67,** 695–708.

Orozco-Cardenas, M. and Ryan, C. A. (1999) Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. *Proc Natl Acad Sci U S A,* **96,** 6553–6557.

 doi:10.20944/preprints202605.1538.v1

Palauqui, J. C., Elmayan, T., Pollien, J. M. and Vaucheret, H. (1997) Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. *Embo j,* **16,** 4738–4745.

Park, C. J. and Ronald, P. C. (2012) Cleavage and nuclear localization of the rice XA21 immune receptor. *Nat Commun,* **3,** 920.

Pearce, G., Moura, D. S., Stratmann, J. and Ryan, C. A., Jr. (2001) RALF, a 5-kDa ubiquitous polypeptide in plants, arrests root growth and development. *Proc Natl Acad Sci U S A,* **98,** 12843–12847.

Pearce, G., Strydom, D., Johnson, S. and Ryan, C. A. (1991) A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science,* **253,** 895–897.

Peters, N. K., Frost, J. W. and Long, S. R. (1986) A plant flavone, luteolin, induces expression of rhizobium meliloti nodulation genes. *Science,* **233,** 977–980.

Pham, A. Q., Cho, S. H., Nguyen, C. T. and Stacey, G. (2020) *Arabidopsis* lectin receptor kinase P2K2 is a second plant receptor for extracellular ATP and contributes to innate immunity. *Plant Physiol,* **183,** 1364–1375.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M. and Bakker, P. (2014) Induced systemic resistance by beneficial microbes. *Annu Rev Phytopathol,* **52,** 347–375.

Pitaksaringkarn, W., Matsuoka, K., Asahina, M., Miura, K., Sage-Ono, K., Ono, M., *et al.* (2014) XTH20 and XTH19 regulated by ANAC071 under auxin flow are involved in cell proliferation in incised *Arabidopsis* inflorescence stems. *Plant J,* **80,** 604–614.

Ranjan, A., Ichihashi, Y., Farhi, M., Zumstein, K., Townsley, B., David-Schwartz, R., *et al.* (2014) De novo assembly and characterization of the transcriptome of the parasitic weed dodder identifies genes associated with plant parasitism. *Plant Physiol,* **166,** 1186–1199.

Rhodes, J., Yang, H., Moussu, S., Boutrot, F., Santiago, J. and Zipfel, C. (2021) Perception of a divergent family of phytocytokines by the *Arabidopsis* receptor kinase MIK2. *Nat Commun,* **12,** 705.

Runyon, J. B., Mescher, M. C., Felton, G. W. and De Moraes, C. M. (2010) Parasitism by *Cuscuta* pentagona sequentially induces JA and SA defence pathways in tomato. *Plant Cell Environ,* **33,** 290–303.

Samuel, M. A., Chong, Y. T., Haasen, K. E., Aldea-Brydges, M. G., Stone, S. L. and Goring, D. R. (2009) Cellular pathways regulating responses to compatible and self-incompatible pollen in Brassica and Arabidopsis stigmas intersect at Exo70A1, a putative component of the exocyst complex. *Plant Cell,* **21,** 2655–2671.

Sanabria, N. M., Huang, J. C. and Dubery, I. A. (2010) Self/nonself perception in plants in innate immunity and defense. *Self Nonself,* **1,** 40–54.

Sánchez-Pérez, R., Belmonte, F. S., Borch, J., Dicenta, F., Moller, B. L. and Jorgensen, K. (2012) Prunasin hydrolases during fruit development in sweet and bitter almonds. *Plant Physiol,* **158,** 1916–1932.

Sankaranarayanan, S., Jamshed, M. and Samuel, M. (2015) Degradation of glyoxalase I in Brassica napus stigma leads to self-incompatibility response. *Nature Plants,* **1**.

Scandola, S. and Samuel, M. A. (2019) A Flower-Specific Phospholipase D Is a Stigmatic Compatibility Factor Targeted by the Self-Incompatibility Response in Brassica napus. *Current Biology,* **29,** 506–512.e504.

Schandry, N. and Becker, C. (2020) Allelopathic plants: Models for studying plant–interkingdom interactions. *Trends Plant Sci,* **25,** 176–185.

Schnake, A., Hartmann, M., Schreiber, S., Malik, J., Brahmann, L., Yildiz, I., *et al.* (2020) Inducible biosynthesis and immune function of the systemic acquired resistance inducer N-hydroxypipecolic acid in monocotyledonous and dicotyledonous plants. *J Exp Bot,* **71,** 6444–6459.

Sehr, E. M., Agusti, J., Lehner, R., Farmer, E. E., Schwarz, M. and Greb, T. (2010) Analysis of secondary growth in the *Arabidopsis* shoot reveals a positive role of jasmonate signalling in cambium formation. *Plant J,* **63,** 811–822.

Serivichyaswat, P. T., Kareem, A., Feng, M. and Melnyk, C. W. (2024) Auxin signaling in the cambium promotes tissue adhesion and vascular formation during *Arabidopsis* graft healing. *Plant Physiol,* **196,** 754–762.

Shen, G., Zhang, J., Lei, Y., Xu, Y. and Wu, J. (2023) Between-plant signaling. *Annu Rev Plant Biol,* **74,** 367–386.

Shimizu, K., Hozumi, A. and Aoki, K. (2018) Organization of vascular cells in the haustorium of the parasitic flowering plant *Cuscuta japonica*. *Plant Cell Physiol,* **59,** 715–723.

 doi:10.20944/preprints202605.1538.v1

Si, T., Yang, L., Lu, J., Lin, Y., Yu, X., Zhang, X., *et al.* (2025) Application of root exudates derived from peanut/maize intercropping system promotes peanut growth and yield via modulating nitrogen turnover processes. *BMC Plant Biol,* **25,** 977.

Sicard, A., Kappel, C., Josephs, E. B., Lee, Y. W., Marona, C., Stinchcombe, J. R., *et al.* (2015) Divergent sorting of a balanced ancestral polymorphism underlies the establishment of gene-flow barriers in *Capsella*. *Nat Commun,* **6**.

Song, W. Y., Wang, G. L., Chen, L. L., Kim, H. S., Pi, L. Y., Holsten, T., *et al.* (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science,* **270,** 1804–1806.

Sorty, A. M., Kudjordjie, E. N., Meena, K. K., Nicolaisen, M. and Stougaard, P. (2025) Plant root exudates: Advances in belowground signaling networks, resilience, and ecosystem functioning for sustainable agriculture. *Plant Stress,* **17**.

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

Stegmann, M., Monaghan, J., Smakowska-Luzan, E., Rovenich, H., Lehner, A., Holton, N., *et al.* (2017) The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science,* **355,** 287–289.

Steinauer, K., Chatzinotas, A. and Eisenhauer, N. (2016) Root exudate cocktails: the link between plant diversity and soil microorganisms? *Ecol Evol,* **6,** 7387–7396.

Stirnemann, E. M. and Sasse, J. (2025) How to harness the effects of exudates and microbes that support beneficial plant-plant interactions for sustainable agriculture. *PLOS Biol,* **23**.

Stringlis, I. A., Yu, K., Feussner, K., de Jonge, R., van Bentum, S., Van Verk, M. C., *et al.* (2018) MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc Natl Acad Sci U S A,* **115,** E5213–E5222.

Su, C., Liu, H., Wafula, E. K., Honaas, L., de Pamphilis, C. W. and Timko, M. P. (2020) SHR4z, a novel decoy effector from the haustorium of the parasitic weed *Striga gesnerioides*, suppresses host plant immunity. *New Phytol,* **226,** 891–908.

Takasaki, T., Hatakeyama, K., Suzuki, G., Watanabe, M., Isogai, A. and Hinata, K. (2000) The S receptor kinase determines self-incompatibility in *Brassica stigma*. *Nature,* **403,** 913–916.

Takayama, S. and Isogai, A. (2005) SELF-INCOMPATIBILITY IN PLANTS. *Annual Review of Plant Biology,* **56,** 467–489.

Takayama, S., Shiba, H., Iwano, M., Shimosato, H., Che, F. S., Kai, N., *et al.* (2000) The pollen determinant of self-incompatibility in *Brassica campestris*. *Proc Natl Acad Sci U S A,* **97,** 1920–1925.

Tan, T. T., Endo, H., Sano, R., Kurata, T., Yamaguchi, M., Ohtani, M., *et al.* (2018) Transcription factors VND1-VND3 contribute to cotyledon xylem vessel formation. *Plant Physiol,* **176,** 773–789.

Tanaka, K., Choi, J., Cao, Y. and Stacey, G. (2014) Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. *Front Plant Sci,* **5,** 446.

Tanaka, K. and Heil, M. (2021) Damage-associated molecular patterns (DAMPs) in plant innate immunity: Applying the danger model and evolutionary perspectives. *Annu Rev Phytopathol,* **59,** 53–75.

Thieme, C. J., Rojas-Triana, M., Stecyk, E., Schudoma, C., Zhang, W. N., Yang, L., *et al.* (2015) Endogenous *Arabidopsis* messenger RNAs transported to distant tissues. *Nat Plants,* **1**.

Thomas, H. R., Gevorgyan, A. and Frank, M. H. (2023) Anatomical and biophysical basis for graft incompatibility within the *Solanaceae*. *J Exp Bot,* **74,** 4461–4470.

Thomas, H. R., Gevorgyan, A., Hermanson, A., Yanders, S., Erndwein, L., Norman-Ariztía, M., *et al.* (2024) Graft incompatibility between pepper and tomato elicits an immune response and triggers localized cell death. *Hortic Res,* **11,** uhae255.

Thomas, H. R., Van den Broeck, L., Spurney, R., Sozzani, R. and Frank, M. (2022) Gene regulatory networks for compatible versus incompatible grafts identify a role for SlWOX4 during junction formation. *Plant Cell,* **34,** 535–556.

Thomma, B. P., Cammue, B. P. and Thevissen, K. (2002) Plant defensins. *Planta,* **216,** 193–202.

Timko., M. P., Gowda., B. S., Ouedraogo., J. and Ousmane, B. (2007) *Integrating new technologies for Striga control*. World Scientific, Singapore.

 doi:10.20944/preprints202605.1538.v1

Toh, S., Inoue, S., Toda, Y., Yuki, T., Suzuki, K., Hamamoto, S., *et al.* (2018) Identification and characterization of compounds that affect stomatal movements. *Plant Cell Physiol,* **59,** 1568–1580.

Tomaz, Z. F. P., Rodrigues, A. C., Veríssimo, V., Marafon, A. C., Herter, F. G. and Rufato, A. D. (2009) Compatibilidade de enxertia de cultivares de marmeleiros compereiras. *Rev Bras Frutic,* **31,** 1211–1217.

Tomilov, A. A., Tomilova, N. B., Abdallah, I. and Yoder, J. I. (2005) Localized hormone fluxes and early haustorium development in the hemiparasitic plant *Triphysaria versicolor*. *Plant Physiol,* **138,** 1469–1480.

Toyota, M., Spencer, D., Sawai-Toyota, S., Jiaqi, W., Zhang, T., Koo, A. J., *et al.* (2018) Glutamate triggers long-distance, calcium-based plant defense signaling. *Science,* **361,** 1112–1115.

Umemoto, N., Kakitani, M., Iwamatsu, A., Yoshikawa, M., Yamaoka, N. and Ishida, I. (1997) The structure and function of a soybean beta-glucan-elicitor-binding protein. *Proc Natl Acad Sci U S A,* **94,** 1029–1034.

Venema, J. H., Dijk, B. E., Bax, J. M., van Hasselt, P. R. and Elzenga, J. T. M. (2008) Grafting tomato (*Solanum lycopersicum*) onto the rootstock of a high-altitude accession of *Solanum habrochaites* improves suboptimal-temperature tolerance. *Environ Exp Bot,* **63,** 359–367.

Wakatake, T., Yoshida, S. and Shirasu, K. (2018) Induced cell fate transitions at multiple cell layers configure haustorium development in parasitic plants. *Development,* **145**.

Walters, D. R., Ratsep, J. and Havis, N. D. (2013) Controlling crop diseases using induced resistance: challenges for the future. *J Exp Bot,* **64,** 1263–1280.

Wang, D., Wei, L., Liu, T., Ma, J., Huang, K., Guo, H., *et al.* (2023) Suppression of ETI by PTI priming to balance plant growth and defense through an MPK3/MPK6-WRKYs-PP2Cs module. *Mol Plant,* **16,** 903–918.

Wang, G., Hu, C., Zhou, J., Liu, Y., Cai, J., Pan, C., *et al.* (2019) Systemic root-shoot signaling drives Jasmonate-based root defense against *Nematodes*. *Curr Biol,* **29,** 3430–3438.e3434.

Wang, J., Jin, Z., Yin, H., Yan, B., Ren, Z., Xu, J., *et al.* (2014) Auxin redistribution and shifts in PIN gene expression during *Arabidopsis* grafting. *Russ J Plant Physiol,* **61,** 688–696.

Wang, L., Einig, E., Almeida-Trapp, M., Albert, M., Fliegmann, J., Mithöfer, A., *et al.* (2018) The systemin receptor SYR1 enhances resistance of tomato against herbivorous insects. *Nat Plants,* **4,** 152–156.

Wang, L., Lin, Z., Carli, J., Gladala-Kostarz, A., Davies, J., Franklin-Tong, V., *et al.* (2022) ATP depletion plays a pivotal role in self-incompatibility, revealing a link between cellular energy status, cytosolic acidification and actin remodelling in pollen tubes. *New Phytologist,* **236,** 1691–1707.

Weiberg, A., Wang, M., Lin, F. M., Zhao, H. W., Zhang, Z. H., Kaloshian, I., *et al.* (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science,* **342,** 118–123.

Wheeler, M. J., de Graaf, B. H., Hadjiosif, N., Perry, R. M., Poulter, N. S., Osman, K., *et al.* (2009) Identification of the pollen self-incompatibility determinant in *Papaver rhoeas*. *Nature,* **459,** 992–995.

Wheeler, M. J., Vatovec, S. and Franklin-Tong, V. E. (2010) The pollen S-determinant in Papaver: comparisons with known plant receptors and protein ligand partners. *J Exp Bot,* **61,** 2015–2025.

Williams, B., Ahsan, M. U. and Frank, M. H. (2021) Getting to the root of grafting-induced traits. *Curr Opin Plant Biol,* **59**.

Wu, Y., Gao, Y., Zhan, Y., Kui, H., Liu, H., Yan, L., *et al.* (2020) Loss of the common immune coreceptor BAK1 leads to NLR-dependent cell death. *Proc Natl Acad Sci U S A,* **117,** 27044–27053.

Xiong, M., Zhang, T., Qian, X., Kadeer, A., Kurotani, K. I., Li, L., *et al.* (2026) Xyloglucan endotransglucosylase/hydrolase family genes are required for the plant graft union formation through callus proliferation. *Plant Physiol*.

Yamada, T. and Marubashi, W. (2003) Overproduced ethylene causes programmed cell death leading to temperature-sensitive lethality in hybrid seedlings from the cross *Nicotiana suaveolens* x *N-tabacum*. *Planta,* **217,** 690–698.

Yamaguchi, Y., Huffaker, A., Bryan, A. C., Tax, F. E. and Ryan, C. A. (2010) PEPR2 is a second receptor for the Pep1 and Pep2 peptides and contributes to defense responses in *Arabidopsis*. *Plant Cell,* **22,** 508–522.

Yamaguchi, Y., Pearce, G. and Ryan, C. A. (2006) The cell surface leucine-rich repeat receptor for AtPep1, an endogenous peptide elicitor in *Arabidopsis*, is functional in transgenic tobacco cells. *Proc Natl Acad Sci U S A,* **103,** 10104–10109.

 doi:10.20944/preprints202605.1538.v1

Yamamoto, E., Takashi, T., Morinaka, Y., Lin, S. Y., Wu, J. Z., Matsumoto, T., *et al.* (2010) Gain of deleterious function causes an autoimmune response and Bateson-Dobzhansky-Muller incompatibility in rice. *Mol Genet Genomics,* **283,** 305–315.

Yang, H., Matsubayashi, Y., Hanai, H. and Sakagami, Y. (2000) Phytosulfokine-alpha, a peptide growth factor found in higher plants: its structure, functions, precursor and receptors. *Plant Cell Physiol,* **41,** 825–830.

Yang, L., Machin, F., Wang, S. F., Saplaoura, E. and Kragler, F. (2023) Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks. *Nat Biotechnol,* **41,** 958–967.

Yang, Z., Wafula, E. K., Honaas, L. A., Zhang, H., Das, M., Fernandez-Aparicio, M., *et al.* (2015) Comparative transcriptome analyses reveal core parasitism genes and suggest gene duplication and repurposing as sources of structural novelty. *Mol Biol Evol,* **32,** 767–790.

Yu, X. Q., Niu, H. Q., Liu, C., Wang, H. L., Yin, W. and Xia, X. (2024) PTI-ETI synergistic signal mechanisms in plant immunity. *Plant Biotechnol J,* **22,** 2113–2128.

Zeidler, D., Dubery, I. A., Schmitt-Kopplin, P., Von Rad, U. and Durner, J. (2010) Lipopolysaccharide mobility in leaf tissue of *Arabidopsis thaliana*. *Mol Plant Pathol,* **11,** 747–755.

Zhai, L., Wang, X., Tang, D., Qi, Q., Yer, H., Jiang, X., *et al.* (2021) Molecular and physiological characterization of the effects of auxin-enriched rootstock on grafting. *Hortic Res,* **8,** 74.

Zhan, C., Xue, N., Su, Z., Zheng, T. and Wu, J. (2025) RBOHD, GLR3.3, and GLR3.6 cooperatively control wounding hypocotyl-induced systemic Ca^{2+} signals, jasmonic acid, and glucosinolates in *Arabidopsis* leaves. *Plant Divers,* **47,** 690–701.

Zhang, A., Matsuoka, K., Kareem, A., Robert, M., Roszak, P., Blob, B., *et al.* (2022) Cell-wall damage activates DOF transcription factors to promote wound healing and tissue regeneration in *Arabidopsis thaliana*. *Curr Biol,* **32,** 1883–1894.e1887.

Zhang, A. J., Wang, T. J., Yuan, L., Shen, Y. X., Liu, K., Liu, B., *et al.* (2024) Horizontal transfer of plasmid-like extrachromosomal circular DNAs across graft junctions in *Solanaceae*. *Mol Genet Genomics,* **4**.

Zhang, H., Hu, Z., Lei, C., Zheng, C., Wang, J., Shao, S., *et al.* (2018) A plant phytosulfokine peptide initiates auxin-dependent immunity through cytosolic Ca^{2+} signaling in tomato. *Plant Cell,* **30,** 652–667.

Zhang, W. N., Kollwig, G., Stecyk, E., Apelt, F., Dirks, R. and Kragler, F. (2014) Graft-transmissible movement of inverted-repeat-induced siRNA signals into flowers. *Plant J,* **80,** 106–121.

Zhang, Y. X., Xu, S. H., Ding, P. T., Wang, D. M., Cheng, Y. T., He, J., *et al.* (2010) Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *Proc Natl Acad Sci U S A,* **107,** 18220–18225.