



Getting to the root of grafting-induced traits

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Grafting is an ancient technique that involves the physical joining of genotypically distinct shoot and root systems, in order to achieve a desirable compound plant. This practice is widely used in modern agriculture to improve biotic and abiotic stress tolerance, modify plant architecture, induce precocious flowering and rejuvenate old perennial varieties, boost yield, and more. Beneficial new rootstock-scion combinations are currently identified through an inefficient trial and error process, which presents a significant bottleneck for the application of grafting to combat new environmental challenges. Identifying the mechanisms that underlie beneficial grafting-induced traits will facilitate rapid breeding and genetic engineering of new rootstock x scion combinations that exhibit superior performance across varying agricultural environments.

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Current Opinion in Plant Biology 2021, 59:101988

This review comes from a themed issue on **Growth and development**

Edited by **Courtney Hollender** and **Zhongchi Liu**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 31st December 2020

<https://doi.org/10.1016/j.pbi.2020.101988>

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Introduction

Farmers cultivate plants under a tremendous variety of environmental conditions, each of which presents a unique suite of abiotic and biotic challenges. Grafting, the process in which the shoot ('scion') of one plant is combined with the root system ('rootstock') of another plant, is a powerful tool that can be used to create a dual plant system that rapidly addresses these challenges. Historically, grafting was predominantly used to protect susceptible varieties from soil-borne pathogens. Over the past 100 years there has been a substantial expansion in grafting applications for both annual and perennial crop improvement. While this is by no means an exhaustive list, we wanted to provide the reader with a brief snapshot into the diversity of grafted crops. Fruit and nut trees are routinely grafted to promote abiotic and biotic

stress tolerance as well as enhance harvesting efficiency through modified shoot architecture (e.g. apples, pears, cherries, grapes, citrus, coffee, cacao, durian, papaya, mango, avocado, walnut, hazelnuts, and olives [1]), woody ornamentals are often propagated and sculpted through grafting (e.g. ash, beech, birch, camellia, weeping cedars, dogwoods, fir, hawthorn, pines, and maples [2]), and vegetable crops, particularly from the Cucurbitaceae and Solanaceae families, are routinely grafted for increased yield and enhanced stress tolerance [3]. Our ability to improve and expand on grafting based applications is largely hindered by a lack of knowledge regarding the fundamental properties that underlie successful graft combinations. In this review, we work to bridge this knowledge gap by identifying unifying developmental principles that can be applied to systematically study, breed for, and build grafting-induced phenotypes.

Grafting to enhance crop performance through graft 'ultra-compatibility'

Graft compatibility versus incompatibility is a concept that broadly applies across all grafting contexts. These terms refer to the capacity for rootstock and scion genotypes to heal together, forming new vascular bridges that span the graft junction [4]. In this review, we introduce another universal concept called graft 'ultra-compatibility' (UC). We use this term to refer to grafted plants that exhibit superior traits in comparison with their independent rootstock and scion genotypes. One logical framework for dissecting the mechanisms that underlie UC grafts is to break down the expression of beneficial traits based on where they occur within the grafted organism. Autonomous and non-autonomous assignment of gene function is a classic framework applied to genetic mosaics [5], wherein genes with autonomous function directly influence the phenotype of the cells in which they are expressed and genes with non-autonomous functions influence cellular phenotypes beyond the cellular context of expression, implying the presence of a mobile signal. Likewise, autonomous UC traits in grafting are isolated to the organ systems in which they are expressed, and non-autonomous traits are transmitted across the graft junction, influencing the reciprocal organ system. A generic example of autonomous UC that is recapitulated in many agricultural contexts is the grafting of elite scion cultivars onto wild rootstocks in order to achieve a dual plant system that exhibits both elite fruit traits in the scion and superior stress tolerance in the rootstock. There are numerous agricultural applications for autonomous UC, we list a handful here: grafting for enhanced salinity

tolerance [6–8], adaptation to flooding [9] and drought stress [10–14], growth in contaminated soils [15–17], nutrient deficient soils [18], and toxic nutrient soils [19], as well as grafting for localized biotic resistance to a broad range of soilborne pathogens [20] (for example, in tomato, elite scions are commonly grafted onto *Ralstonia solanacearum* resistant rootstocks to protect plants from bacterial wilt [21**]). In all of these examples, the superior rootstock and scion traits are expressed within separate root and shoot organ systems, respectively. Many of these autonomous traits may be directly linked to the architecture of the adaptive rootstocks [22], rootstock-mediated modifications to the rhizosphere and endosphere microbiomes [23–25], and localized, genotype-specific adaptations for disease resistance [26] and ion uptake [27]. The extent to which beneficial autonomous traits can be translated across diverse grafted species is largely dependent on the degree to which these mechanisms for abiotic and biotic stress tolerance are conserved across species.

Non-autonomous traits, on the other hand, are more complicated to track on a mechanistic level, as they imply the putative existence of an underlying mobile signal. Furthermore, UC non-autonomous traits often arise as a synergistic interaction between specific genotypic rootstocks x scion combinations, similar to heterotic interactions in hybrid breeding [28]; however, rather than crossing two genotypes together, the genomes are surgically united in a single organism through grafting. Here, we delve into a few examples of non-autonomous UC traits and current progress on understanding their underlying mechanisms.

Grafting-induced modifications to shoot architecture is a prevalent non-autonomous UC trait that is sought out in both perennial and vegetable cropping systems. In some perennial fruit crops such as apples, pears, and mangoes, dwarfing rootstocks are used to condense plant stature in order to promote harvesting efficiency and increase planting density. The genetic basis for rootstock-induced dwarfing is starting to take shape thanks to recent molecular and physiological studies in apple. A comparative transcriptomics and targeted metabolomics approach between two dwarfing rootstocks ('M27' and 'M9') and a vigorous rootstock ('M793') indicate that these dwarfing rootstocks overaccumulate starch, inducing a perpetual state of sugar starvation in dwarfed trees [29]. Work on a different, semi-dwarfing rootstock ('M26') points to a brassinosteroid (BR)-based mechanism for altered architecture, wherein M26 rootstocks overexpress MdWRK9, an inhibitor of BR synthetase, which ultimately leads to reduced BR accumulation. Impressively, the dwarfing phenotype can be reversed following exogenous brassinolide application [30**].

In contrast to perennial fruit trees, grafting is used to invigorate vegetative and reproductive growth in

vegetable crop scions, which ultimately leads to significant boosts in yield. In tomato, for example, a popular invigorating rootstock called 'Maxifort' can, in some cases, double scion yield [31]. Precisely how grafting-induced vigor works is an active area of investigation. Evidence for a potential phytohormone-based method comes from a study where genetically engineered rootstocks overexpressing the cytokinin biosynthesis enzyme, IPT, exhibit significantly higher yields than wildtype controls, under suboptimal conditions [32]. Two recent publications point towards a different, epigenomic-based mechanism for grafting-induced vigor [33,34**]. A new study in eggplant demonstrates that grafting with invigorating hybrid rootstocks leads to hypomethylation, particularly around LTR-retrotransposons in the scion [33]. At this point it is unclear whether these marks are correlatively or causatively linked to grafting-induced vigor. While agricultural grafting-induced vigor results in within-generation yield boosts that are not thought to be heritable, there is a new, intriguing study showing that heritable, second generation invigorating phenotypes can be induced by grafting wildtype scions onto msh1 (DNA mismatch repair protein1) mutant rootstocks [34**]. Again, an epigenomic mechanism that is likely controlled by mobile short-interfering RNAs (siRNAs) is implicated in this grafting-induced vigor phenotype, as rootstocks carrying mutations for msh1 and siRNA biogenesis machinery fail to transmit heritable vigor into scion progeny [34**].

Another fascinating goal of UC grafting is rootstock-induced alterations to scion maturity. Older scions from long-lived perennials can be grafted onto young rootstocks to rejuvenate growth, restore juvenile features, and enhance successful scion propagation [35–37]. The molecular and physiological mechanisms underlying graft-induced rejuvenation are not well studied; however work in apple, juglans, and pinus indicate that rejuvenation is associated with increased auxin and in some cases, decreased abscisic acid levels [38–40]. Further work from Arabidopsis demonstrates that induced expression of microRNA156, a microRNA that promotes vegetative juvenility, is able to restore some juvenile features in adult plants [41]. For long-lived perennials, the reverse grafting-induced effect is often desired. Long periods of juvenility can be circumvented by grafting young scions onto mature rootstocks that induce precocious flowering [42–44]. Again, the mechanisms underlying this grafting-induced process in non-transgenic cropping systems remain unresolved at the molecular level. A recent study in avocado demonstrated that although rootstocks can influence scion precocity, the expression of age-related molecular markers, including microRNA156, is largely controlled by the scion, not the rootstock [45]. Other studies investigating the interaction between dwarfing rootstocks and flowering time in apple indicate that dwarfing rootstocks (e.g. the

commonly used rootstock, ‘M9’) may induce early flowering by altering carbohydrate balance, which supports a physiological mechanism for grafting-induced precocity [46]. An alternative approach, involving the transgenic expression of the mobile flower-promoting hormone FLOWERING LOCUS-T (FT) demonstrates that overexpression of FT in blueberry and jatropha rootstocks is sufficient to induce precocious flowering in the scion [47^{••},48]. We will return to the topic of FT in the molecular signaling section, below.

An additional example of non-autonomous UC grafting that remains contentious, is whether grafting can be used to confer disease resistance and/or enhanced tolerance in the reciprocal half of the plant. One case study that supports the potential for non-autonomous disease resistance through grafting is in apple where particular rootstock cultivars have been shown to increase scion tolerance to specific strains of *Erwinia amylovora*, the causative agent of fire blight [49–51]. The precise mechanism for how this grafting-enhanced resistance works is unclear; however, a recent study demonstrated that there is a positive correlation between rootstock biomass and scion resistance to *E. amylovora* [52^{••}], indicating that root system vigor may be involved in enhanced graft-induced disease resistance.

Finally, there are complex traits that elude unique autonomous or non-autonomous assignments because the adaptive trait may function solely in the scion, solely in the rootstock, or in both halves of the plant. An example of this problem includes grafting-induced temperature tolerance [53–56]. Controlled soil versus aerial temperature treatments would be necessary to determine where these complex, grafting-induced traits are expressed. Nevertheless, the majority of UC grafting traits can be broken down into autonomous and non-autonomous functions, and this systematic categorization is a useful step towards identifying the mechanisms that underlie beneficial graft combinations.

Mobile molecular signals that underlie non-autonomous traits

In the context of genetic mosaics, non-autonomous phenotypes imply the involvement of mobile signals [5]. Likewise, non-autonomous grafting-induced traits may involve underlying root-to-shoot signals that impact scion phenotypes. While we have a rather extensive knowledge of the mobile RNAs, proteins, and hormones that travel between grafted rootstocks and scions, very few of these mobile molecules have been directly implicated in grafting-induced traits in an agricultural context [57–63]. One exception that has gained considerable traction in perennial crop production, is the overexpression of Flowering Locus T (FT) to promote precocious flowering in woody plants [47^{••},64–66,67^{••}]; however, this approach yields variable success when FT overexpression is restricted

to rootstocks. Indeed, only a small subset of species with engineered FT overexpression (for example blueberry and Jatropha) have been shown in the published literature to accelerate reproductive development when the transgenic line is used solely as the rootstock [47^{••},48], while other overexpression lines, for example in apple, fail to transmit precocious flowering phenotypes from the rootstock into the scion [68^{••}].

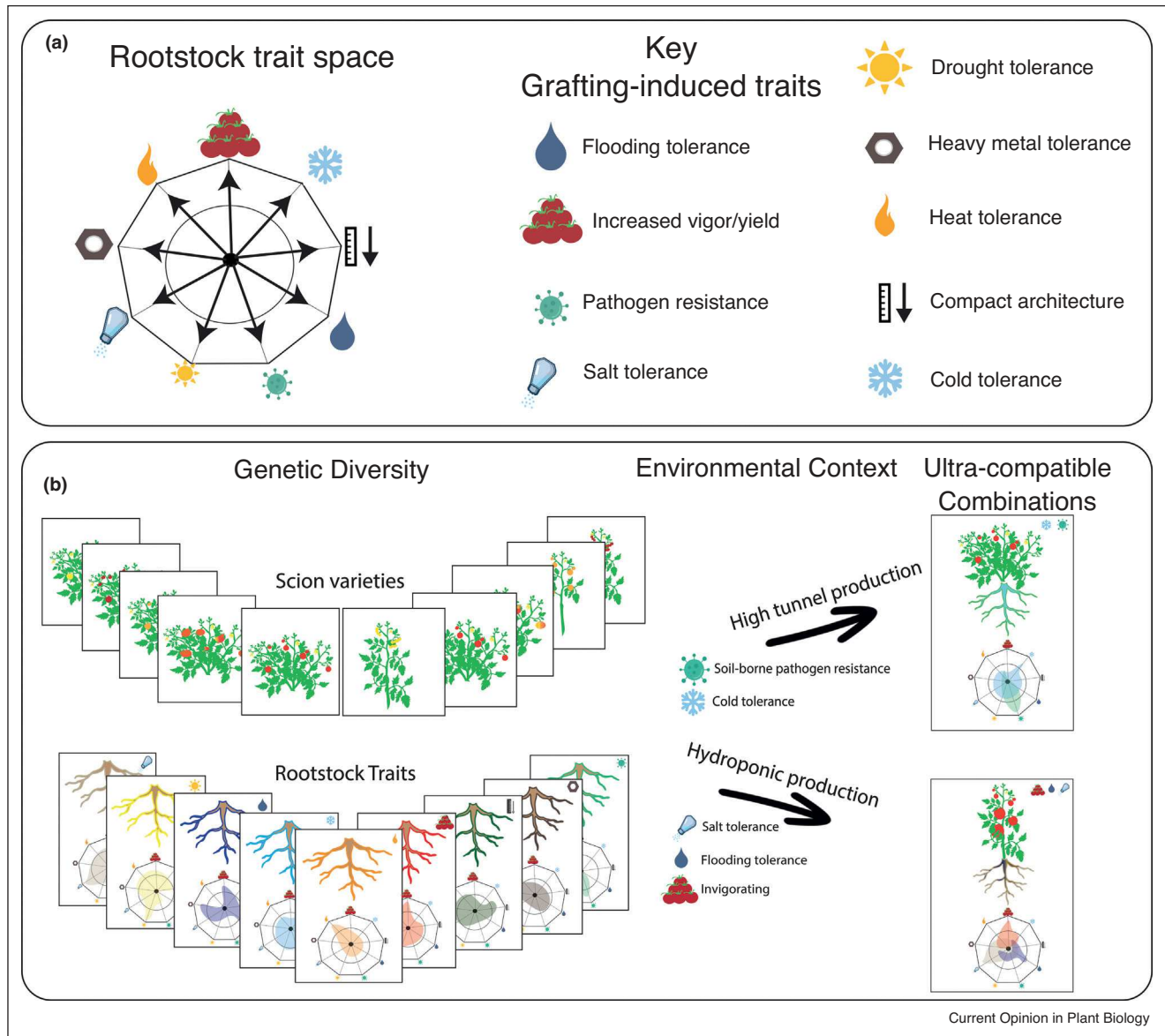
Understanding and circumventing this road block for delivering rootstock-expressed transgenic traits could facilitate the adoption of genetic engineering in perennial crop production. Notably, whether the fruit harvested from a non-transgenic scion grafted onto a genetically engineered rootstock is considered genetically modified falls into a gray area when it comes to regulations and marketing, and thus presents a potential alternative to directly modifying scion varieties [67^{••}]. Another, recent development in graft-mobile signaling involves the graft delivery of mobilized CRISPR guide RNAs from transgenic CRISPR donor lines into non-transgenic editing lines, offering the potential to edit the scion germline without the introduction of a transgene [69^{••}]. Perhaps it is not surprising to point out that these guide RNAs are mobilized through the addition of native FT sequences [69^{••}]. These exciting advancements in graft-donor molecular biology opens up tremendous flexibility for engineering new ultra-compatible rootstock x scion combinations that can address the challenges of our changing environment.

Challenges and opportunities for discovering new rootstocks

Agricultural challenges vary from one environment to the next. For example, high-tunnel production systems (similar to greenhouses placed over open ground) offer an affordable option for buffering vegetable crops from cold aerial temperatures, effectively extending the growing season by several months [70]. Some of the unique challenges presented in high tunnel systems include suboptimal ground temperatures since the high-tunnel only buffers aerial conditions, nutrient leaching from the soils, and increased soil pathogen load, due to repeated production cycles in the same earth. High tunnel growers would benefit from grafting to rootstocks that confer below ground cold tolerance, offer protection from soil-borne pathogens, and maximize output by increasing yield (Figure 1); however, the majority of commercially available rootstocks are optimized for controlled, soilless environments. These contextual challenges coupled with the additional variability in water availability, temperature, and stress brought on by climate change, presents an immediate need for expanded rootstock options for rapid environmental adaptation.

Our ability to introduce novel, UC graft combinations has been fueled by active rootstock breeding programs that have

Figure 1



Accelerating ultra-compatible graft selection by cataloging the rootstock trait space.

Identifying the fundamental mechanisms that underlie beneficial grafts will accelerate the application of new graft combinations in agriculture.

Here, we provide an example in tomato where rootstock traits can be catalogued along a 2-dimensional grid quantifying beneficial trait space (a), which can be systematically combined with selected scion cultivars to produce optimal performance in distinct environmental contexts (b).

transformed the way in which woody perennials and vegetable crops are cultivated around the globe. However, growers are currently limited by the availability of rootstocks that can be used to address their unique environmental challenges, and the performance of a given rootstock has been shown to vary based on both rootstock x scion combinations and environmental context [71]. Unlike standard breeding programs that are aimed at identifying individual genotypes that exhibit optimal performance within particular environmental contexts, rootstock breeding requires an evaluation of genome-by-genome interactions, in the form of

rootstock x scion combinations. This interactive breeding equation can quickly approach what is known as a combinatorial explosion, where a problem rapidly grows in complexity due to the addition of input variables. This rootstock breeding problem is further compounded by the slow generation time of perennial crops that are typically grafted, which can add decades to the rootstock selection process [72]. All of these issues taken together results in a time consuming, laborious, and expensive breeding process that presents a significant bottleneck when it comes to adopting novel graft combinations in agriculture.

Here, we envision the development of a workflow for the identification and documentation of high-performing rootstocks and ultra-compatible graft combinations. In this systematic approach, the base stats and performance of rootstocks for specific traits would be measured and scored on a quantitative scale. This data can be visualized utilizing a ‘rootstock trait space’ radar plot system, which is a useful way of comparing multiple quantitative variables (Figure 1a). Genotypes with high-performance traits can then be systematically stacked using traditional breeding, in order to address and target specific environmental challenges. Finally, non-autonomous UC traits can be incorporated into the breeding process by extensively testing rootstock x scion combinations across diverse germplasm (Figure 1b).

Conclusion

Grafting is a technique that has been around for over a millennium [73]. It has found its place in modern agriculture for its utility in conferring rapid adaptation to biotic and abiotic stresses, boosting yield, and ensuring that farmers are obtaining maximum output from the crops that they plant. Despite the widespread utility of grafting, the identification of new rootstock-scion combinations is limited by our lack of knowledge regarding the fundamental mechanisms that underlie ultra-compatible grafting-induced traits. Breaking these beneficial traits into an autonomous versus non-autonomous framework and pinning down the potential long-distance signals that facilitate non-autonomous traits will allow us to move rootstock breeding out of the black box, and access predictive rootstock x scion combinations that help sustain and improve agricultural output.

Acknowledgements

We are grateful to two anonymous reviewers whose constructive comments improved this manuscript. Funding for related work on tomato grafting in the Frank Lab is supported by a grant from the USDA-NIFA Federal Capacity Fund: 1021188 and an NSF CAREER award (IOS-1942437).

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Declaration of Competing Interest

The authors report no declarations of interest.

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