

# Making the connection: Gene regulatory networks reveal a new regulator of graft formation

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In Brief

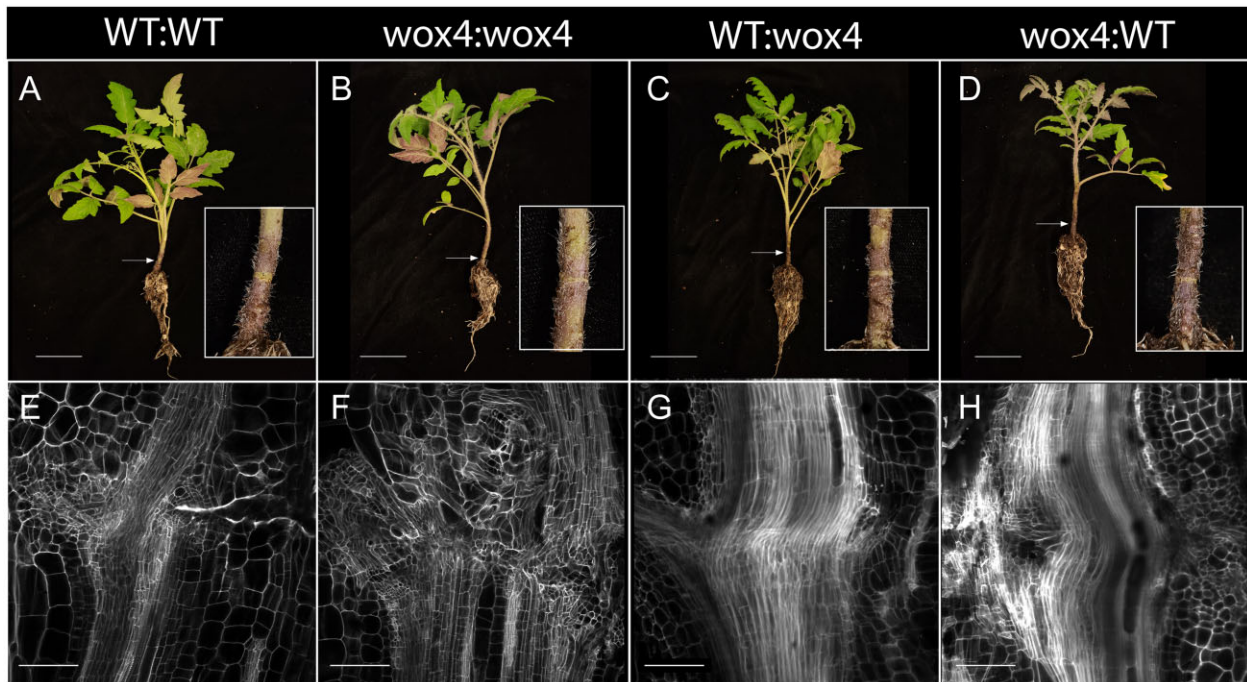
Grafting is a vegetative propagation technique wherein the root system of one species (stock) is joined with the shoot (scion) of another species to form a new plant variety. Successful grafting involves the joining of both vascular (xylem and phloem) and non-vascular tissues (epidermis, cortex/pith) between the stock and scion. Grafting is generally used to develop plant varieties that are resistant to diseases, abiotic stresses, and for desired plant stature (Willingham et al., 2001; Sánchez-Rodríguez et al., 2012). Generally, grafts tend to be more compatible between species of the same genus than between more distantly related species (Goldschmidt, 2014). In certain cases, an incompatible graft can survive for months without establishing a proper vascular connection, called delayed graft incompatibility. Delayed graft incompatibility causes economic burden and time loss to farmers and commercial growers. Hence, it is important to know the molecular events that occur during delayed graft incompatibility.

In this issue of *The Plant Cell*, **Hannah Thomas, Lisa Van den Broeck, and colleagues** (Thomas et al., 2021) inferred gene regulatory networks (GRNs) for compatible versus incompatible grafts using tomato (*Solanum lycopersicum* var. M82) and pepper (*Capsicum annum* var. Big Dipper) plants correlated with major anatomical changes during grafting. The authors established that self-grafted tomato or pepper plants exhibited 100% survival and 0–6% fragility across the graft junctions. By contrast, hetero-grafted pepper:tomato (scion:stock) and tomato:pepper plants exhibited 75% and 37% survival at 30 days after grafting (DAG), respectively, and the majority of the heterografts broke in the fragility test. Anatomical observations at 30 DAG showed a successful vascular connection in self-grafted plants and discontinuous vascular connection consistent with delayed graft incompatibility in heterografted plants.

To understand delayed graft incompatibility, the authors constructed an anatomical timeline for the compatible self-grafts and delayed graft incompatibility heterografts. This timeline showed that self-grafted plants developed callus around the stem periphery at the graft junction by 3 DAG, which eventually differentiated into xylem between 3 and 6 DAG. However, the heterografted plants developed callus and differentiated into xylem only at the tomato half of junction suggesting that the pepper half stalled in establishing a vascular connection even at 6 DAG.

To identify the genes involved in a successful vascular connection at the graft junction, the authors performed RNA sequencing with graft junction tissue at 1, 3, and 5 DAG for both self- and hetero-grafts and generated GRNs. Comparing the GRNs between the self-grafted tomato and pepper plants revealed that these two plants use a distinct gene network for the successful graft connection, even though these networks converge to similar downstream genes. To further identify the shared genetic components between the self- and hetero-grafted plants, the authors identified orthologs of graft-related transcription factors from Arabidopsis in both tomato and pepper and observed that their dynamics are perturbed in heterografts compared to self-grafted plants.

Based on the observation that some of the common transcription factors between the self- and hetero-grafted plants are regulated by *WUSCHEL RELATED HOMEODOMAIN 4* (*WOX4*), a gene involved in procambial specification, the authors studied *SIWOX4* and *CaWOX4* in more detail. While the expression of the *WOX4* orthologs in self-grafted plants shows a steady increase, their expression in heterografts is disrupted, suggesting that *WOX4* plays a crucial role in establishing the vascular connection. To test this, the authors obtained a



**Figure** *WOX4* is essential for graft compatibility. A–D, Representative images for the self- (A and B) and hetero-grafts (C and D) between the WT and *wox4* mutant. E–H, Representative anatomical sections through the graft junction for the graft combinations shown above at 30 DAG showing a discontinuous vascular connection in *wox4* self-grafts (F) and a continuous vascular connection in WT self-grafts (A) and WT and *wox4* hetero-grafts (C and D). Reprinted from [Thomas et al. \(2021\)](#), Figure 7.

CRISPR-Cas9 mutant of tomato *WOX4* and made self- and hetero-grafts with wild-type (WT) plants. While *Slwox4* self-grafts failed to form a successful graft junction, heterografts between *Slwox4*:WT and WT:*Slwox4* succeeded in establishing vascular connections, suggesting a requirement for *WOX4* in at least one of the graft partners (see [Figure](#)). It will be interesting to determine if *WOX4* homologs play a key role in successful graft formation in other species.

In a nutshell, this study shows that delayed graft incompatibility may be related to distinct GRNs employed by incompatible species in the graft junction connection. As pointed out by the authors, the observation that grafting is not controlled by a genetically conserved regulatory pathway is consistent with the fact that grafting is a human intervention exploiting plants' innate ability to regenerate after healing. Thus, it follows that certain components

related to the wound response and regeneration may be conserved, but regulation of a successful graft junction may be species-specific.

## References

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