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Wound-Induced Rooting in Plants—A big BIG ROle Emerges for Calcium and Auxin

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Commentary

As a graduate student, I would root a supermarket-bought basil plant in water to propagate it in order to satisfy my herb-craving needs. In fact, many plants are capable of rooting in water since, unlike animals, they are capable of (re)gaining pluripotency under the right conditions to recover from loss or damage (Ikeuchi et al. 2019). In this way, plants can grow new tissues or organs in response to different environmental and developmental cues due to their indeterminate growth.

Roots are a key innovation of plants, as they enable sufficient adaptation to different environmental settings (Birnbaum 2016). In particular, adventitious roots (ARs) are involved in multiple physiological roles (Bellini et al. 2014, Mhimdi and Pérez-Pérez 2020), such as in response to wounding or during nutrient acquisition. Moreover, ARs can be formed from multiple sites, including shoots and hypocotyls, and are classified as shoot-borne roots (SBRs) or wound-induced roots (WiRs) depending on specific developmental contexts and injuries, respectively. Understanding the molecular mechanisms of WiR formation is particularly important for the clonal propagation of certain horticultural species, especially for tubers, fruit trees and ornamentals. Such practice can help preserve agronomically important traits in plants that are difficult to propagate sexually.

The phytohormone auxin is crucial for many aspects of root development, including WiR formation (Bellini et al. 2014, Omary et al. 2022). Auxin effective concentration is perceived by cells and is determined by their rate of biosynthesis (local and distal; Zhao 2010) and how it is distributed (for instance, by polar auxin transport systems mediated by the PIN_FORMED (PIN) proteins; Hammes et al. 2021). During WiR formation, some local auxin is made near the wound site, while the majority is shoot-derived and transported in a basipetal orientation to the wound site. The resulting auxin accumulation at the wound site activates cell proliferation and eventually WiR formation (Omary et al. 2022). Auxin, however, may not be the only player in the WiR process, and there are numerous examples where calcium—together with auxin—dictates many cellular processes (Vanneste and Friml 2013), including AR formation in cucumber (Lanteri et al. 2006). In addition, while auxin-induced cytosolic free calcium signaling ($[Ca^{2+}]_{cyt}$) has been shown to be important for root development (De Vriese et al. 2019), until recently, it is not yet known if auxin and calcium together regulated WiR formation.

In this issue of *Plant and Cell Physiology*, Modrego et al. (2023) identify and describe how the BIG/ROSETTE (RO) protein, which is a large callosin-like protein with unknown molecular function, bridges the gap between auxin and calcium signaling in WiR formation. First, the authors re-examined the classical tomato *rosette* (*ro*) mutant, which has been known for almost half a century to be incapable of producing SBRs (Zobel 1975). The authors confirmed that, in addition to SBR production, *ro* mutants also showed defects in WiR production and that this was dependent on auxin response build-up at the hypocotyl and/or cotyledon base as a result of a concerted action by the auxin transport network. To better understand why *ro* mutants were defective in WiR production at the molecular level, they first identified the causal *ro* mutation by sequence-based mapping. The mutation was located in a gene encoding a very large protein closely homologous to both *Arabidopsis* BIG—previously shown to be involved in polar auxin transport (Ruegger et al. 1997, Gil et al. 2001)—and mammalian UBR4/p600, which functions to ameliorate the deleterious effects of elevated calcium concentrations during neuron activation (Belzil et al. 2013). The authors confirmed that the tomato *BIG* homolog was the casual gene by generating a clustered regularly interspaced short palindromic repeats (CRISPR) induced insertion (i.e. a frameshift mutation of this gene) in tomato plants, which displayed a similar WiR phenotype to that of *ro* plants.

Modrego et al. (2023) next attempted to understand where BIG fits into the pathway for WiR production by turning their attention to the *Arabidopsis big* mutants given their ease of manipulation. They confirmed that *Arabidopsis big* were also defective in WiR formation, similar to tomato *ro* mutants, suggesting that BIG plays a similar role in both species. However, the exogenous application of auxin alone could not fully rescue the *big* WiR initiation defects in *Arabidopsis*, suggesting the involvement of additional components. Given that the BIG/RO proteins contain a non-conical calmodulin domain and that previous work has linked calcium to AR formation, the authors speculated that calcium could be another key player in WiR production. By manipulating external calcium levels, they confirmed

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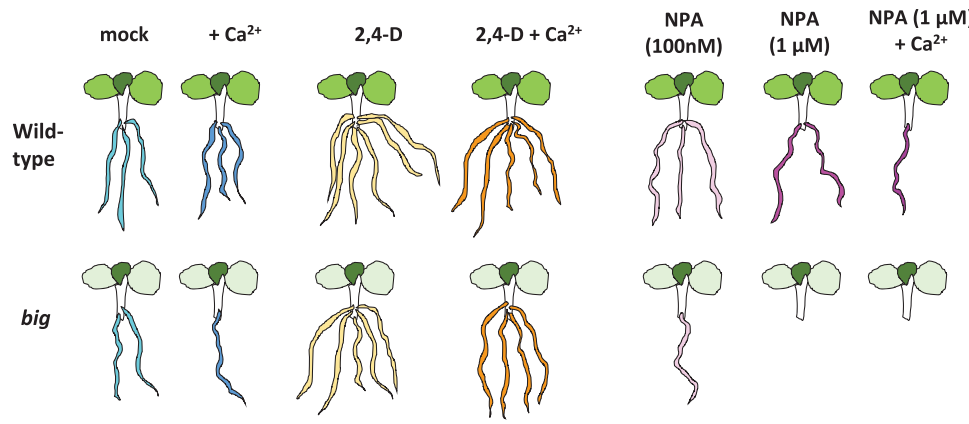

Fig. 1 A simplified illustration to show how exogenous calcium and auxin transport inhibition synergistically affect wound-induced root formation in the *ro*/*big* mutants. WiR formation is compared between the *Arabidopsis thaliana* WT and the *big* mutant under different chemical treatment regimes. The WiR process is inhibited by a high concentration of external calcium, which likely interferes with auxin transport and thereby reduces auxin accumulation required for WiR formation. *big* mutants are hypersensitive to external calcium. A synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) is not transported by conventional auxin transporters (i.e. PIN proteins), and its effects on WiRs are not influenced by external calcium. Simultaneous treatment of WT cuttings with a high concentration of calcium and an auxin transport inhibitor, NPA, can phenocopy the response of the *big* mutant to either the high concentration of calcium or NPA treatments alone.

that high concentrations disrupted PIN accumulation more profoundly in the tomato *ro* and *Arabidopsis big* mutants when compared with the wild types (WTs), likely interfering with the polar auxin transport that is required for WiR production. Furthermore, simultaneous treatment of auxin transport inhibitors, such as *N*-1-naphthylphthalamic acid (NPA), under a high external calcium concentration synergistically affected WiR formation in *Arabidopsis* only (**Fig. 1**). This is a somewhat surprising finding, given that *big* was originally isolated from a genetic screen for NPA-resistant mutants (Ruegger et al. 1997). This may be explained by differences in developmental context between the two studies, since Modrego et al. (2023) focused on WiR formation, while Ruegger et al. (1997) screened for plants that were resistant to NPA in a primary root growth assay.

How, then, does BIG/RO regulate auxin transport and accumulation during wounding? A clue came from the predominant localization of BIG to the endoplasmic reticulum (ER). Remarkably, there were no obvious structural phenotypic differences in ER between WT and *big* plants. However, ER and Golgi movement within cells of the *big* mutant was greatly impaired, occurring at a slower rate. The authors attempted to draw some parallels from animal neuroscience research and hypothesized that BIG/RO could regulate auxin-induced or wound-induced $[Ca^{2+}]_{cyt}$ elevation to allow for faster cytoplasmic streaming of PIN proteins to the plasma membrane and thereby influence auxin fluxes. Such a hypothesis is attractive, given that auxin-induced cytoplasmic streaming is a highly conserved response across land plants and likely plays an ancestral role of auxin in plants (Kuhn et al. 2022). Cytoplasmic streaming as a result of auxin signal transduction is likely to influence the localization of PIN proteins and thus auxin fluxes during auxin canalization—a process in which auxin promotes its own directional transport through a well-controlled localization of auxin transporters. Recently, it was demonstrated that the cell surface auxin perception module (auxin-binding protein 1 (ABP1)/ transmembrane kinase 1 (TMK1)) (ABP1/TMK1) in *Arabidopsis* controls cytoplasmic streaming by differential protein phosphorylation during auxin canalization (Friml et al. 2022), as well as directly controlling PIN-mediated auxin fluxes required for root gravitropism (Rodriguez et al. 2022). Therefore, it is possible that BIG/RO may act, together with ABP1/TMK1, to control PIN localization via cytoplasmic streaming and thus auxin transport during WiR production.

Taken together, Modrego et al. (2023) demonstrate that the BIG/RO protein regulates auxin transport and its accumulation in a calcium-dependent manner during WiR formation, shedding further light on the complex relationship between both processes. To precisely position, BIG/RO in the control of auxin transport is challenging since BIG has been shown to be involved in a plethora of plant processes. While the first important step of uncovering the role of BIG in WiRs has now been achieved, other questions remain open-ended. For instance, whether auxin or other wounding signals (such as but not limited to extracellular ATP, glutamate and mechanical stresses) is the primary contributor of $[Ca^{2+}]_{cyt}$ signaling upon WiR induction needs to be clarified. Other questions that need addressing in the future include the following: exactly how does $[Ca^{2+}]_{cyt}$ exert its action on PIN localization and how does BIG/RO maintain $[Ca^{2+}]_{cyt}$? Also, does BIG/RO protein act primarily through (or in parallel with) the ABP1/TMK1 module during WiR production and does it contribute to other ABP1-TMK1-dependent processes, such as wound-induced vasculature formation and auxin canalization? Addressing such questions will pave the way for a deeper understanding of the role played by BIG/RO in WiR formation and in other plant developmental processes.

A better understanding of WiR formation should, in turn, facilitate the improved clonal propagation of agronomically important plant species (for instance, apples) whose desirable characteristics cannot be preserved by conventional methods or when grown from seeds. It may also help to conserve recalcitrant or endangered plant species that would otherwise be struggling to thrive in their native habitats due to perturbations in their environment.

Data Availability

Data sharing not applicable as no datasets were generated or analysed for this commentary.

Disclosures

The author has no conflicts of interest to declare.

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