

Commentary

How plants get round problems: new insights into the root obstacle avoidance response

Plants have a wonderful ability to modulate their growth and development to suit their environment. Morphogenesis can be altered in response to external cues like gravity, light, water gradient, and obstacles. Responses that involve growth towards or away from a given stimulus are called tropisms and their combined effects define the architecture of the adult plant. Mechanical impedance generated by obstacles or by the mechanical properties of the growth medium can have dramatic effects on the root. Frontal tip impedance inhibits root growth and increases root hair formation, whereas lateral impedance induces root thickening and can even change stem cell niche organization (Potocka *et al.*, 2011; Feng *et al.*, 2020). In this issue of *New Phytologist*, Jacobsen *et al.* (2021; pp. 225–242) deepen our understanding of the spatiotemporal regulation of the root developmental response to mechanical impedance.

‘The response to mechanical impedance is a complex interaction of differently timed responses.’

Currently, it is believed that the root tip response to encountering a physical barrier is a combination of negative thigmotropism (a response to touch) and positive gravitropism (Massa & Gilroy, 2003). Interestingly, both responses are mediated by redistribution of the plant hormone auxin via PIN auxin efflux carriers (Lee *et al.*, 2020). In addition to auxin, several other signals, such as reactive oxygen species (ROS), calcium ion (Ca^{2+}) and pH, have been shown to be involved in the regulation of both root thigmotropism and gravitropism (Nakagawa *et al.*, 2007). Moreover, mechanical impedance causes a decrease in cell elongation, which is caused by a combination of auxin, calcium, and ethylene signalling (Okamoto *et al.*, 2021).

To analyse the response of Arabidopsis root to an *in vitro* artificial barrier system, Jacobsen *et al.* investigated changes in the root transcriptome and hormone signalling. It was found that ROS biosynthesis-related terms were overrepresented among the genes regulated after root tip barrier stimulation. Moreover, ethylene biosynthesis genes were dynamically regulated during the mechanical impedance response. The authors also found that key

ethylene response genes, such as *ETHYLENE INSENSITIVE LIKE 2 (EIL2)* and the *ETHYLENE RESPONSE FACTOR (ERF)* genes *ERF1A* and *ERF4*, were upregulated in the early phases of the response. In addition to these ethylene-related genes, Jacobsen *et al.* found a dynamic regulation of genes involved in auxin signalling (*IAA30*, *IAA14*) and auxin conjugation (*GH3s*, *BRU6*, *DFL1/2*) that were differentially expressed after the onset of the obstacle avoidance response. Together, these data give new insights into the role of the ROS, ethylene, and auxin pathways in the obstacle avoidance response and also provided a platform for the authors to proceed to dissect the spatiotemporal dimensions of the process.

Spatiotemporal resolution in the obstacle avoidance response

Stimuli perceived by the root columella can affect cell expansion in the elongation/differentiation zone (EDZ) to change the direction of root growth. During the graviresponse, the columella cells sense the change in the inclination of the organ within the gravitational field thanks to the sedimentation of dense starch-filled amyloplasts that act as statoliths (Su *et al.*, 2017). This triggers a shift in the lateral transport of the hormone auxin towards the lower side, via PIN-transporters, resulting in differential cell elongation and causing the root to bend. Thigmotropism is also perceived by the columella cells, via a still unknown mechanism, and stimulus also causes asymmetrical redistribution of auxin towards the EDZ and changes in cell elongation (Zhang & Friml, 2020). To give a spatial characterization to ROS signalling, Jacobsen *et al.* performed confocal analysis of the hydrogen peroxide (H_2O_2) reporter HyPer. The reporter line showed upregulation of ROS in the lateral root cap cells during the early phases of obstacle avoidance. Interestingly, by using CellROX Deep Red staining to reveal ROS levels, the authors also found a decrease in ROS content in the EDZ. Jacobsen *et al.* found that root length was not inhibited by barrier impedance in the ethylene resistant mutant *etr1-1* and in the ethylene-insensitive mutants (*ein2*, *aux1*, *eir1*), in accordance with the literature (Okamoto *et al.*, 2008, 2021). Moreover, the authors found that the increase in root hair growth was inhibited in the presence of chemical inhibitors of ethylene biosynthesis (aminoethoxyvinylglycine, AVG) and signalling (silver thiosulphate, STS). These data all reinforce the role of ethylene in the EDZ during the barrier avoidance response. Recent work has shown the dynamic regulation of auxin signalling and transport during the obstacle avoidance response (Lee *et al.*, 2020). Through the analysis of auxin marker lines mDII-ndTomato/DII-m3xVenus, Jacobsen *et al.* found an overall increase in auxin concentration. A similar trend was detected in the DR5rev::3xVENUS-N7 marker line, but the difference was only significant in the stele, which could be due to tissue-specific

This article is a Commentary on Jacobsen *et al.* (2021), 231: 225–242.

variation in auxin response. The authors also confirmed the asymmetry of auxin distribution across the root.

The response to mechanical impedance is a complex interaction of differently timed responses. The initial bend is caused by thigmotropism, whereas the second downward bend is caused by gravitropism. Notably, work done in *Arabidopsis* has revealed that root bending takes place within 20 min after encountering the barrier (Lee *et al.*, 2020), indicating that root obstacle avoidance is a rapid process. Through a detailed analysis of root tip angle variations, Jacobsen *et al.* give a great insight on the differential role of the specific hormones through the response. Transcriptomic analysis data suggest an early and transient upregulation in ethylene biosynthesis during the barrier response. In addition, the analysis of the loss-of-function *etr1-1* mutant indicates that ethylene signalling is necessary from the very early phases of the avoidance, with the mutant showing phenotype already at 10 min after barrier encounter. Similarly, the loss-of-function *atrbohD/F* double mutant, which is impaired in the ROS signalling pathway, showed a hindered early obstacle avoidance response, albeit after the *etr1-1* mutant. Previously, it has been shown that PIN-mediated redistribution has specific roles during the two phases of the response. However, time course analysis of angle variation revealed that neither AUX1 nor PIN2 auxin transporters, both of which are essential for the gravitropic response, are involved in the early phases of the obstacle avoidance response.

Taken together, the data corroborate a model by which the stimuli perceived in the columella trigger an accumulation of auxin. These signals then propagate through the lateral root cap via ROS and auxin redistribution. At the EDZ, auxin, ROS and ethylene work to regulate asymmetrical cell expansion. However, it appears that ethylene signalling also plays a pivotal role in the earlier stages of obstacle avoidance and that auxin transporters involved in the gravitropic response might not be important for the initial thigmotropic response, despite auxin transport being involved (Lee *et al.*, 2020). Despite attempts to link ROS signalling to ethylene and auxin pathways, details of ROS–ethylene–auxin crosstalk remain to be discovered. A very recent paper has shown that ethylene signalling follows a transient increase in cytoplasmic Ca²⁺ during mechanical impedance response (Okamoto *et al.*, 2021). Since ROS production in response to mechanical stimuli requires Ca²⁺ signalling (Monshausen *et al.*, 2009) and auxin and Ca²⁺ pathways are highly connected (Vanneste & Friml, 2013), Ca²⁺ could be the link between these pathways during the obstacle avoidance response.

Root hair regulation during the obstacle avoidance response and the importance of understanding mechanical impedance in a changing climate

Understanding the root response to mechanical impedance is of great agronomic relevance, since soil drying and compaction can reduce crop yields (Whalley *et al.*, 2008). One potentially relevant mechanism, seldomly considered in relation to root penetration, is the anchorage of the root tip, which is necessary for the tip to advance into new soil. Genotypes with root hairs were found to have an advantage for root penetration into high-strength layers

relative to root hairless genotypes (Haling *et al.*, 2013). Root hairs anchor the root tip by increasing friction between the root and the soil and mechanical impedance of the root tip has been shown to induce root hair growth (Bengough *et al.*, 2011). Jacobson *et al.* show that root hairs are longer, denser, and emerge closer to the root tip after a barrier is encountered. Moreover, these effects were completely lost in the presence of inhibitors of ethylene biosynthesis and signalling. This shows that normal ethylene biosynthesis and signalling are essential for root hair elongation and formation. Root hair morphogenesis is regulated by a highly complex hormonal crosstalk (Shibata *et al.*, 2019). In addition to shedding light on the role of ethylene in mediating root hair induction upon mechanical impedance, Jacobson *et al.* showed the upregulation of *ROOT HAIR DEFECTIVE 2 (RHD2)*, an enzyme required for the production of ROS and which has been linked to root hair growth (Foreman *et al.*, 2003), hinting at a possible cross talk during the obstacle avoidance response.

Climate change increases the odds of worsening drought, which results in water stress for the plant but also increases the mechanical impedance of the soil. Interestingly, a recent paper has shown how compact soil can modulate ethylene accumulation in a direct manner by limiting ethylene diffusion away from the root (Pandey *et al.*, 2021). Understanding the intricate mechanisms regulating root hair growth by mechanical impedance could guide breeding for crops that are more resilient to these harsh conditions and increase the prospects of food security.

Acknowledgements

The authors wish to thank The Leverhulme Trust (RPG-2018-137) and the Biotechnology and Biological Sciences Research Council (BBSRC) (BB/N010124/1) for their support of relevant projects in the Kepinski laboratory.

Marta Del Bianco^{1*} and Stefan Kepinski^{2*}

¹Italian Space Agency, Via del Politecnico snc, Rome 00133, Italy;

²School of Biology, University of Leeds, Leeds, LS2 9JT, UK

(*Authors for correspondence: emails marta.delbianco@asi.it (MDB); S.Kepinski@leeds.ac.uk (SK))

References

- Bengough AG, McKenzie BM, Hallett PD, Valentine TA. 2011. Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *Journal of Experimental Botany* 62: 59–68.
- Feng X, Xiong J, Hu Y, Pan L, Liao Z, Zhang X, Guo W, Wu F, Xu J, Hu E *et al.* 2020. Lateral mechanical impedance rather than frontal promotes cortical expansion of roots. *Plant Signaling and Behavior* 15. doi: 10.1080/15592324.2020.1757918.
- Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Angel Torres M, Linstead P, Costa S, Brownlee C, Jones JDG *et al.* 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 422: 442–446.
- Haling RE, Brown LK, Bengough AG, Young IM, Hallett PD, White PJ, George TS. 2013. Root hairs improve root penetration, root-soil contact, and phosphorus acquisition in soils of different strength. *Journal of Experimental Botany* 64: 3711–3721.

- Jacobsen AGR, Jervis G, Xu J, Topping JF, Lindsey K. 2021. Root growth responses to mechanical impedance are regulated by a network of ROS, ethylene and auxin signalling in *Arabidopsis*. *New Phytologist* 231: 225–242.
- Lee HJ, Kim HS, Park JM, Cho HS, Jeon JH. 2020. PIN-mediated polar auxin transport facilitates root-obstacle avoidance. *New Phytologist* 225: 1285–1296.
- Massa GD, Gilroy S. 2003. Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*. *The Plant Journal* 33: 435–445.
- Monshausen GB, Bibikova TN, Weisenseel MH, Gilroy S. 2009. Ca²⁺ regulates reactive oxygen species production and pH during mechanosensing in *Arabidopsis* roots. *Plant Cell* 21: 2341–2356.
- Nakagawa Y, Katagiri T, Shinozaki K, Qi Z, Tatsumi H, Furuichi T, Kishigami A, Sokabe M, Kojima I, Sato S *et al.* 2007. *Arabidopsis* plasma membrane protein crucial for Ca²⁺ influx and touch sensing in roots. *Proceedings of the National Academy of Sciences, USA* 104: 3639–3644.
- Okamoto T, Takatani S, Motoe H, Iida H, Takahashi T. 2021. The root growth reduction in response to mechanical stress involves ethylene-mediated microtubule reorganization and transmembrane receptor-mediated signal transduction in *Arabidopsis*. *Plant Cell Reports* 40: 575–582.
- Okamoto T, Tsurumi S, Shibasaki K, Obana Y, Takaji H, Oono Y, Rahman A. 2008. Genetic dissection of hormonal responses in the roots of *Arabidopsis* grown under continuous mechanical impedance. *Plant Physiology* 146: 1651–1662.
- Pandey BK, Huang G, Bhosale R, Hartman S, Sturrock CJ, Jose L, Martin OC, Karady M, Voeselek LACJ, Ljung K *et al.* 2021. Plant roots sense soil compaction through restricted ethylene diffusion. *Science* 371: 276–280.
- Potocka I, Szymanowska-Pułka J, Karczewski J, Nakielski J. 2011. Effect of mechanical stress on *Zea* root apex. I. Mechanical stress leads to the switch from closed to open meristem organization. *Journal of Experimental Botany* 62: 4583–4593.
- Shibata M, Sugimoto K. 2019. A gene regulatory network for root hair development. *Journal of Plant Research* 132: 301–309.
- Su SH, Gibbs NM, Jancewicz AL, Masson PH. 2017. Molecular mechanisms of root gravitropism. *Current Biology* 27: R964–R972.
- Vanneste S, Friml J. 2013. Calcium: the missing link in auxin action. *Plants* 2: 650–675.
- Whalley WR, Watts CW, Gregory AS, Mooney SJ, Clark LJ, Whitmore AP. 2008. The effect of soil strength on the yield of wheat. *Plant and Soil* 306: 237–247.
- Zhang Y, Friml J. 2020. Auxin guides roots to avoid obstacles during gravitropic growth. *New Phytologist* 225: 1049–1052.

Key words: auxin, Ca²⁺ signalling, ethylene, gravitropism and thigmotropism, mechanical impedance, obstacle avoidance, reactive oxygen species (ROS), root growth.



About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Foundation, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.
- Regular papers, Letters, Viewpoints, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**