Branching out underground: brassinosteroid signaling promotes lateral root development in rice

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Though hidden below the ground, roots are an essential organ system for plant fitness, allowing the uptake of water and nutrients and anchoring plants in the soil. Root system architecture is a highly plastic trait, allowing plants to adjust root number, length, and position in response to environmental conditions (Khan et al., 2016). This architecture is shaped by lateral roots (LRs), which grow at an angle from the axis of other roots. LR development, which has been extensively studied in Arabidopsis (*Arabidopsis thaliana*), is mediated by auxin signal transduction in specific cells that rewires their global transcriptional profiles to promote organogenesis (Gala et al., 2021). Whether this developmental module is conserved in other species, particularly monocot crop species with complex root systems, is less studied, and the contribution of other hormone and environmental signaling pathways into LR growth is still an area of active research.

In this issue of *Plant Physiology*, Hou et al (2022) report a pathway that promotes lateral root growth in rice (*Oryza sativa*), expanding on our understanding of this key developmental program. The authors initially observed that HISTONE DEACETYLASE 1 (OsHDAC1) promotes LR development, as transgenic rice containing an RNAi construct targeting OsHDAC1 had decreased LR density and transgenic plants overexpressing *OsHDAC1* under a ubiquitin promoter had increased LR density (Figure 1A). HDACs remove acetyl groups from histones, making chromatin less accessible, but HDACs can also act on non-histone proteins (Narita et al., 2019). A previous study in Arabidopsis found that the kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2) is deacetylated by HDAC proteins, reducing its kinase activity (Hao et al., 2016). Hou et al (2022) hypothesized that a similar interaction may occur in rice. Using *in vitro* and *in vivo* techniques they demonstrated the physical interaction of OsHDAC1 and OsGSK3/SHAGGY-LIKE KINASE 2 (OsGSK2), the rice ortholog of BIN2 (Yoo et al., 2006). They then analyzed the

acetylation levels of OsGSK2 and found that OsGSK2 acetylation decreased when incubated with OsHDAC1. Furthermore, this deacetylated OsGSK2 showed reduced kinase activity. Finally, they observed that CRISPR knockout of *OsGSK2* caused increased LR density and overexpression of *OsGSK2* caused decreased LR density (Figure 1B), suggesting that OsGSK2 inhibits LR development. OsHDAC1 relieves this inhibition by deacetylating OsGSK2, decreasing its kinase activity.

A study several years ago observed that treating Arabidopsis seedlings with synthetic brassinosteroids increases LR density (Bao et al., 2004). In this article Hou et al (2022) replicated this result in rice and further found the increased density depends on OsHDAC1 activity, as brassinosteroid treatment did not have an effect in OsHDAC1 RNAi plants (Figure 1C). As active OsGSK2 phosphorylates the transcription factor OsBRASSINAZOLE-RESISTANT1 (OsBZR1) and targets it for proteasome-mediated degradation (Tong et al., 2012), Hou et al (2022) proposed that OsGSK2's inactivation by OsHDAC1 allows for a sustained brassinosteroid response that promotes LR development. Through yeast-three-hybrid and *in vitro* pull down approaches they showed that indeed the presence of OsHDAC1 disrupted OsGSK2-OsBZR1 interactions. They further found that *OsBZR1* expression increased in their *OsHDAC1* overexpression lines and decreased in the OsHDAC1 RNAi lines, while expression of known genes promoting LR development decreased in OsHDAC1 and OsBZR1 RNAi lines. Notably, external brassinosteroid treatment increased *OsHDAC1* transcript levels, adding a positive feedback loop to this system (Figure 1D).

Through extensive characterization of transgenic rice and molecular phenotyping, this work establishes a genetic pathway by which brassinosteroid signaling promotes LR development in a highly agriculturally relevant system. A recent large scale transcriptional analysis of LR primordia in Arabidopsis found HDACs were upregulated in these cells (Gala et al., 2021). Cell-type specific repression of these genes impacted LR development, suggesting the role of HDACs in LR development may be conserved across rice and Arabidopsis. The pleiotropic functions of OsHDAC1 required Hou et al (2022) to take a similarly hypomorphic approach. They initially

found they could not generate homozygous CRISPR knockout mutants of OsHDAC1 in rice, suggesting these mutants were embryonic lethal. Their RNAi lines reduced *OsHDAC1* expression but did not completely abolish it, allowing for normal seedling development. Further work using cell-type-specific and inducible activation and repression of *OsHDAC1*, in contrast to the global approaches employed in this study, may further refine our understanding of the timing and spatial dependence of this developmental program.

The highly pleiotropic nature of the genes under analysis also leads to the question of what other pathways these genes may impinge upon to regulate LR development. For example, OsHDAC1 likely also influences histone acetylation and chromatin accessibility in LR primordia. As LR organogenesis requires a complete rewiring of cellular transcriptional profiles, this canonical role of OsHDAC1 could promote LR development in addition to its role in brassinosteroid signaling. OsGSK2's Arabidopsis ortholog BIN2 directly phosphorylates transcription factors AUXIN RESPONSE FACTORS 7 and 19 (ARF7 and ARF19) (Cho et al., 2014), master regulators of LR development, and increases their activity by preventing the binding of repressor cofactors. It would be interesting to determine whether this mechanism is conserved in rice, especially as this potential interaction between OsGSK2 and the ARFs promotes LR initiation whereas the mechanism by which OsGSK2 targets OsBZR1 for degradation elucidated here represses LR initiation. This type of complex feedback regulation is a hallmark of LR development, which, as an essential but environmentally-dependent developmental process, must be both robustly inducible while also tunable. This balance between genetic redundancy and tunability is often achieved by the complex interactions of multiple genetic players and pathways (Alon, 2006), as exemplified by the developmental program elucidated in this paper.

Figure Legends

Figure 1: Brassinosteroid signaling through OsHDAC1 and OsGSK2 promotes lateral root (LR) development in rice. A) RNAi repression of OsHDAC1 (Ri2, Ri3) causes decreased LR density, while ubiquitin promoter-driven overexpression (OE5, OE6) causes increased LR density. Values are means ± SDs (n = 20 plants). Asterisks mark significant changes compared with WT based on

Student's t-test: *P < 0.05, **P < 0.01. B) CRISPR knockout of OsGSK2 (*osgsk2-1*, *osgsk2-2*) causes increased LR density, while ubiquitin promoter-driven overexpression (OE5, OE10) causes decreased LR density. Values are means ± SDs (n = 20 plants). Asterisks mark significant changes compared with WT based on Student's t-test: *P < 0.05, **P < 0.01, ***P < 0.001. C) Treatment of rice seedlings with the synthetic brassinosteroid brassinolide (BL) and the GSK3/SHAGGY-LIKE kinase inhibitor Bikinin causes increased LR density. In OsHDAC1 RNAi transgenic seedlings (Ri2, Ri3) Bikinin treatment increased LR density, but BL treatment had no effect. Asterisks mark significant changes compared with WT, Ri2, or Ri3 without 10 nM BL and 5 µM Bikinin treatment based on Student's t-test: **P < 0.01. n.s., no significant change. D) Proposed model by which brassinosteroid (BR) treatment promotes LR development through OsHDAC1 and OsGSK2. BR signaling upregulates expression of *OsHDAC1*, which deacetylates OsGSK2, decreasing its kinase activity. OsGSK2 in the absence of OsHDAC1 phosphorylates the BR-response transcription factor OsBZR1, targeting it for proteasomal-mediated degradation. Consequently, the inactivation of OsGSK2 by OsHDAC1 causes OsBZR1 accumulation and increased BR signaling, which upregulates the expression of genes that promote LR development. Figures adapted from Hou et al (2022).

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