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Molecular mechanism of crown root initiation and the different mechanisms between crown root and radicle in rice

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Monocot plants produce numerous adventitious (crown) roots. The plant hormone auxin has positive effects on crown root formation, while cytokinin suppresses it. We have demonstrated that auxin-induced *CROWN ROOTLESS5* (*CRL5*) regulates crown root initiation in rice through the induction of *OsRR1*, a negative regulator of cytokinin signaling. *CRL5* overexpressing calli formed adventitious roots, although *CRL5* overexpressing plants did not induce ectopic roots, suggesting that *CRL5*, which promotes de novo root initiation, might function only in de-differentiated cells. A radicle initiated normally in a *crl5* mutant, in spite of the defect in crown root initiation, whereas crown roots, but not a radicle, were produced in a *radicleless1* (*ral1*) mutant. A *crl5 ral1* double mutant displayed an additive phenotype, showing that the formation of each root is regulated by different genetic mechanisms in rice.

Roots play essential roles in plant development such as absorbing water and nutrients and supporting the plant body. Recent progress in our understanding of the molecular mechanisms of root formation has revealed an elaborate system of regulation by plant hormones and their interactions.[1,2] However, these analyses were mostly performed in Arabidopsis, a model dicot.

Monocot plants produce numerous adventitious (crown) roots from nodes (**Fig. 1A**) and form a fibrous root system (**Fig. 1B**). We reported a rice mutant,[3] termed *crl5*, which produced fewer crown roots and displayed impaired initiation of crown root primordia. *CRL5* encodes a member of the APETALA2 (AP2)/EHYLENE RESPONSIVE FACTOR (ERF) transcription factor family. We found that *CRL5* functions downstream of the AUXIN (AUX)/INDOLE-3-ACETIC ACID (IAA) and AUXIN RESPONSE FACTOR (ARF)-mediated auxin signaling pathways involved in crown root initiation. Further analysis revealed that *CRL5* upregulates a type-A response regulator (RR) of cytokinin signaling, *OsRR1*. These results indicated that auxin-induced *CRL5* functions as a positive regulator of crown root initiation through repression of cytokinin signaling.

The Ability of *CRL5* for de novo Root Initiation

We showed that CRL5 shares significant sequence similarity with AINTEGUMENTA (ANT) containing 2 AP2 domains in Arabidopsis.[4] Seven AINTEGUMENTA-like (AIL)/PLETHORA (PLT) proteins were reported to share high sequence similarity with ANT.[5] Among them, *PLT1*, *PLT2*, *AIL6*/*PLT3* and *AtBABY BOOM* (*AtBBM*) have been shown to act redundantly in root development and are thought to function as master switches for root initiation and development because of their ability, when overexpressed, to induce ectopic roots in cells that do not possess competence to produce roots (like shoot organs) in Arabidopsis.[6,7] In spite of the similarity, *CRL5* overexpressing plants did not induce ectopic roots, with the exception of the strong ability of *CRL5* overexpressing calli to form adventitious roots (**Fig. 1C–F**). Therefore, it is possible that *CRL5* is not involved in the de-differentiation process, but its involvement in root initiation might be restricted to de-differentiated cells (**Fig. 1G**). We used in situ hybridization to examine the expression of a gene similar to Arabidopsis *PLT*s in rice, *OsPLT*, at the base of shoots. This gene was expressed in the parenchyma cells adjacent to the peripheral cylinder of vascular bundles of the stem and the tip of crown root primordia (**Fig. 1H–J**). The expression of this gene in the root tip was similar to that of Arabidopsis *PLT*s (**Fig. 1J**), and overlapped the region where crown root initiation occurs (**Fig. 1H**). We also made transgenic plants that overexpressed this gene; however, ectopic root formation was not observed (**Fig. 1K**). We suggested that the function of *PLT* in rice may be different from that of Arabidopsis *PLT*s in root initiation and that other gene(s) may function as a master switch in rice root initiation.

Difference between Crown Root and Radicle Initiation in Rice

Roots are largely classified as embryonic roots and post-embryonic roots. Embryonic roots initiate during embryogenesis and

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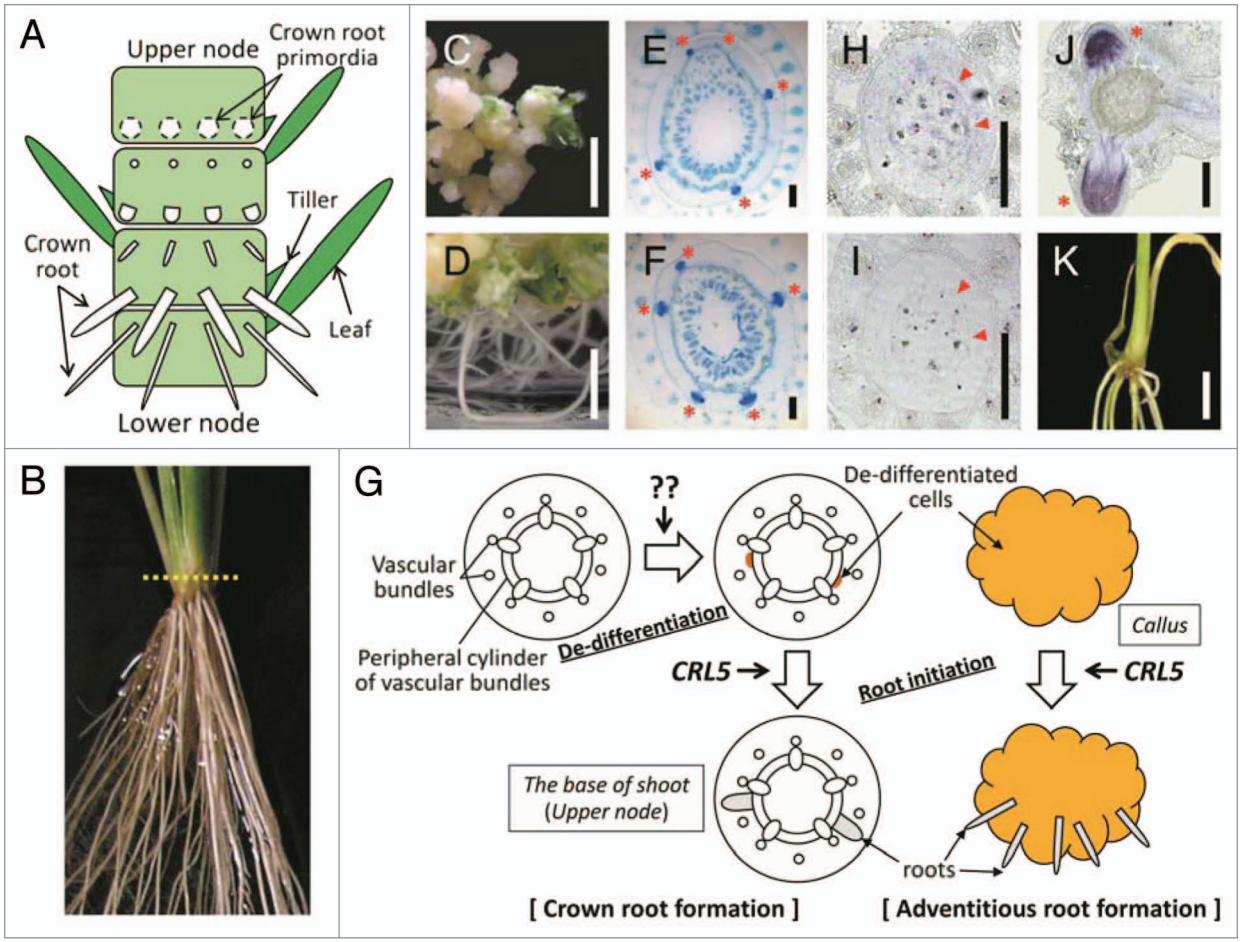

Figure 1. The ability of *CRL5* for de novo root initiation. (A) Schematic diagram of the base of shoot in rice. Nodes are not seen from outside because they are coverd by leaves. (B) One-month-old wild-type plant. Cross sections indicated in (E–J) were made associating the dotted line. (C and D) Wild-type calli containing empty vector (C) and *CRL5* overexpressing calli (D) on regeneration media containing 1 μM NAA and 10 μM kinetin. Scale bars = 1 cm. (E and F) Cross sections through the nodes of 1-month-old wild-type containing empty vector (E) and *CRL5* overexpressing plant (F). Asterisks indicate the crown root primordia. Scale bars = 200 μm. (G) Schematic diagram of the de novo root initiation. *CRL5* may function in de-differentiated cells (highlighted) to promote root initiation, not in the de-differentiation process. The factor inducing the de-differentiation is unknown. (H–J) In situ hybridization with cross sections through the nodes of 7-d-old wild-type seedling. Localization of one of the *OsPLT* genes in the region where crown root initiation occurs using antisense (H) or sense probes (I) and in the region where crown root emergence occurs using antisense probe (J). Arrowheads and asterisks indicate the peripheral cylinder of vascular bundles and crown root primordia, respectively. Scale Bars = 200 μm. (K) Transgenic plant overexpressing one of the *OsPLT* genes. Scale bar = 1 cm.

post-embryonic roots initiate after embryogenesis. In rice, a radicle (a seminal root) is an embryonic root and crown roots are post-embryonic (**Fig. 2A**). Although the number of crown roots in *crl5* was clearly lower than that in wild-type, *crl5* normally produced a radicle (**Fig. 2B**).[3] On the other hand, the crown root number was not decreased in *radicleless1* (*ral1*), but *ral1* cannot produce a radicle (seminal root) (**Fig. 2C**).[8] In the *crl5 ral1* double mutant, a seminal root was not produced and the crown root number was also clearly lower than in the wild type (**Fig. 2D**). However, the double mutant produced almost the same number of crown roots as *crl5*, showing that crown root initiation is not affected by *ral1* mutation. The additive phenotype displayed by the *crl5 ral1* double mutant indicated that formation of each root is regulated by different genetic mechanisms in rice.

We demonstrated that interaction between *OsARF* and *OsRR1* is mediated by *CRL5* in rice crown root initiation (**Fig. 2E**).[3] On the other hand, it has been reported that auxin signaling directly induces transcription of type-A *ARABIDOPSIS RESPONSE REGULATOR7* (*ARR7*) and *ARR15* genes, which negatively regulate cytokinin signaling through conserved TGTC elements and whose mutations prevent the establishment of a normal embryonic root.[9] In addition, interaction between auxin signaling and these *ARR*s is essential for control of the shoot stem-cell niche.[10] In the shoot apical meristem, MONOPTEROS (MP)/ARF5 directly relays auxin signals to *ARR15* by binding TGTC elements in its promoter.[10] Based on these reports, we hypothesized that MP/ARF5 is the most likely candidate to regulate *ARR7* and *ARR15* directly in Arabidopsis embryonic root initiation (**Fig. 2F**). We suspected that *CRL5* might be a key factor to define the different genetic mechanisms between embryonic root (radicle) initiation and post-embryonic root (crown root) initiation in rice. Comprehensive understanding of root initiation in

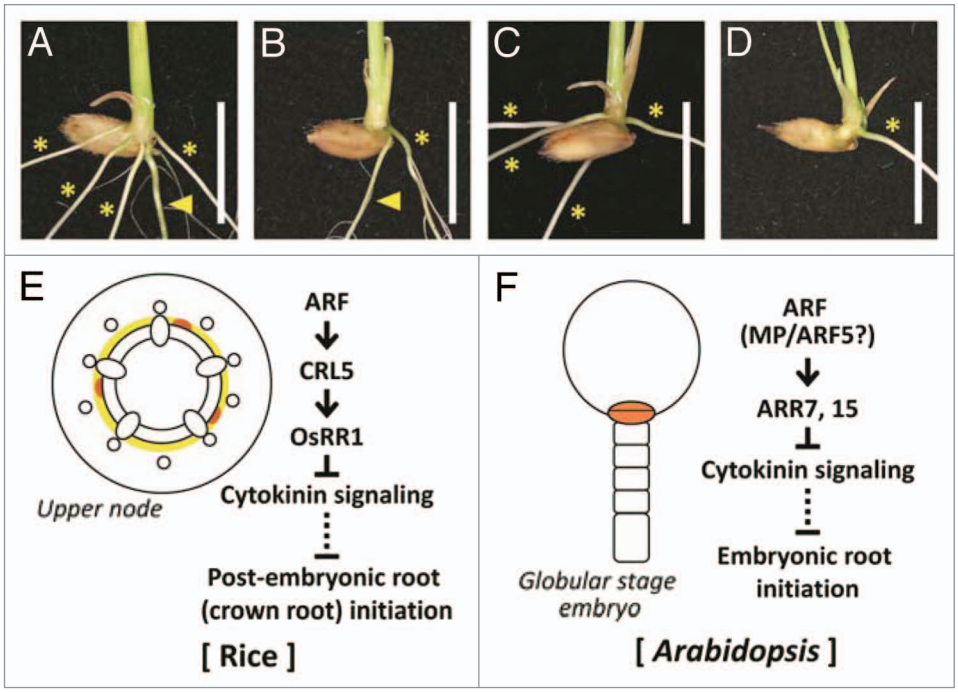

Figure 2. Difference between embryonic root and post-embryonic root initiation in rice. (A–D) Phenotype of wild-type (A), *crl5* (B), *ral1* (C) and *crl5 ral1* double mutant (D). Arrowheads and asterisks indicate seminal roots (radicles, embryonic roots) and crown roots (post-embryonic roots), respectively. Scale bars = 1 cm. (E and F) Schematic diagram of post-embryonic root (crown root) initiation in rice (E) and embryonic root initiation in Arabidopsis (F). The regions where root initiation occurs are highlighted.

rice may progress by revealing the mechanism of embryonic and post-embryonic root initiation.

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