

9. Characterization and mapping of the *CROWN ROOTLESS2* gene, *CRL2*, in rice.

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The root system, which mainly consists of seminal, adventitious, and lateral roots, plays an important part in plant growth. In contrast with *Arabidopsis*, in which adventitious roots are rarely formed, adventitious (crown) roots are dominant in the root system of cereals. In order to study the molecular mechanism of crown root formation in rice, we previously reported rice mutants defective in crown root formation, crown rootless1 (*crl1*) and *crl2* (Inukai et al., 2001 Inukai et al., 2005). Here, we further characterized the *crl2* mutant and determined its map position on the rice chromosome.

The *crl2* mutant was obtained from a cultivar, Blue Rose, mutagenized with γ -ray treatment. Genetic analysis indicated that the *crl2* mutation is recessive. As the homozygous *crl2* plants are sterile, we maintained this mutant as heterozygous plants. As shown in Fig. 1A, the number of crown roots of this mutant was dramatically lower than that of the wild type, while the length of seminal root of the *crl2* was not different or slightly longer than the wild type. As another rice *crl* mutant, *crl1* shows some auxin-related abnormal phenotypes, such as auxin insensitivity in lateral root formation and impaired root gravitropism. We investigated the gravitropism of shoot and root of *crl2* by measuring the curvature after gravistimulation at 90° to the vertical. Wild-type roots responded sharply to the change in the gravity vector, whereas the response of *crl2* roots was apparently impaired (Fig. 1B). Actually, approximately 90% of the wild-type roots had root tip angles of 70-90° (θ° in Fig. 1C), and no plants had an angle of < 50°; by contrast, 80% of *crl2* roots had angles of < 50°. As for the shoot, the *crl2* was interestingly much more sharply responded to the gravity vector than the wild-type (Fig. 1B). Results showed that approximately 60% of the wild-type shoots had shoot tip angles of 10-30° (θ° in Fig. 1D), and no plants had an angle of > 50°; by contrast, all *crl2* shoots had angles of > 70°. Impaired gravitropism of the *crl2* root suggest that the *CRL2* gene might be involved in the auxin-related signaling in root similar to the *CRL1* gene, which encodes a member of the plant-specific ASYMMETRIC LEAVES2/LATERAL ORGAN BOUNDARIES protein family and functions as a key regulator for auxin signaling pathway (Inukai et al., 2005). However, it is not likely that *CRL2* is involved in the same pathway as *CRL1*, because the *crl1* shoots did not show hyper-gravitropism, which was observed in the *crl2* mutant. Consequently, *CRL2* might function in a novel unknown pathway related to gravitropic response in rice.

To isolate the *CRL2* gene, we used the F2 plants from a cross between the *crl2* mutant (japonica) and Kasalath (indica) in this study. We screened 32 F2 plants showing the crown rootless phenotype and extracted their genomic DNAs from leaf tissue for the linkage analysis. We found that the *CRL2* locus was roughly mapped on the long arm of chromosome 1, between the molecular marker RM493 (64.9cM) and C175 (73.4cM) (Fig. 2). For fine mapping, we used 342 *crl2* homozygous plants and found that the *CRL2* locus was located between the molecular marker C2690-2 (69.3cM) and C2134-2 (70.1cM) (Fig. 2). In this region, 23 genes are predicted (Rice Annotation Project Database). Now, we are determining the more precise location of *CRL2*.

References

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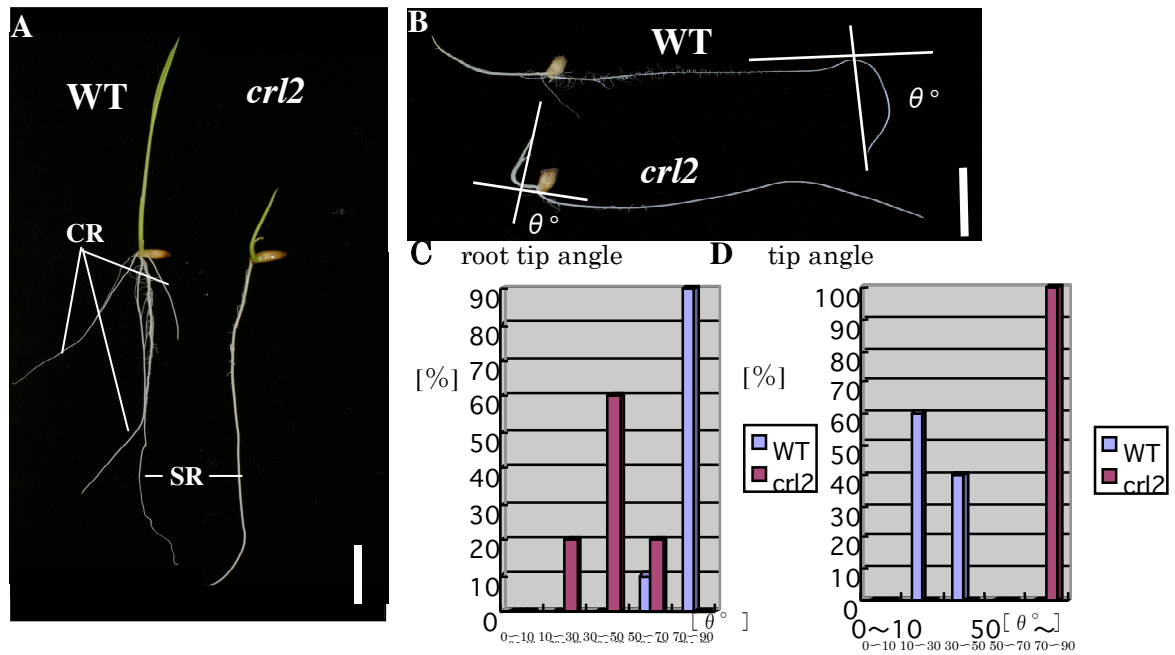


Fig. 1 Phenotypes of *crl2* and its abnormal gravitropic response of roots and shoots. (A) Two-week-old wild-type (left) and *crl2* (right) seedlings. SR, seminal root; CR, crown root; Bar = 1 cm. (B) Wild-type and *crl2* seedlings were grown vertically for 3d and then rotated 90°. Bar = 1 cm (C) The root and shoot tip angle (θ°) in (B) was measured 24h after reorientation. Each data point is the average of 15 plants.

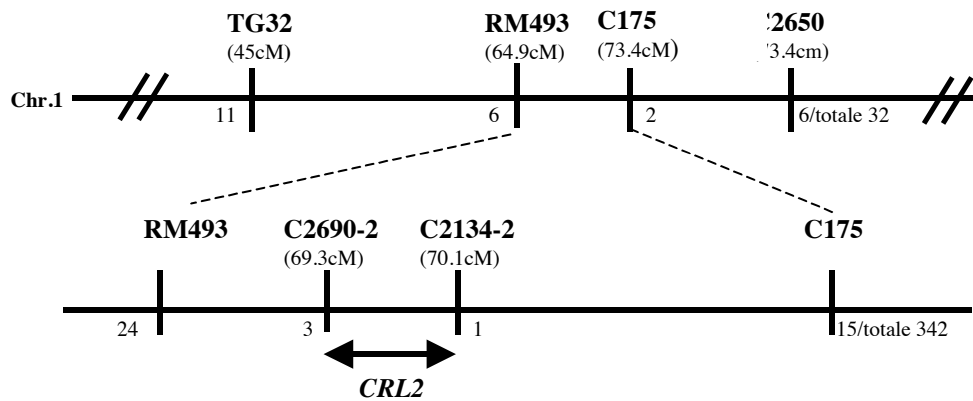


Fig. 2 The linkage map of *crl2* with molecular markers on chromosome 1. The *crl2* is located within 0.8cM between C2690-2 and C2134-2.