9. Mapping of the *CROWN ROOTLESS3* gene, *CRL3*, in rice.

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We have made a lot of progress in the analysis of the shoot system up to the present, but our knowledge of the root system is limited in comparison. Roots play an important part in plant growth, and adventitious (crown) roots hold a majority of the root system in cereals. 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 characterized a new mutant involved in the crown root formation, *crl3*.

The *crl3* mutant was obtained from Taichung 65 (T65) mutagenized with N-methyl-N-nitrosourea (MNU) treatment. Genetic analysis indicated that the *crl3* mutation is recessive. As shown in Fig.1A, the number of crown roots of *crl3* mutant was clearly lower than that of the wild type. To determine at which stage of root development the defect in crown root formation became apparent in the *crl3* mutant, we examined serial cross-sections of the stem regions of the *crl3* mutant and the wild type seedlings. The result was that the cells constructing the crown root primordia of *crl3* mutant apparently differentiated; they were already vacuolated compared with those of the wild type (Fig.1B-C). Despite the abnormality of crown root primordia, the *crl3* mutant produced the lateral root primordial normally (Fig.1D-E). In addition, we found no phenotypic differences between the *crl3* mutant and the wild type on seminal root (radicle) primordia and shoot apical meristem. These results indicate that *CRL3* is especially involved in the formation of the crown root primordia, but not in the formation of other types of root primordia and shoot apical meristem.

To isolate the *CRL3* gene, we used the F_2 plants from a cross between the *crl3* mutant (*japonica*) and Kasalath (*indica*) in this study. We screened 32 F_2 plants showing the crown rootless phenotype and extracted their genomic DNAs from leaf tissue for the linkage analysis. We found that the *CRL3* locus was roughly mapped on the long arm of chromosome 3, between the molecular marker I03_F (94.9cM) and H15_F (96.6cM) (Fig.2). For fine mapping, we used 256 *crl3* homozygous plants and found that the *CRL3* locus was located between the molecular marker I03_F and K08-20 (Fig.2), which are covered by two bacterial artificial chromosome (BAC) clones in this region. Now, we are determining the more precise location of *CRL3*.

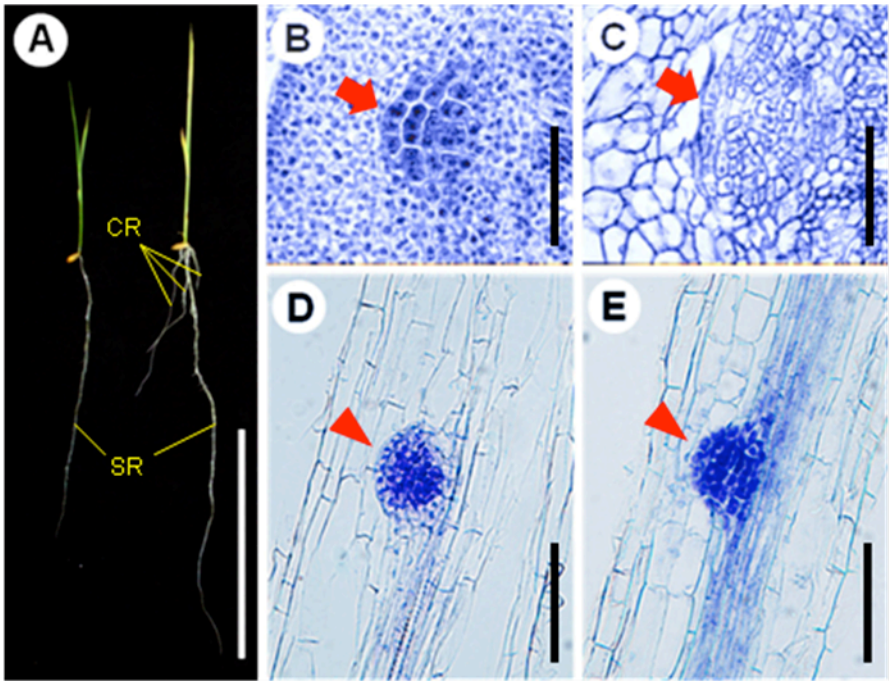

Fig.1. Phenotypes of the *crl3* mutant. (A) Two-week-old seedlings of the *crl3* mutant (left) and the wild type (T65, right). SR, seminal root; CR, crown roots. Bar=10cm. (B) and (C) Cross-sections through the second nodes of the wild type (B) and *crl3* mutant (C). Arrows indicate crown root primordium. Bars=100μm. (D) and (E) Longitudinal sections through the seminal roots of the wild type (D) and the *crl3* mutant (E) in two-week-old seedlings. Arrowheads indicate lateral root primordia. Bars=10μm.

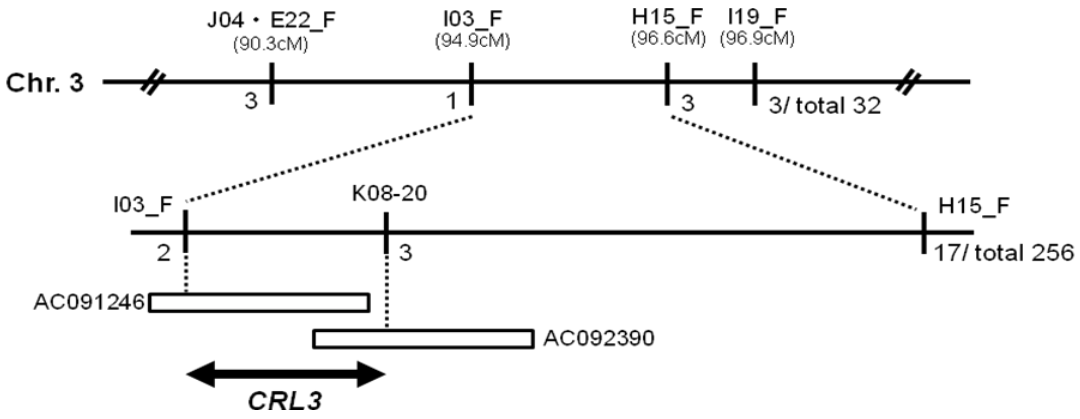

Fig.2. The linkage map of *CRL3* and nearby molecular markers. The horizontal bar and the vertical bars show chromosome 3 and molecular markers, respectively. The numbers of recombinant plants are indicated under the linkage map. The upper part is the result of rough mapping from 32 F_2 recombinants, and lower part is that of fine mapping from 256 F_2 recombinants. White boxes show BAC clones.

References

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