6. Mapping and Analysis of the *CLUB-SHAPED EMBRYO1*, *CLE1*

K. IMAMURA[2], H. IMAI[2], Y. NAGATO[3], M. MATSUOKA[1], H. KITANO[1,4] and T. SAZUKA[1,4]

1)Bioscience Center, Nagoya University, Nagoya, 464-8601 Japan

2)Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601 Japan

3)Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, 113-8657 Japan

4) These corresponding authors equally contributed to this work.

The *club-shaped embryo* (*cle*) mutant fails to develop most organs and tissues, and is characterized by the unique embryo shape (*club-shape*, see Fig. 1A-F), which is not observed in wild-type and the other organless-mutant embryos in rice (Hong et al. 1995). Three NMU-induced *cle1* mutant alleles (*cle1-1*, *cle1-2*, and *cle1-3*) have been briefly reported previously (Hong et al. 1995).

Firstly, we studied a detailed histological analysis of the three *cle1* alleles. It was revealed that these showed the same phenotype, which failed to develop shoot apical meristem (SAM) and root apical meristem (RAM), but developed the vascular-like tissue and the scutellar epitherium-like tissue (see Fig. 1B-D).

Next, we screened the other *cle*-like lines in a callus-culture induced mutant library. Of them, the embryos of the two lines (*cle1-4*, and *cle1-5*, see Fig. 1E and F respectively) also showed the *cle* phenotype, but the embryos were obviously larger (Fig. 1E and F compared with Fig. 1B-D). We carried out the allelism test, and the result showed that the two lines are allelic to *cle1* (data not shown). The difference of the phenotype should depend on the each mutation, which causes the different activity of the gene product.

A rice homeobox gene *OSH1*, which is specifically expressed in the shoot meristem region before the morphological development in wild-type (Fig. 1G), was misexpressed in *cle1-1* (the *cle* small-allele) embryo; the expression was observed in the basal region of the embryo on both the dorsal and the ventral sides (Fig. 1H). The same expression pattern was also observed in *cle1-5* (the *cle* large-allele, Fig. 1I). These observations imply that the pattern formation is impaired both in the small- and the large-allele embryos. It should be noted that this pattern is obviously different from that of another organless-mutant, *organless1 (orl1)*, in which the expression pattern of *OSH1* is reported to be similar to that in the wild-type embryo (Kamiya et al. 2001).

In the aim of *cle1* gene mapping, we used the genomic DNAs of F_2 heterozygous plants (*CLE1/cle1-1*), which were identified by the segregation of the *cle* phenotype in F_3 embryos. We used 46 F_2 heterozygous plants for the linkage analysis. The result demonstrated that *CLE1* is located between K0432D01 and TG1020 on chromosome 3 (Fig. 2). According to the RGP (Rice Genome Project) map (Harushima et al. 1998), the genetic distance between them is 5.2 cM (Fig. 2). We are narrowing down of this region to determine the *CLE1* gene.

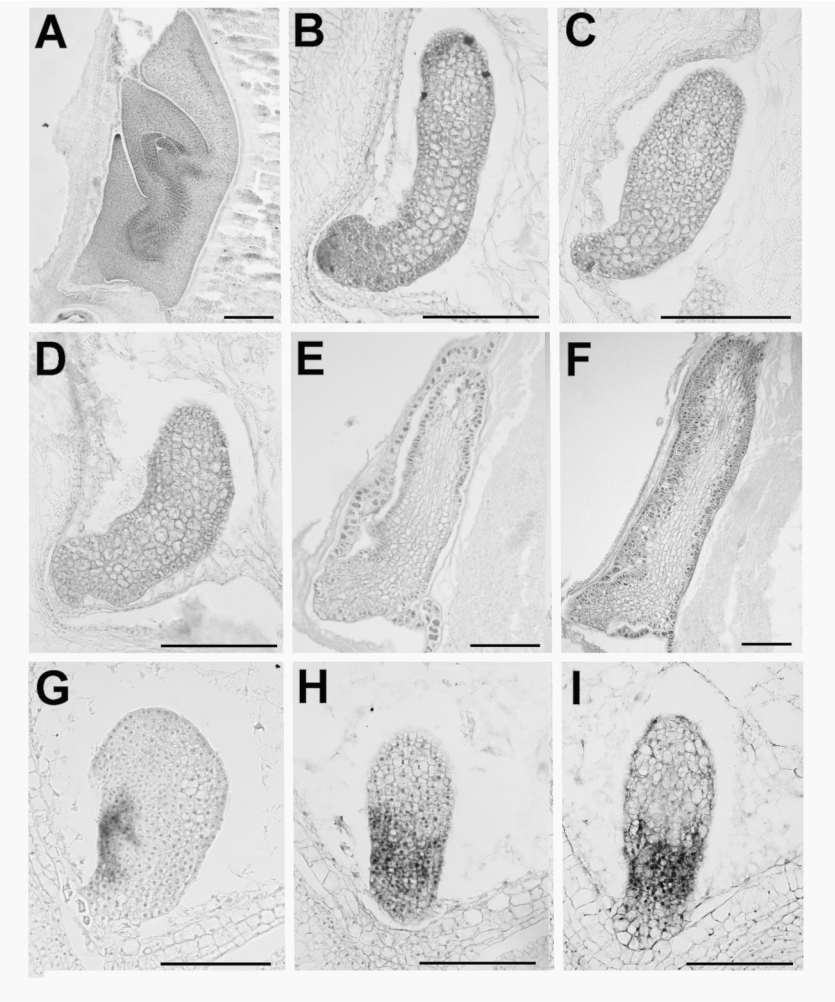

Fig. 1. Phenotypes and expression patterns of *OSH1* in wild-type and *cle1* mutant embryos. (A-F) Phenotype of embryos at 10 days after pollination (DAP). (A) Wild-type. (B) *cle1-1*. (C) *cle1-2*. (D) *cle1-3*. *cle1-1*, *cle1-2* and *cle1-3* are the *cle*-small alleles. (E) *cle1-4*. (F) *cle1-5*. *cle1-4* and *cle1*-5 are the *cle*-large alleles. Expression pattern of *OSH1* in wild-type (G), in *cle1-1* (H), and in *cle1-5* (I) at 4 DAP are shown. Bars = 200 μm in (A-F) and 100 μm in (G-H).

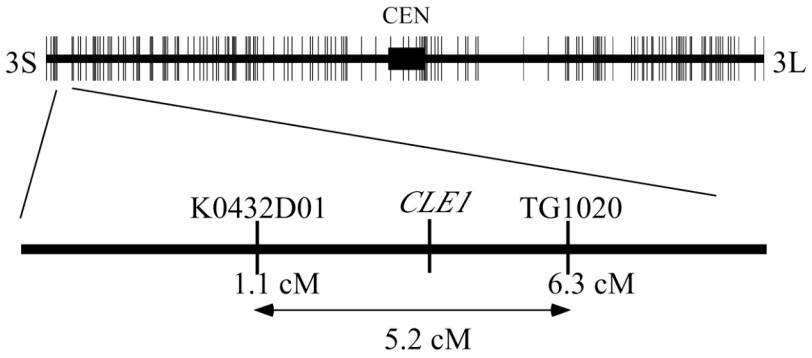

Fig. 2. Linkage map of *CLE1* with RFLP makers on chromosome 3.

References

Harushima Y., M. Yano, A. Shomura, M. Sato, T. Shimono, Y. Kuboki, T. Yamamoto, S. Y. Lin, B. A. Antonio, A. Parco, H. Kajiya, N. Huang, K. Yamamoto, Y. Nagamura, N. Kurata, G. S. Khush and T. Sasaki, 1998. A high-density rice genetic linkage map with 2275 makaers using a single F_2 population. Genetics 148: 479-494.

Hong S. K., T. Aoki, H. Kitano, H. Satoh and Y. Nagato, 1995. Phenotypic diversity of 188 rice embryo mutant. Dev. Genet. 16: 298-310.

Kamiya N., M. Ashikari, Y. Sato, Y. Nagato and H. Kitano and M. Matsuoka, 2001. Mapping of the *ORGANLESS1* gene, *ORL1*. Rice Genet. Newsletter, 18: 33.