26. **OsNAM and OsCUC3 are expressed specifically in organ boundaries**

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Separation of organs from the neighboring tissue, e.g. leaf primordia from shoot apical meristem (SAM), is prerequisite for normal organ development. Cells at the boundary display characteristic patterns of cell division, morphology and gene expression (Aida and Tasaka 2006). Recent studies in dicots have revealed that members of the NAC gene family such as *CUP-SHAPED COTYLEDON1* (*CUC1*), *CUC2* and *CUC3* of *Arabidopsis* contribute to the establishment of shoot meristem and organ separation (Hibara et al. 2006). In this study, we have examined in detail the expression pattern of OsNAM and OsCUC3 genes that are orthologs of *CUC2* and *CUC3*, respectively, and are supposed to have roles on organ separation (Hibara and Nagato 2006).

We examined the expression patterns of OsCUC3 and OsNAM. The OsCUC3 mRNA was detected in embryo at four days after pollination (4 DAP) at the boundary between the SAM and coleoptile (Fig. 1A). The two expression sites in Fig. 1A correspond to two sites of coleoptile-SAM boundaries because the coleoptile encircles the SAM. OsCUC3 expression was stronger on the basal side of the SAM than the apical (scutellar) side, while OsNAM transcripts were more abundant at the apical boundary (Fig. 1A, B). At 5-6 DAP, expression of both OsNAM and OsCUC3 was observed at the boundary between SAM and first leaf primordium (Fig. 1C-E). In addition, OsNAM transcripts were detected in several adaxial cell layers of coleoptile (Fig. 1E) but this expression was not observed in case of OsCUC3.

After germination, OsNAM transcripts remained detectable at the boundaries between SAM and P1 leaf primordium and between leaves (Fig.2 A). The similar expression pattern was observed in the case of OsCUC3 except that the expression was undetectable between P1 and SAM (Fig.2 B). In the cross sections, OsCUC3 was expressed in the leaf margin of P1 primordium and/or at the boundary between SAM and leaf margin. Such expression in leaf margin was not detected with OsNAM probe.

The present study has revealed that both OsNAM and OsCUC3 are expressed specifically at the organ boundaries during embryogenesis and vegetative development. These results suggest that OsNAM and OsCUC3 are involved in the organ separation in rice. Different expression patterns of OsNAM and OsCUC3 suggest that OsNAM and OsCUC3 have their own local roles on the organ boundary formation.
Fig. 1. Localization of OsCUC3 and OsNAM transcripts during embryogenesis. (A, C, D) OsCUC3 expression in wild-type embryos at 4 DAP (A) and 6 DAP (C, D). (B, E) OsNAM expression in wild-type embryos at 4 DAP (B) and 6 DAP (E). Arrowheads: SAM. Arrows: coleoptile.

Fig. 2. Localization of OsNAM and OsCUC3 transcripts in vegetative shoot at 10 days after germination. Longitudinal sections of OsNAM (A) and OsCUC3 (B) shoot apices. In (B), OsNAM expression is observed at the boundary between P1 and SAM (arrow in A), but OsCUC3 is not expressed at the boundary between P1 and SAM (arrow in B). Arrowheads indicate the boundary between P1 and SAM. (C-F) Serial transverse sections of a shoot apex showing OsCUC3 expression in lateral leaf margin (arrows). Pink color marks P2 primordia and green color P1 primordia.

References