

24. *Petunia NO APICAL MERISTEM* ortholog, *OsNAM*, is expressed in the embryonic SAM and organ boundary in rice

K. HIBARA and Y. NAGATO

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

Elaboration of the body plan in flowering plants depends on the activity of the shoot apical meristem (SAM), which is formed during embryogenesis. Genetic and molecular studies in dicots have revealed that members of the NAC gene family such as *NO APICAL MERISTEM* (*NAM*) of *Petunia* and *CUP-SHAPED COTYLEDON* (*CUC*) of *Arabidopsis* contribute to the establishment of the embryonic SAM (Souer et al. 1996, Aida et al. 1999). For understanding the embryonic SAM formation in rice, we isolated and characterized *OsNAM* gene that is an ortholog of the *NAM/CUC* genes.

We found two predicted genes (*OSJNBa0021N09.15* and *OSJNBa0016N23.129*) that were highly related to the *NAM/CUC* genes from the rice genome database using BLAST search. Phylogenetic analyses based on the amino acid sequence of NAC domain revealed that *OSJNBa0021N09.15* was orthologous to *NAM/CUC1-2* and *OSJNBa0016N23.15* was orthologous to *CUC3*, thus they were designated *OsNAM* and *OsCUC3*, respectively (Fig.1). The NAC domain of *OsNAM* is 76.0%, 80.3% and 83.6% identical to those of *CUC1*, *CUC2* and *NAM*, respectively. *NAM/CUC1-2* class genes in dicots have micro RNA (miR164) recognition sites. *OsNAM* also have a potential miR164 site.

We investigated the expression of *OsNAM* by *in situ* hybridization. *OsNAM* mRNA was detected at three days after pollination (3 DAP) in the predicted region of embryonic SAM (Fig. 2A). When the SAM was initiated at 4 DAP, however, *OsNAM* expression disappeared from the SAM, and was detected in the boundary between the SAM and coleoptile (Fig. 2B). These expression patterns during 3-4 DAP embryos were analogous to those of *NAM* and *CUC* genes. At 5-6 DAP, the expression was observed also in the boundary between SAM and the first leaf primordium (Fig. 2C, D). In addition, the signal was detected in several adaxial cell layers of coleoptile.

After germination, *OsNAM* expression was maintained in organ boundaries (Fig. 2E). Interestingly, *OsNAM* mRNA was also detected in part of the vegetative SAM (Fig. 2E), in contrast to the case of dicots in which *NAM/CUC* was not expressed in the SAM. This suggests that the function of monocot *NAM/CUC* gene may be partially different from that in dicots.

In conclusion, *OsNAM* that is the ortholog of *NAM/CUC1-2* would play an important role in SAM initiation and organ separation.

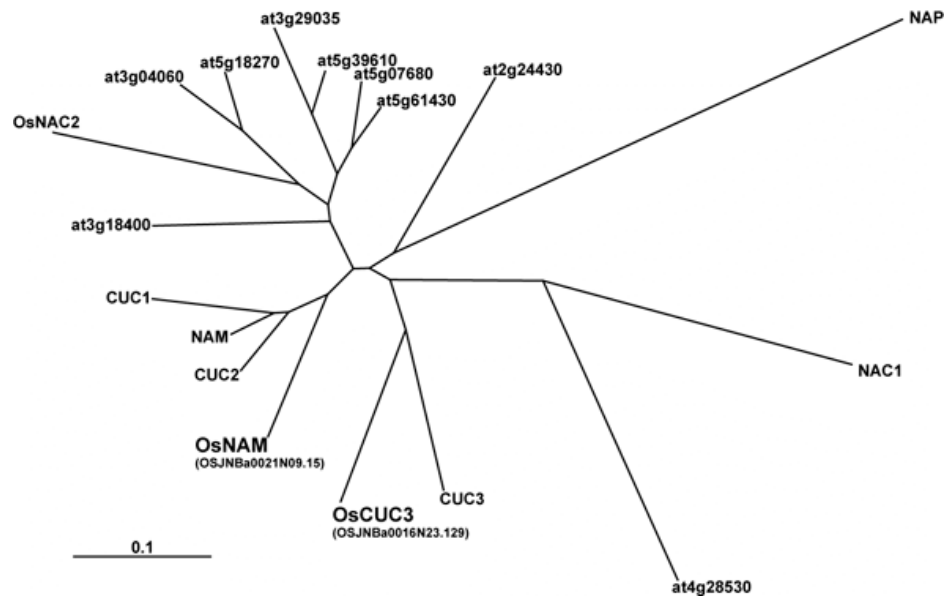


Fig. 1. The phylogenetic tree of NAC family genes deduced from amino acid sequences of NAC domains.

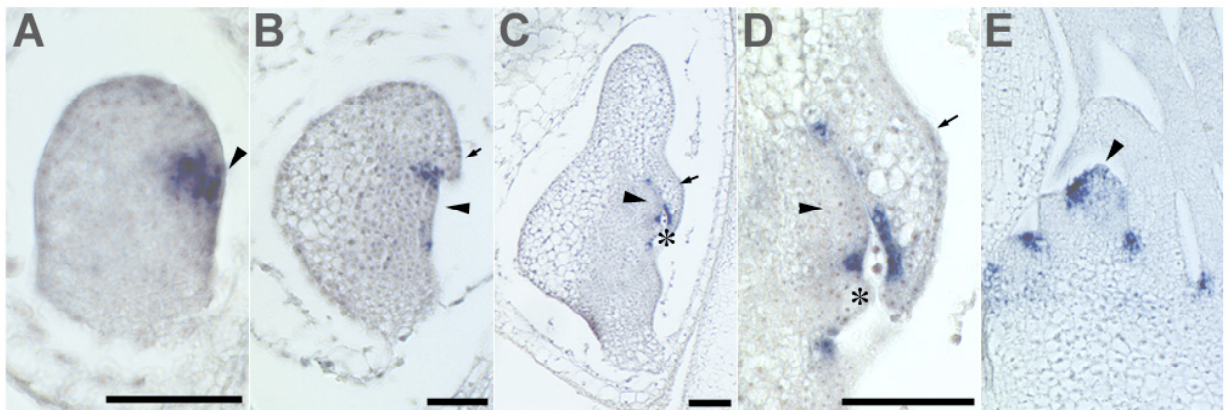


Fig. 2. Localization of *OsNAM* transcripts during embryogenesis and in vegetative shoot. Longitudinal sections of embryo at 3 DAP (A), 4 DAP (B), 6 DAP (C, D) and vegetative shoot at 10 DAG (E). Arrowheads: SAM. Arrows: coleoptile. Asterisks: first leaf primordium. Bar: 50 μ m.

References

- Souer, E., A. van Houwelingen, D. Kloos, J. Mol and R. Koes, 1996. The *no apical meristem* gene of *Petunia* is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell* 85: 159-170.

Aida, M., T. Ishida and M. Tasaka, 1999. Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development* 126: 1563-1570.