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Apical displacement1 gene regulates apical–basal pattern formation in rice embryo

Takahide Kinae, Soon-Kwang Hong [1], Yasuo Nagato *

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

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Abstract

We analyzed an embryo mutant *apical displacement1* (*apd1*) that formed an underdeveloped shoot at the apex of the embryo and one or two radicles in the enlarged basal region. At the early embryonic stage, *apd1* initiated shoot and radicle in almost normal positions. However, in later stages, reduction of the apical region and enlargement of the basal region became gradually more pronounced. Consequently, a shoot and a radicle were shifted toward the apical position; the second radicle was formed below the first one. The *apd1* plant showed pleiotropic phenotypes in shoots and roots: many tillers, narrow leaves, short roots and small inflorescences. In *shl1-2 apd1* double mutant, a shoot was deleted, as in *shl1-2*. In addition, the number of radicles was more than that of *apd1*. This result suggests that *APD1* and *SHL1* function redundantly in the regulation of basal region size. Taken together, these results suggest that *APD1* gene regulates the development of shoot and the regionalization along apical–basal axis. Moreover, we infer that in rice embryogenesis, sizes of apical and basal regions are under compensatory regulation.

Keywords: Rice; *Apical displacement1*; Embryo; Pattern formation; Shoot; Radicle

1. Introduction

In most higher plants, shoot apical meristem (SAM) and root apical meristem (RAM) are initiated during embryogenesis. The position and the number of these meristems are stable in an embryo. Thus, the basic body plan of plants is established during embryogenesis. Embryo pattern formation is an interesting issue for understanding plant ontogenesis.

Pattern formation during embryogenesis has been studied vigorously in *Drosophila*, and genetic models have been proposed [1–3]. On the contrary, pattern formation in plants remains almost unknown. In dicotyledonous plants, embryo mutants affecting pattern formation have been reported in *Arabidopsis*. Mayer et al. [4] reported mutants that showed the loss of apical (SAM and cotyledons in *gurke* (*gk*)), central (hypocotyl in *fackel*), basal (hypocotyl and radicle in the *monopteros* (*mp*)) or terminal (SAM, cotyledons and radicle in *gnom* (*gn*)) region. Based on phenotypic analysis, they concluded that the *Arabidopsis* embryo comprises three regions (apical, central and basal) along the apical–basal axis. These mutant genes have been cloned. However, their expression domain does not correspond to the region that is missing in the respective mutant [5–10]. However, the plant hormone auxin was recently clarified as playing an important role on embryonic pattern formation that involves some of the above mutants [11].

Apart from the genetic studies, experiments in which auxin or auxin transport inhibitor was applied to young embryos of Indian mustard and wheat have yielded interesting results concerning embryonic regionalization. Globular embryos treated with high concentrations of auxin tended to arrest at the globular stage before organ differentiation [12,13]. An auxin transport inhibitor frequently caused fused cotyledons and duplicated organs in

* Corresponding author. Tel.: +81 3 5841 5063; fax: +81 3 5841 5063.
E-mail address: anagato@mail.ecc.u-tokyo.ac.jp (Y. Nagato).
[1] Present address: College of Agriculture and Life Sciences, Kangwon National, University, Chuncheon, Kangwon-Do 200-701, South Korea.

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Indian mustard embryos [13,14], and the multiplication of organs (multiple scutella, SAMs or embryos) in wheat embryos [12,15,16]. Therefore, the auxin polar transport appears to be involved in embryo pattern regionalization.

Regarding monocots, a number of embryo mutants have been identified in rice and maize [17–19], including those lacking embryo organ(s), those showing aberrant organ morphology, those with modified embryo size, and so on. Among them, shootless and embryo size mutants have been analyzed in detail [20–23]. However, almost no studies have addressed embryonic pattern formation. In rice embryos, SAM and radicle are differentiated in the apical and basal region, respectively. Along the dorsoventral axis, the scutellum and SAM are formed in the dorsal and ventral regions, respectively. This pattern is sufficiently stable that it suggests a strict regulation of embryo regionalization is operating. Hong et al. [19] identified many rice embryo mutants including putative pattern mutants. These mutants would be useful for addressing mechanisms of embryonic pattern formation.

We analyzed a unique mutant *apical displacement1* (*apd1*) identified by Hong et al. [19] to obtain information about the pattern formation in rice embryo. The *apd1* formed one underdeveloped shoot at the apex and one or two radicles in the basal region of embryo. The analysis of *apd1* embryo suggests that the sizes of apical and basal regions are under compensatory regulation. Possible functions of *APD1* and embryo pattern formation are discussed.

2. Materials and methods

2.1. Plant materials

We used a single recessive mutant of rice (*Oryza sativa* L.) in the M_2 population of cv. Taichung 65 mutagenized with *N*-methyl-*N*-nitrosourea [19]. Because the shoot apex of the mutant was positioned at the apex of embryo, it was designated *apd1*. We sterilized seeds of the mutant and wild-type siblings in 2% hypochlorite. They were germinated in plant boxes containing MS medium [24] supplemented with 6% sucrose and 1% agar at pH 5.8 at 28 °C. After 1 month of germination, seedlings were transplanted to pots.

2.2. Paraffin sections

Developing embryos at various stages were fixed in FAA (formalin:acetic acid:50% ethanol = 1:1:18), then dehydrated in a graded ethanol series. After substitution with xylene, they were embedded in paraffin: Paraplast Plus (Oxford Labware, St. Louis, MO). Subsequently, they were cut into 8-μm-thick sections using a rotary microtome. Sections were stained with hematoxylin and observed with a light microscope (AX-80; Olympus Optical Co., Tokyo, Japan).

2.3. In situ hybridization

The paraffin sections prepared as above were applied onto glass slides coated with Vectabond (Vector Laboratories, Inc., Burlingame, CA). Digoxygenin-labeled antisense probes were prepared from the coding region of *OSH1* without a poly A end. We conducted in situ hybridization and detection of signals by the methods of Kouchi and Hata [25].

2.4. Preparation of double mutant

To elucidated the interaction of *APD1* with *SHOOTLESS1* (*SHL1*) that is required for shoot formation in embryo, we crossed heterozygous *shl1-2* plants with pollen of heterozygous *apd1* plants. The *shl1-2* mutant fails to form SAM in embryo but forms a radicle normally [20]. We examined the double mutant phenotype in F_3 seeds.

3. Results

3.1. Phenotype of mature apd1 embryo

Wild-type embryos differentiate one shoot with three leaf primordia in the apical region and one radicle in the basal region on the lemma side (Fig. 1A). However, in *apd1* embryos, one immature shoot was positioned at the apex; one or two radicles were in the basal region (Fig. 1B and C). Most *apd1* embryos had only two leaf primordia. In the basal region, about 40% of *apd1* embryos had two radicles (Fig. 1C). When two radicles were present, the upper one was directed toward the suspensor as the wild-type radicle; the basal one was directed toward the ventral (lemma) side (Fig. 1C). Embryos with three radicles were not observed. The scutellum and coleoptile were underdeveloped (Fig. 1B and C). The cells in the scuttelum were vacuolated (Fig. 1B and C).

The above results suggest that sizes of apical and basal regions were modified in *apd1*. Therefore, we measured the lengths of apical and basal regions. This study defines the boundary of apical and basal regions as the center line between the shoot and radicle. The wild-type embryo was 1926 μm long, on average, comprising a 1414-μm long apical region and a 512-μm long basal region (Fig. 1D). In contrast, the *apd1* embryo with one radicle was 1373 μm long, consisting of a 547 μm apical region and an 826 μm basal region (Fig. 1D). The length of the *apd1* embryo with two radicles was 1237 μm divided into a 408 μm apical region and and 829 μm basal region (Fig. 1D). Therefore, the apical region was reduced and basal region was enlarged in the *apd1* embryo.

3.2. Developments of apd1 embryo

Wild-type embryos typically remain globular for 3 days after pollination (3 DAP). At 4 DAP, SAM and coleoptile