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Characterization of the Rice L2 Layer Aberrant Differentiation Mutant *lad1*

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Abstract

A mutagenesis rice mutant L2 layer aberrant differentiation *lad1* have the differentiation of L2 layers of male and female reproductive organs. Genetic analysis indicated *lad1* was a single recessive gene. By microscopic observation, we discovered the *lad1* mutant had one or two regular layers in the anther wall and at least seven archespores or archespore-like cells in the ovules in stage Ov2. Because of seed setting by being pollinated with the pollen of wild type, at least one MMC can undergo meiosis and produce functional megaspores. By compared with *nzz*/*spl* mutants in Arabidopsis and *mps1* mutant in rice and microscopic observation, we purpose that *LAD1* gene should be an allele or upstream gene of *MSP1* and regulate the development and differentiation of L2 layers in anthers and ovules by cell communication between cell layers.

Keywords: Differentiation; Aberrant; L2 layer; Mutant; Rice (*Oryza sativa L.*)

1. Introduction

The plant body develops from groups of dividing cells, the meristems. Each of the meristems is able to produce certain structures. After floral induction, the shoot apical meristem (SAM) produces the floral primordial. Then the floral primordial goes through a series of anticlinal and cell divisions to form the four whorl organs (one lemma and one palea, two lodicules, six stamens and one gynoecium with two stigmas from outside to inside in rice). The process of forming the organs goes with anticlinal cell divisions to produce regular layers in wild type, especially in anther and carpel of wild type.

The cell lineages produced by each meristem layer usually contribute to distinct regions with each organ in a way, but the instances show that there are invasion between the different layers in certain organ, i.e. cells in different layers are not restricted in the nature fate. The development fate of cells is decided by position rather than by cell lineage [1]. In Antirrhinum, the second whorl of def mutant is sepal rather than petal, and the third is carpels rather than stamens [2]. As mentioned above, the similar phenotype exists in rice, too. DEF gene may play a role in generating a signal that moves from L2 layer to L1layer and determines the pattern of differentiation [3]. In rice, msp1 mutant produces multispore at the expense of the tapetum in anther and have at least four megasporocytes [4]. This is, the cells with distinct function from the L2 layer are capable of communicating. In the present study, we describe the characterization of the L2 layer development defect mutant, lad1. We compared lad1 with msp1in rice and nzz in Arabidopsis and discuss the possible relationship among these genes.

2. Materials and Methods

2.1 Plant materials

Rice mutant materials were screened in Wenjiang, China, and were observed continuously in Wenjiang and Hainan, respectively. Inflorescences and flower buds of all sizes and stages were fixed in FAA (formalin: acetic acid:70% ethanol 5:5:90, by volume).

2.2 Paraffin sectioning

Flowers were fixed in FAA for approximately 1d at room temperature (RM), dehydrated in a graded ethanol series, and mounted in paraffin. Samples were sectioned at 8 um, stained with 0.5%Tiluiodine blue in 4-5 min, rinsed with distilled water twice and air-dried at RM. Slides were dewaxed with dimethylbenzenexylene. Images were taken under Olympus light microscopes.

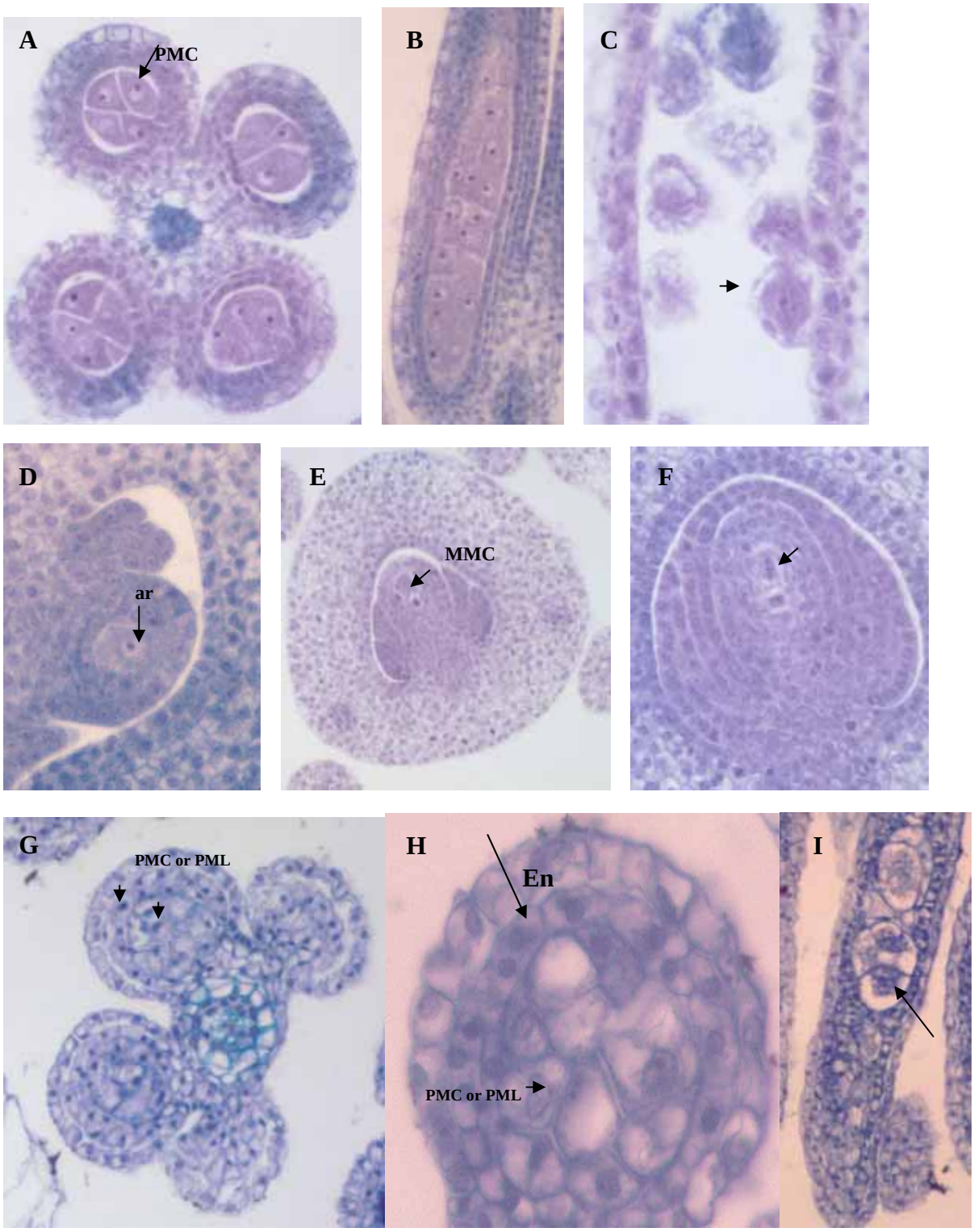
2.3 Scanning electron microscopy (SEM)

Procedures for the scanning electron microscopy were modifed according to Feng et al. [5] and Mizukami and Ma [6]. Young panicles at various

stages were fixed in glutaraldehyde buffer (3%) for 12h at 4°C, and dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90%, 100%, 100%, each for 15min), and incubated in isoamyl acetate twice for 30min. Then, samples were dried, pasted on the specimen stage and coated by gold film. The samples were photographed with SEM (KYKY-1000B) at 25kV.

3. Results and discussion

3.1 Screening and genetic analysis of the *Idd1* mutant



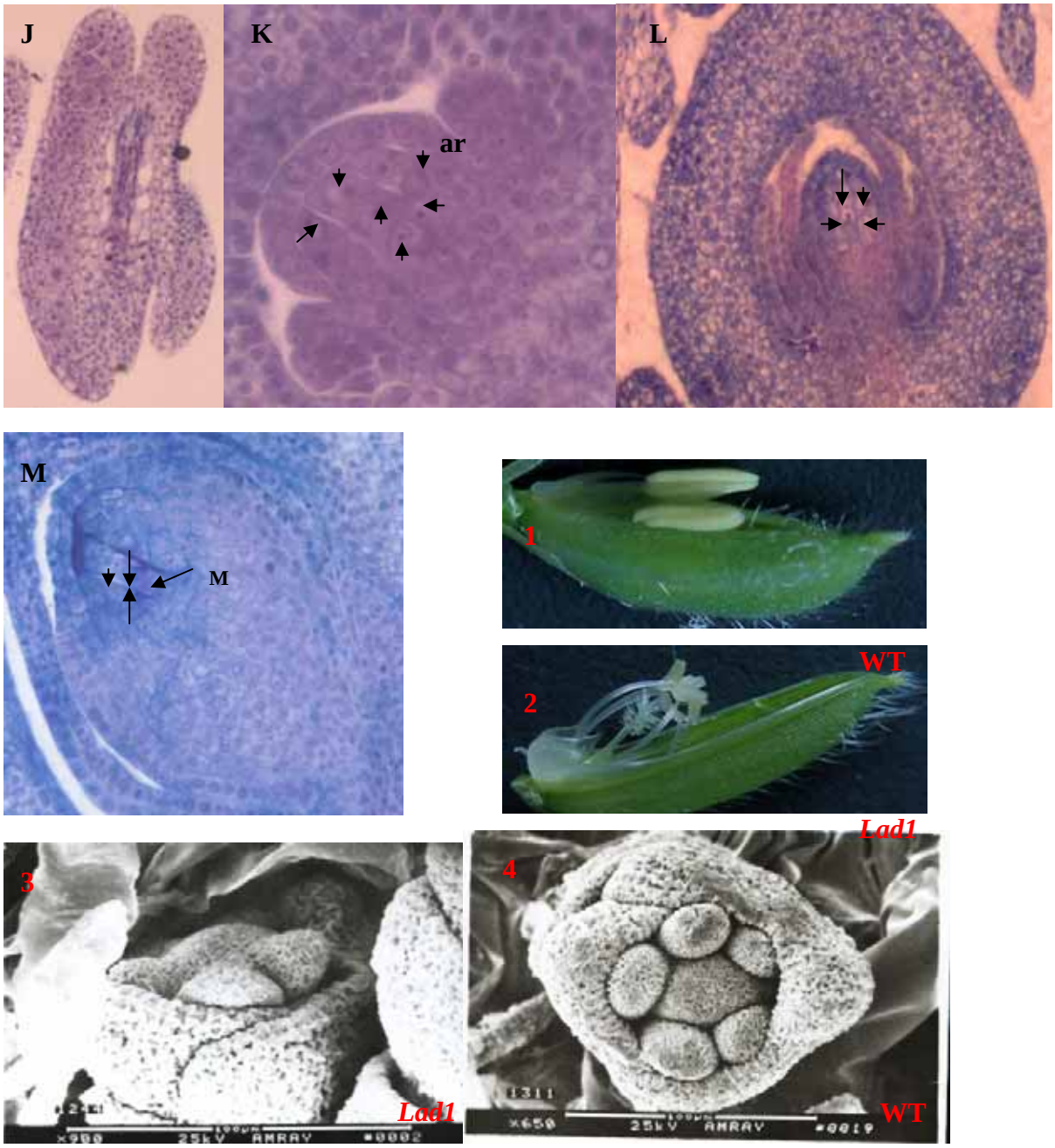


Figure 1. Histological features of male and female reproductive organ development in the wild type and the lad1 mutant (A) and (B)Transverse and longitudinal sections of anther of the wild type. There are four regular layers in the anther wall and PMCs.. (C) Longitudinal section of anther of the wild type. PMCs undergo meiosis. (D) to (F) Longitudinal sections of ovule of the wild type. (D) There is an archespore (ar) in ovule. (E) There is a megaspore in ovule. (F) A megaspore undergoes meiosis. (G) to (J) The transverse and longitudinal sections of anther of the lad1 mutant. (G) and (H) The one and two regular layers are observed in anther wall of lad1 mutant, respectively. (I) PMCs vacuolates. (J) the cells in anther of *lad1* mutant are much more than those of anther of the wild type. (K) to (M) Longitudinal sections of ovule of the *lad1* mutant.(K) At least seven archespores are observed at stage Ov2. (L) At least four MMCs are observed at stage Ov3. (M) four megaspores (M) are observed.

The lad1 mutant was screened in the T-DNA inserted mutant library with Zhonghua11 (ZH11) as receptor, but had not T-DNA by PCR testing. Hence, the mutant was considered as a mutagenesis mutant in the course of tissue culture. The length of the mutant anthers was about one fourth of the length of the ZH11 anthers, and the anther appeared crimped, twisted, white and transparent (Figure1-1,2). The phenotype was segregated as a single recessive mutation (sterile: fertile, 16:56, X 2=0.3 for 1:3). Observation of the mutant phenotype revealed that the differentiation of L2 layers in anther and ovule showed aberrant. Hence, we designated this mutant lad1 according to the phenotype.

3.2The phenotype of the *lad1*mutant anther

In this study, we compared histological features of the anthers between wild type ZH11 and the lad1 mutant. Refered as Sanders P.M. et al [7] and Nonomura K. I. et al [4], stamen primordia consist of the three layers, L1, L2 and L3. In wild type, archesporial cells arising in four "corners" of L2 layer divided periclinally into primary parietal cell (PPC) and primary sporogenous cell (PSC) lineages that differentiated into the endothecium, middle layer, tapetum, and microspore mother cells from stage 1 to 4. At stage 5, four clearly defined locules established and microspore mother cells appeared. From stage 1 to 2, the size of stamen primordial of the lad1 mutant was almost the same as that of the wild type (Figure1-3,4). The smaller anther of lad1 mutant was the result of the following differentiation of stamen primordial. In stage 3, the distinct difference lied in the result of the differentiation of L2 layer. Namely, archesporial cells did divide in PSC and PSC-like lineages instead of PPC lineage. In the anther of the lad1 mutant, there are pollen-mother-cell-like (PMC-like) cells and epidermal layer cells, but not the regular layers of endothecium cells, middle layer and tapetum cell and callose accumulation (Figure1G, H). This is, the anther wall of the lad1 mutant consisted of one regular layer and disorder PMC-like cells, instead of four regular layers in wild type. The epidermal layer cells of lad1 mutant anthers fail to elongate. In stage 5, the PMC or PMC-like cells (PML) began to vacuolated and did not enter meiosis to show no pollen in anthesis (Figure1 I). At the same time, there are more cells in anther of the lad1 mutant than those of the wild type(Figure1 J), which suggests that the cells of anther of lad1 mutant continue to divide when the cells of anther of the wild type stop dividing. Hence, these observations suggested that the lad1 mutant altered the differentiation of L2 layer and influenced the elongation of epidermal layer cells and the deposition of callose to show smaller, twist and transparent aberrant phenotype in anther.

3.3 The phenotype of the *lad1* mutant ovule

In wild type, an ovule consist of a nucellus (megasporangium), two integuments commonly, and the funiculus [8]. In common, ovules arise from three cell layers (L1, L2 and L3) of the placental region [9].The outer integument develops from both epidermal and subepidermal cells, and the inner integument originates from the L1layer. Nucellus which consist of both vegetative and sporogenous cells and funiculus arise from L2 and L3 layer, respectively. Refered as Lopez-Dee Z.D. et al [10], the development of rice ovule is divided into nine stages. In stage 2, the archespore forms from one of the subepidermal cells in nucellus(Figure1 D). In stage 3, the archespore differentiates into the megaspore mother cell (MMC)(Figure1 E). As the megaspore mother cells expands, its cytoplasm becomes less dense than those of the surrounding nucellus cells. In stage 4, meiotic division of the megaspore mother cell results in a linear tetrad of megaspores (Figure1 F).

In wild type ovule, only one archespore and oneMMC are observed. But in the lad1 mutant ovule, at least seven archespores or archespore-like cells are observed with normally developing inner and outer integuments (Figure1 K). Archespores are able to differentiate into MMCs, at least four MMCs are observed (Figure1 L). MMCs enter meiosis and produce four megaspores (Figure1 K). Since the mutant is capable of setting seeds when it is pollinated with wild-type pollen, this suggests at least one of megaspores is functional. In nucellus of the lad1 mutant, the tendency of transforming vegetative cells to MMC-like cells from proximal to distal is stronger gradually.

3.4 The lad1 mutant and other mutants affecting cell differentiation

As far, it has been reported that a few mutants for example, spl/nzz mutants in Arabidopsis [11-13] and msp1mutant in rice [4] affect the differentiation and development of not only microsporocytes but also megasporocytes. The nzz mutants mainly show the alterations of anthers and ovules. In early stages, MMCs in ovule and the initial periclinal division of the archesporial cells are not observed. Inner integument is reduced or missing and the funiculus is longer. So, in later stages, nuclellus , embryo sac and microsporocytes are not observed. In spl mutants, except no alteration of integument and funiculus, the other alteration is same to the nzz mutants. Hence, SPL and NZZ genes are considered as alleles. In rice, compared with msp1 mutants, we found that the difference between lad1 and msp1mutants included the number of anther wall layers, the size of anther and the number of MMCs. Hence, at the base of the relationship of NZZ and SPL, we purpose that the relationship of MSP1 and LAD1may be similar to that of NZZ and SPL, allele. The difference between the phenotype of msp1 and lad1 mutants may be the result of allele-specific effects or the genetic background [12]. But we could not exclude the possibility that LAD1 was the upstream gene of MSP1 in the pathway of floral differentiation and development. In addition, In msp1 mutants, some seeds obtained by pollinated with the pollen of wild type have two embryos. Because we don't obtain enough seeds by pollinated with the pollen of wild type, the phenomenon of two embryos is not observed. But the rate of seed-setting is lower than that of general cross.

In lad1 mutant, nuclellus and embryo sac can be observed, and inner and outer integuments are normal. Because there is the conservation of genes regulating floral differentiation and development among different species, we assume that the genes homologous to SPL/NZZ genes in rice should be an upstream gene of LAD1.

3.5 the function of LAD1 gene in rice

Cell division and differentiation are essential for the development of multicellular organisms, and cell signal communication between cell layers is important for the regulation of cell division and differentiation. In recent years, some genes relative to cell communication such as CLAVTA gene family BAM1/BAM2 [14], ERECTA [15], TPD1 [16,17] EXS/EMS1 [18,19], UDT1 [20], BEL1 [21], SIN [21], and so on are studied. These genes only regulate the differentiation and development of male or female organ. But the LAD1gene regulates the differentiation and development of not only male but female organ, which suggest the LAD1 gene have equivalent functions during the early establishment of the two types of sporangia in the respect of evolution. With coupled with heterosporangy, higher plants exhibit heterospory, which indicates heterospory evolved from homospory [22]. This is, the ancestor gene of LAD1 regulates the differentiation of L2 layer of homosporous species by cell communication between cell layers. So the mutation of LAD1 lead to the dysfunction of L2 layers, which causes the cells from L2 layers to show uniform tendence (many PMC-like cells in anther and several MMC-like cells in ovules).

In anther development, the function of *LAD1* may be similar to those of *CLV1* and *BAM1/BAM2*. Namely, *LAD1* may regulate the balance between the central zone cells and peripheral zone cells in a manner that it may negatively the number of sporogenous cells by promoting the differentiation of the adjacent somatic cells or reducing the division of sporogenous cells [14,23-25]. By compared with *ems1/exe*, *bam1/bam2*, *msp1* and *lad1* mutants, it is possible that the formation of PMCs and MMCs may result from a default pathway and the differentiation and development of the other somatic cell layers and vegetable cells require the media of additional signaling. After the formation of PMCs or PMC-like cells, these PMCs or PMC-like cells followed to vacuolate and degenerate. In addition, because the epidermal cells originating from L1 layer in *lad1* mutant fail to elongate, we purpose that *LAD1* may regulate the elongation of cells of L1 layer indirectly by the communication of cell layers.

Conclusions

In the study, the *lad1* mutant shows the defect of anther, ovule and the abnormity of the number of MMCs and megaspores, which suggests that the *LAD1* gene may have equivalent functions during the early establishment of the two types of sporangia in the respect of evolution or regulate the development and differentiation of anther and ovule by the default pathway. Due to the aberrant development of L2 layer in anther and ovule, the rice mutant was named as *lad1* mutant.

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