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Developmental mutants showing abnormal organ differentiation in rice embryos

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Summary. Zygotes of rice (*Oryza sativa* L. cv Taichung 65) were treated with 1.0 m*M* solution of the chemical mutagen N-methyl-N-nitrosourea. Out of 1420 M_2 lines, 28 single-locus recessive mutants on embryogenesis were identified. Among them, we analyzed 11 mutants in the present study, which differentiated the shoot (plumule) and/or root (radicle) with abnormality. Of the 11 mutants, two showed no shoot differentiation with normal root. On the other hand, we could not detect any mutant which exhibited a normal shoot without a root. This suggests that shoot and root are genetically controlled by different loci and that the alleles associated with shoot formation mutate more frequently than do those of the root. Five mutants showed aberrant morphology of shoot when both the shoot and root developed. One of them, odm 5 (organ differententiation mutant 5) was germinable, but produced many fine and twisted leaves. This mutant was, however, lethal at the early post-germination stage under the usual cultural conditions. In another mutant (odm 4), shoot differentiation seemed to be initiated at an arbitrary position, resulting in a very abnormal morphology of the shoot when the position fronted the endosperm. The other two mutants showed abnormal morphology of both the shoot and root. One (odm 11) of the remaining two mutants showed a wide variation of abnormalities including no organ differentiation, either shoot or root differentiation and the development of both shoot and root with abnormalities. The last one (odm 16) was unique. It had an embryo with normal shoot and root but the embryo size was only one-third to one-half of normal embryos in length. Of course, the shoot and root are also small but viable. Therefore, odm 16 is considered to be a mutant in the size regulation of the embryo. Although an allelism test has not yet been done, most of these mutants are probably non-allelic, as the phenotypic abnormality differs largely with each one. In rice, the shoot and root highly differentiate in contrast to dicotyledonous embryo. Accordingly, these developmental mutants are very useful materials for investigating the regulatory mechanism of gene expression in organ differentiation.

Key words: Developmental mutants – Organ differentiation – Rice embryogenesis

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Introduction

Organ differentiation is a very important phenomenon for many developmental biologists. Although anatomical, nutritional and biochemical information on embryogenesis has accumulated (Raghavan 1986), the genetic regulation of organ differentiation during embryogenesis is still poorly understood. Recently, the regulation of gene expression during animal embryogenesis has been extensively studied. In *Drosophila,* many segmentation genes and homoeotic genes controlling early morphogenesis have been isolated, leading to the discovery of homoeobox common to many animal species, including humans, mice, chicken, fish, annelid, etc . . . (Lewis 1978; McGinnis et al. 1984a, b; Scott and Weiner 1984). It should be noted that these recent findings in animals have been made using various developmental mutants. Therefore, it is conceivable that plant embryogenesis and organ differentiation can be genetically analyzed and un-

derstood if appropriate mutants of organ differentiation are available.

In *Arabidopsis thaliana* (Meinke and Sussex 1979; Meinke 1985) and maize (Neuffer and Sheridan 1980; Sheridan and Clark 1987), lethal mutants affecting organ differentiation in the embryo were isolated through the screening of defective or aborted seeds. In spite of the above worker's efforts, the regulatory mechanism of organ development during embryogenesis is not understood. Gramineous species produce complex embryos containing plumule with more than one foliar leaf, radicle, scutellum, epiblast, etc. This means that most morphogenetic events in plant development are concentrated into embryogenesis, indicating that gramineous plants are good material to study the mechanism of organ differentiation. Further, the presence of an abnormal endosperm may deleteriously affect the development of a genetically normal embryo resulting in the lethal or abnormal embryo, as suggested in maize (Neuffer and Sheridan 1980). Accordingly, in the present study with rice, we describe mutants which alter organ differentiation in the embryo of the seed but do not affect normal endosperm development.

Materials and methods

Rice (*Oryza sativa* L. cv Taichung 65) was used. A 1.0 m*M* solution of chemical mutagen N-methyl-N-nitrosourea was applied to zygotes set on the normally grown plants as described in Satoh and Omura (1986). M_1 plants obtained from the chemical treatment were grown and self-pollinated. In the next year, we randomly collected single plants from 1420 M_2 lines and screened embryogenic mutants on M_3 seeds set on M_2 plants as follows. First, only seeds with normal endosperm were tested for germination and seeds not showing normal germination were collected. Then, these were sectioned by the usual paraffin method and the morphological abnormalities of embryos were examined. Finally, 28 mutants were detected which showed abnormal embryos in about 25% of the seeds, suggesting single-locus, recessive mutants. They were named as odm 1–28 (organ differentiation mutants 1–28).

Among them, two types of mutants were recognized. Eleven mutants differentiated the shoot (plumule) and/or root (radicle) with abnormality. The other 17 mutants failed to differentiate both organs. In the present experiment, we used the former 11 mutants showing abnormal organ differentiation for further analysis.

In the next year, M_3 seeds of 11 mutants were grown under standard cultural conditions. In seven odm lines, mutant embryos could not germinate. Embryos in the remaining four mutants could germinate but failed to develop to maturity under standard cultural conditions because of the weakness or the lack of shoot. As the dominant homozygous plant and heterozygous plant could not be phenotypically distinguished in each mutant, we collected 20 seeds from individual plants at various developmental stages after flowering and sectioned them by the usual paraffin method. After examining for the presence of abnormal embryos, the frequency of heterozygous plants among all the plants grown was calculated in each mutant. We also examined the frequency of mutant embryos in each heterozygous plant.

Table 1. Frequency of heterozygous plants grown from M_3 seeds

Mutant	No. plants examined	No. heterozygous plants (%)	Chi-square (1:2)
odm 4	8	5 (62.5)	0.063
odm 5	42	25 (59.5)	0.965
odm 6	11	7 (63.6)	0.046
odm 11	24	15 (62.5)	0.188
odm 14	15	8 (53.3)	1.201
odm 16	22	12 (54.5)	1.455
odm 18	28	19 (67.9)	0.018
odm 19	23	15 (65.2)	0.022
odm 22	25	16 (64.0)	0.080
odm 24	11	6 (54.5)	0.728
odm 26	15	9 (60.0)	0.300

Table 2. Frequency of mutant embryos set on M_3 heterozygous plants

Mutant	No. embryos examined	No. mutant embryos (%)	Chi-square (3:1)
odm 4	136	35 (25.7)	0.039
odm 5	270	68 (25.2)	0.005
odm 6	115	33 (28.9)	0.838
odm 11	260	64 (24.6)	0.021
odm 14	176	43 (24.4)	0.030
odm 16	255	66 (25.9)	0.106
odm 18	259	57 (22.0)	1.237
odm 19	270	62 (23.0)	0.598
odm 22	256	63 (24.6)	0.021
odm 24	106	33 (31.1)	2.126
odm 26	123	28 (22.8)	0.328

Results

Segregation of mutant embryos and heterozygous plants

First, we examined a segregation ratio of heterozygous M_3 plants grown from the seeds of each 11 mutant. As shown in Table 1, the frequency of heterozygous plants in each mutant did not significantly deviate from a 1 (dominant homozygous): 2 (heterozygous) ratio, which would be expected if the mutant was recessive at a single locus.

Next, the frequency of mutant embryos in the heterozygous plants was calculated (Table 2). In all the mutants, the percentages were ca. 25% and did not significantly deviate from a 3:1 ratio. These results confirmed that all mutants were the result of single-locus, recessive mutations.

Characteristics of 11 developmental mutants

Embryos of the 11 mutants were sectioned for anatomical observation. Also, the germination was tested. Morphological characteristics of each mutant are as follows (Fig. 1).

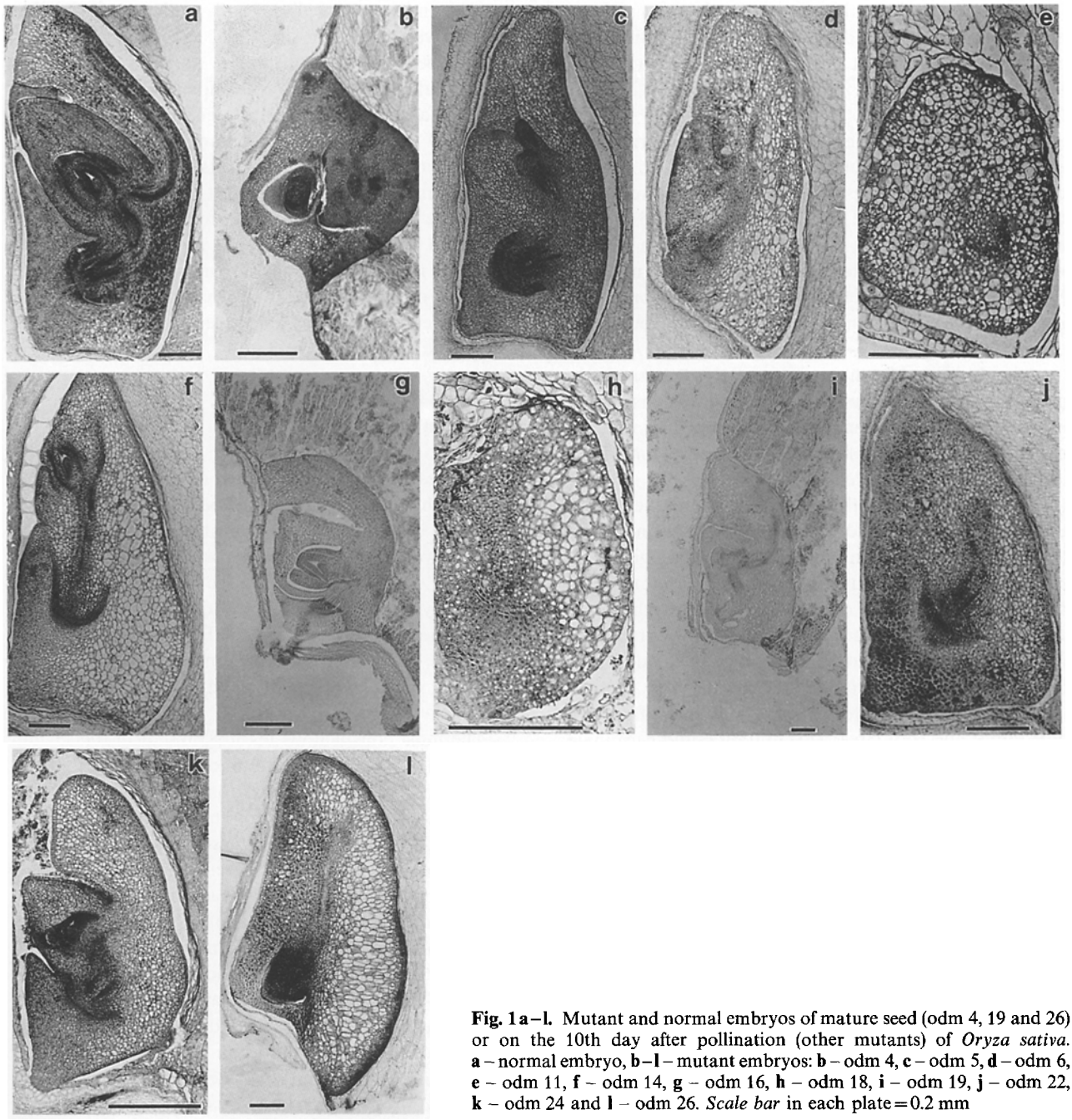

Fig. 1 a–l. Mutant and normal embryos of mature seed (odm 4, 19 and 26) or on the 10th day after pollination (other mutants) of *Oryza sativa*. **a** – normal embryo, **b–l** – mutant embryos: **b** – odm 4, **c** – odm 5, **d** – odm 6, **e** – odm 11, **f** – odm 14, **g** – odm 16, **h** – odm 18, **i** – odm 19, **j** – odm 22, **k** – odm 24 and **l** – odm 26. *Scale bar* in each plate = 0.2 mm

odm 4 (Fig. 1 b): this mutant showed a wide variation of shoot abnormalities. In general, the root developed normally in the mutant seed. On the other hand, shoots in most mutant embryos aborted at various stages or showed gross morphological aberrations. Also observed were embryos in which the position of morphologically normal shoots had somewhat deviated from the normal position. Aborted or morphologically abnormal shoots were positioned on the opposite side of the normal position, i.e. in contact with the endosperm. Therefore, this mutation is considered to randomize the position of shoot differentiation without affecting the differentiation of the root.

odm 5 (Fig. 1 c): embryos of this mutant were viable in mature seed and able to germinate. Many fine and twisted leaves emerged on germination but soon died under standard cultural conditions. In mature embryos, the root was normal and the shoot had three foliar leaves with abnormal morphology. This mutant seems to influence the organization of the shoot meristem.

odm 6 (Fig. 1 d): in this mutant, both shoot and root meristems differentiated but soon aborted during an early stage. As a result, many embryos had no leaves, though some embryos grew vigorously (ca. 2,000 μm long at maturity).

odm 11 (Fig. 1 e): there existed a wide variation of abnormalities in this mutant. The abnormalities could be classified into four types. First, no organ differentiation was recognized. Second, the shoot was formed to some extent but root did not differentiate. Third, in contrast with the second type, a root was produced with no shoot. Fourth, both shoot and root developed but aborted during development. All the mutant embyros grew longer than 1100 μm.

odm 14 (Fig. 1 f): the embryo of this mutant had a normal root but aberrant shoot. The shoot was formed in the apical region of embryo where scutellum was normally expected to develop. The first and second foliar leaves were recognizable.

odm 16 (Fig. 1 g): this mutant was unique in that unlike the other mutations, this mutation did not affect the organ differentiation itself or the morphologies of the shoot and root. The mature embryo was very small (only one-third to one-half of normal embryo in length). Shoot and root meristems were morphologically normal and viable. Although the scutellum did not develop normally, the reduction of embryo size was not only the result of the underdeveloped scutellum but also of the reduction in both shoot and root sizes. This mutant could germinate, producing very fine leaves and roots. We consider that this mutant is caused by the mutation of a gene regulating embryo size.

odm 18 (Fig. 1 h): in this mutant, two types of embryos were present. One showed no organ differentiation and the other showed a normal root formation with a rudimentary shoot. Most of the embryos lacking organ differentiation were relatively small (400–1000 μm in length). On the other hand, embryos developing root were 900–1400 μm long. These embryos were aborted before seed maturity.

odm 19 (Fig. 1 l): in this mutant, both shoot and root developed but showed morphological abnormalities. The abnormalities were more extreme in the root than in the shoot. Mature embryo size was 1300–1800 μm long.

odm 22 and odm 26 (Fig. 1 j, l): these two mutants showed the same abnormality. The embryo had a viable and normal root but no shoot primordium. Accordingly, only the root emerged upon germination and elongated normally without any shoot formation if the seed were sown on a culture medium. This type of mutant clearly shows that the root and shoot are genetically separable from one other. In other words, the root is capable of developing autonomously with no dependence on shoot.

odm 24 (Fig. 1 k): the embryo had both a shoot and a root but the two organs were located very close to one another and were morphologically underdeveloped. The embryo was normal in size (ca. 2000 μm in length) but aborted before seed maturity.

In total, we detected 11 single-locus recessive mutants showing abnormal organ differentiation as described above. Of the 11 mutants, 2 mutants exhibited root formation with no shoot. In 5 mutants, the embryos showed aberrant morphology of the shoot when both shoot and root were produced. In 2 mutants, the embryos contained an underdeveloped shoot and root. There also existed a mutant in which the embryo showed a wide variation of abnormalities. The last mutant was unique in that the mutation was associated with controlling the size of the embryo.

Discussion

In the present study, we screened embryonic mutants in seeds with normal endosperm, because endosperm affects the embryonic development. We sometimes observed severely defective embryos in seeds lacking endosperm. This suggests that the development of the genetically normal embryo is influenced by endosperm development. The developmental mutants reported in this study have normal endosperms. Thus, the deleterious effect of the endosperm on embryonic development is not a factor.

The most conspicuous feature present in the 11 mutants is that no mutant was obtained in which the embryo showed only shoot differentiation without that of the root. Such a situation was also reported in maize (Sheridan and Clark 1987). This suggests that the number of gene loci controlling root differentiation is fewer than that of shoot differentiation, at least in these Gramineae species. On the other hand, a root-less mutant with abnormal cotyledon was detected in *Arabidopsis thaliana* (Meinke et al. 1985), indicating the possibility of obtaining a root-less mutant in rice. It appears, however, that the genetic mechanism associated with shoot development is more subject to mutation than that of the root.

The wide range of mutations detected in organ differentiation during embryogenesis suggests that a large number of gene loci are associated with shoot and root differentiation. Genes controlling organ differentiation may be classified into several groups: genes controlling the onsets of shoot and/or root differentiations as in odm 22 and 26, genes controlling the position of shoot differentiation as in odm 4, genes controlling the morphological development of the shoot and/or root as in odm 5, 6, 14, 18, 19 and 24, and genes controlling the sizes of shoot and/or root as in odm 16. Of course, it should be noted that each group classified above will be composed of many genes, each performing a distinctive role in organ differentiation.

The present results indicate that a spatially and temporally cooperative expression of many genes is required for the normal differentiation of the shoot and root. At this point, we have practically no information on the cooperative expression. The developmental mutants obtained in the present study will be very useful for revealing the regulatory mechanism of organ differentiation, because all are single-locus mutants and cover a wide range of abnormalities. Analytical research using these developmental mutants will be needed from various aspects.

All the present developmental mutants alter the differentiation of the shoot and/or root. There is another type of embryonic mutant in which no organ differentiation is present. Such mutations will be manifested in the early embryogenesis. The entire developmental mechanism of the embryo can be investigated using such mutants and the mutants isolated in the present study.

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