Developmental characterization of embryo size mutant in rice (*Oryza sativa* L.)

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ABSTRACT

In this experiment, three kinds of mutations(*ge, re, and eml*)relating to the size of embryos were used to study their generation, genetic mechanism and developmental characteristics, and the interactions between embryo and endosperm were also examined. Giant embryo mutation comprises 7 kinds including the already isolated *ge*, and *ge-2*, which share an identical gene site. The SAM and the size of radicule for the *ge* showed little difference compared to a normal type. The number of embryo cells did not increased as much as it would affect the size of embryo. Therefore, the enlargement of embryo was due to the enlargement of scutellum that originated from the corpulence of each cell.

Both F_1' s of *re 1* and odm 49 formed reduce embryos, and other combinations of hybridization showed all wild type of embryo sizes. Accordingly, the odm 49 must have an identical gene site of *re 1*, while odm 48 and odm 62 have different gene sites. Their shoots and radicules also shrank by the same ratio, however no sign of physical change was noticed. The size of embryo cell showed no change, while the number of cells was the half of that of wild types. The three gene sites of *re* represent all of them control the size of the entire embryo forming organs.

The *eml 1* was defined to have temperature sensibilities that the generation of endosperms was active at a high temperature while that was hampered at a low temperature.

Key Words : Embryogenesis, Embryo, Endosperm, giant *embryo(ge), reduced embryo(re), embryoless(eml),* Developmental Genetics

INTRODUCTION

Until now, few researches have been made to study whether the embryo size of plants is genetically controlled. Added to that, excluding rice plants, no other spices have been reported to include a mutation related to the embryo size. Nonetheless, the embryo size for edible grains can be seen as one of major forms affecting the ingredient of species. Thus, finding out the genetic control system of their embryo size is a very challenging and interesting in field of study.

So far, there are two recessive *giant embryo (ge)* mutations, which have been reported (Satoh and Omura 1981; Satoh and Iwata 1990; Kitano *et al.* 1993). Besides that, more than five recessive *ge* mutations are

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isolated. Although many researches have been conducted in relation to the growth of embryos mostly for corns (Neuffer and Sheridan 1980; Sheridan and Neuffer 1980; Miller and Chourey 1992), Arabidopsis (Meinke and Sussex 1979; Clark and Sheridan 1991; Errampalli *et al.* 1991; Jurgens *et al.* 1991; Mayer *et al.* 1991; Castle and Meinke 1993), barleys (Felker *et al.* 1985; Bosnes *et al.* 1987), and peas (Marions 1970; Lopes and Larkins 1993), however, no specific report on the embryo size is found from these researches. Therefore, in this reason, the prior mentioned reports are regarded as very important information to specify the genetical mechanism of embryo sizes. The *ge* reported by Satoh and Omura (1981) and the odm (organ developmental mutation) 30 (*ge-2*) of Kitano *et al.* (1993) are defined to have allelism. In addition, *ge-2* also has allelism with odm 44. There was no reason to determine that the three clarified *ge* ′ s are identical mutations of gene site by chance, and actually, up to date, there is no simple way to explain such results. The showing types of *ge* and *ge-2* are relatively quite unique compared to wild type mature embryos; only their scutellums are enormously enlarged while shoots and radicules are not. So far, there is no research reported on the embryogenesis or the growth in the aftermath of germination of these mutations.

In this experiment, odm 44 and other six unexplained mutations are used for allelism tests while studying their physiological characteristics based on various types of rearing to explain the genetically mechanism relating to the giantism of an embryo, and the effects of such mechanism on the rearing after germination.

Contrary to the giant embryo (3.2 mm), four mutations are isolated for the reduced embryo (0.9 mm) mutation that are smaller than a normal embryo (2.2 mm). The reduced embryo herein means that regardless of the development in their organs such as a shoot and radicule, their embryo size becomes quite smaller than normal forms. Accordingly, a mutation having diminished embryo size without experiencing an organ genesis cannot be referred as a reduced embryo (*re*). Among the reduced embryo mutation, only odm 16 (*reduce embryo*; *re 1*) has been reported to some degree of progress in study (Kitano *et al.* 1993). No specific research on the other three family lines has been reported yet. In case of the *ge*, only scutellum cells enormously enlarge while showing a generally entrenched embryo organ in *re 1*. Thus, the *re 1* seems to be the gene determining the size of the entire organs that form an embryo. Furthermore, in case of this *re 1*, the size of an embryo is defined to be affected by controlling the number of cells not by the size of cell. In this experiment, allelism tests were made on four reduced embryos-odm 16 (*re 1*), odm 48, odm 49, and odm 62-and their forms were studied as well.

Meanwhile, it is well known fact that embryoless seeds are rarely occurring among the existing plant breeding. In such cases, no sign of embryo can be found, but only endosperms show full shapes. The embryoless type due to mutation also shows similar trend that shows no trace of an embryo and has a full shape of endosperm. Currently, a few embryoless (*eml*) linage are isolated, but they are always in the state of globular embryos (*gle*) that stop growing in the beginning and then disappears. Among these, *eml 1* is generally in an embryoless shape while some wired shapes of embryos are seldom found, and the frequency of the population of recessive homozygote varies. Therefore, it was hard to explain the functionality of this gene. As a result of the screening of numerous mutations, most seeds were proven to be embryoless. Through the screening, however, a few seeds having various sizes of embryos including tiny *gle* ′ s or *ge* ′ s were found. This means that the seeds have originated from the recessive homozygote and obtained from seeds with germinative embryos in a certain condition. Accordingly, the *eml 1* not seems to be just embryoless mutations but is highly likely to be a mutation of a gene

Table 1. Phenotype of mature embryos in *giant embryo* mutations.

Line	Gene	Embryo size (㎛)		Shoot apex size (㎛)		Radicle size (㎛)		No. of cells ǂ
		Length	Thickness	Height	Diameter	Lenght	Diameter	
odm 30	*ge-2*	2494±109**	1232±53**	81±15	68±21	352±21	314±39	7915
odm 44	*ge-3*	2606±175**	1120±54**	67±13	78±16*	472±48	301±77	7890
odm 90	*ge-4*	2444±102**	1124±76**	66±11	64±13	402±18	276±34	7910
odm 93	*ge-5*	2284±218**	1052±81**	70±15	79±13*	360±41	277±50	8010
odm 124	*ge-6*	2220±364**	1040±77**	59±13	56±6	336±8	304±8	7855
odm 132	*ge-7*	2088±158**	1060±40**	54±3	58±6	346±47	330±56	7830
Wild type T-65		1860±112	996±20	75±12	68±15	299±24	331±22	7810

Size data are presented in ㎛±s.d.

ǂ Counted in medium longitudinal sections.

*.** Significantly deviated from the wild type at 5% and 1% level respectively.

that controls the size of embryo in every aspect. Therefore, in this respect, this experiment used an embryoless unit that is highly likely to be a recessive homozygote to study the temperature sensibility of this mutation and the effects of its characteristics on the size of embryo.

MATERIALS AND METHODS

The mutation linage used in this experiment is selected from M_2 family lines that are obtained by treating 0.1 mM of chemical mutagen MNU (N-methyl-N-nitrosourea) on to the immature embryo of Kinmaze and Taichung 65 (T-65). (Nagato *et al.* 1989; Kitano *et al.* 1993; Hong *et al.* 1995). odm 30 (*ge-2*), odm 44, odm 90, odm 93, odm 124, and odm 132 are the 6 *ge* ′ s used in this experiment. As a result of this experiment, each *ge* is proven to be a recessive mutation of a gene site. Four re ′ s used in the experiment are odm 16 (*re 1*), odm 48, odm 49 and odm 62, and they are also specified to be recessive mutations of a gene site. To test the allelism of each family line, reciprocal cross are carried out by preparing ample units of recessive heterozygote and heterozygote based on from each mutation family line. The size of embryo of F_1 seeds acquired from such process is measured by a microscopic examination.

To examine embryogeneses of each mutation, the embryo length, embryo thickness and the number of cells in the central section are measured by making sample slides prepared through selecting 10 units of glumes in many seasons of flowering for recessive heterozygote and 40 units for heterozygote, fixing the collected glumes with FAA fixation solution (fixation solution; formalin : acetic acid : 50 % ethanol = 1 : 1 : 18), and then dissecting those into general paraffin sections. Furthermore, the matters related to the growth after germination and germination ratios are examined as the following process.

Firstly, 20 granules from each mutation family lineage were selected, and the selected granules were placed in 70 % ethanol for 30 seconds to sterilize them and to eliminate waxes, exposed at 5 ℃ for 4 hours to assimilate water, and arranged on 6 ㎝ diameter petri-dishes lined with 2 sheets of filter. The arranged seeds were placed in an incubator fixed at 30 ℃ for about 12 hours for growth. By using a dissecting microscope, the status of germinations and the length of germinated shoots were measured. Especially for odm 16 (*re 1*) and odm 44, their plant heights had been measured several

times during the 100 days after seeding.

In case for *eml 1*, units regarded as recessive homozygote were selected. The selected units were preserved and nurtured to grow into plants, planted out, and bred under a general concealed rearing condition until the earning season. 2-3 days old ears of the grown units were transferred to a growth chamber and placed under the three kinds of temperature conditions; days / 30 ℃ ~ nights /25 ℃, days / 25 ℃ ~ nights / 20 ℃, days and nights / 20 ℃. The bloomed flowers (1-3 days old flowers during transfer) were marked with a magic marker before moving them to the growth chamber.

Fully matured spikes (1-2 months old ears) were all gathered, and 2 or 4 healthy spikes were selected among them to examine conditions of embryos and endosperms for the entire seeds by using a dissecting microscope. After the microscope examination, inner structures of some seeds were observed with sample slides made by general paraffin sectioning.

RESULTS

1. Analysis of ge mutation

1) Test of the allelism in *ge*

For odm 44, odm 90 and odm 93 with recessive homozygote, and odm 124 and odm 132 with heterozygote, the reciprocal cross has been enforced among the respective giant embryos as well as on the *ge* and *ge-2* of which gene sites has already set. Furthermore, for the control combination, the cross between *ge* and T-65 to study F_1 showed the result that all had normal embryo sizes. Inferring from that, no abnormality could be found in cross and there was no possibility that they were offsprings.

In the cross combination between recessive homozygotes, F_1 showed complete *ge*. F_1 between recessive homozygote and heterozygote showed separation between normal embryos and giant embryos. This result implies that the *ge* used in this test is mutated under the completely same gene site. Hence, total of 7 kinds of mutations including the isolated *ge* and *ge-2* have the same gene site. Therefore, the respective mutations (allele) are called *ge-3* (odm 44), *ge-4* (odm 90), *ge-5* (odm 93), *ge-6* (odm 124) and *ge-7* (odm 132). Also, this gene site is set to be called *GE*.

2) Analysis of embryogenesis in *ge*

Table 1 shows the characteristics of the 6 *ge* family lines and the mature embryo of T-65. The embryo size of 6 *ge* family line completely become bigger than T-65 and the size differences are seen between the family lines. The embryos of *ge-2* and *ge-3* are the biggest and the size of an embryo of *ge-6* is the smallest among the family lines. The size of a shoot apical meristem and radicule showed little difference with the size of normal T-65. The increase of the cell number on the center cut end, enough to influence the size of an embryo, could not be confirmed. Therefore, in case of *ge*, we got to know the giantism of an embryo stems from the giantism of scutellum not by the increase in the number of scutellum cells, but by the giantism of respective scutellum cells. Also, in *ge-6*, the development of a shoot and radicule was unstable and gap was seen in a certain part of inside of an embryo. In case of *ge-7*, many radicules were differentiated or abnormality was seen in the formation of a radicule.

Hence, this *GE* gene site not only controls the size of a scutellum but also influences the differentiation of a shoot and radicule to some degree.

Figure 1 shows the embryo growth, consecutively researched, after flowering over 3 family lines including *ge-3*, *ge-4* and *ge-5*, and T-65. The growth period seems to be affected by summer season, however, in the very early stage of embryogenesis, no difference could be found between a wild type and mutation. It is regarded that the giantism of an embryo makes progress starting from 6 to 8 days after flowering.

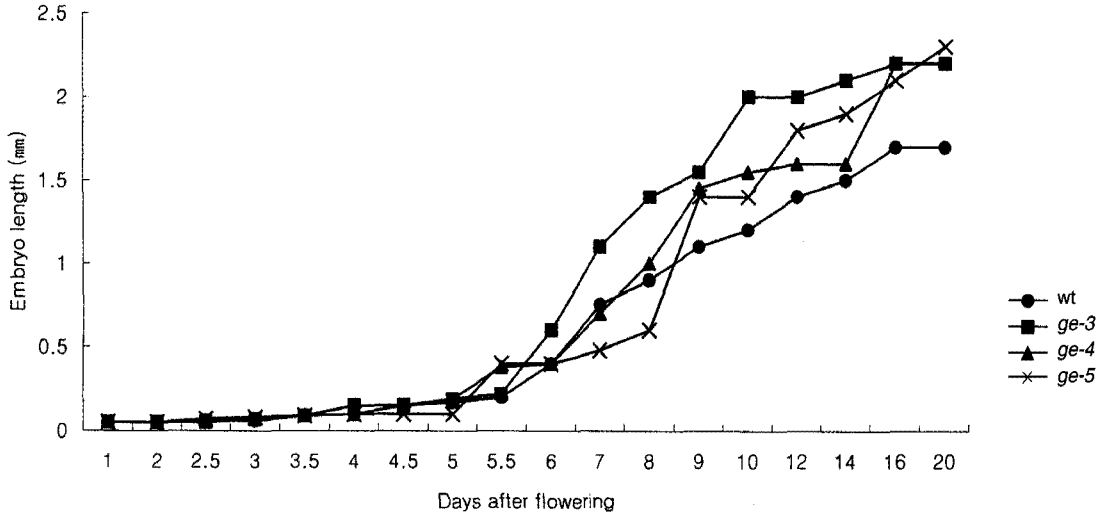

Fig. 1. Gorwth of the embryo length after flowering in *giant embryo* mutants.

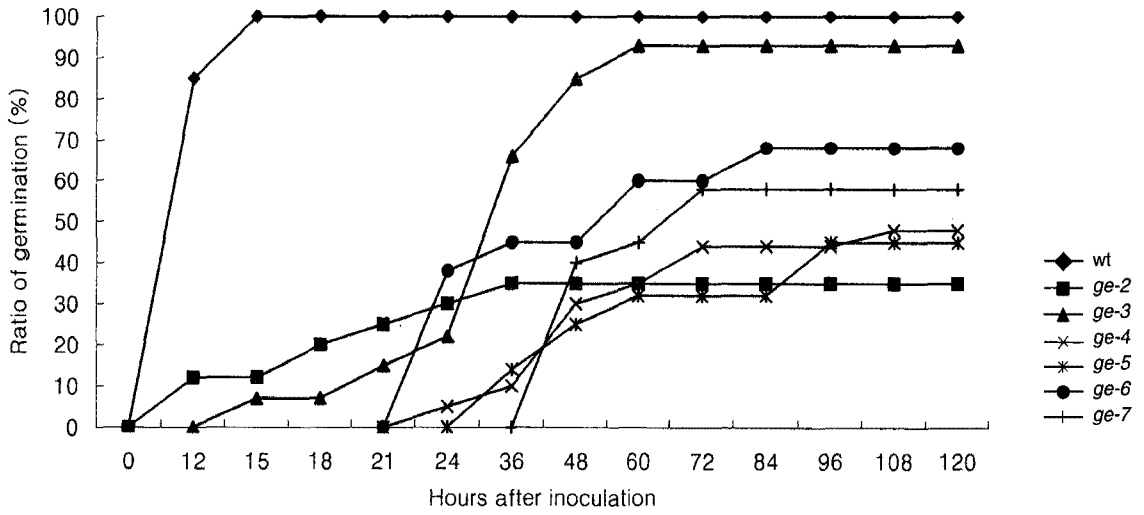

Fig. 2. Ratio of germination in *giant embryo* mutants.

3) Changes after germination of *ge*

Figure 2 shows the changes of the germination ratio after the arrangement of each *ge* mutation family line. The germination ratio of T-65, which is one of the wild type, reached up to 100 % within 18 hours after the arrangement. However, whole *ge*' s germination had been delayed and showed the result that the germination ratio finally decreased. There was the gap between giant embryos. *ge-3* showed very high germination ratio, however, *ge-2* displayed the low ratio of 35 %. There was also the gap among giant embryos in germination start point. In particular, the germination start point was far delayed in *ge-4*, *ge-5*, *ge-6* and *ge-7*.

The impediment and delay of the *ge* are not only stemming from the giantism of scutellum but also influencing to the development of a shoot and radicule. In addition, the fact, that the degree of the impediment

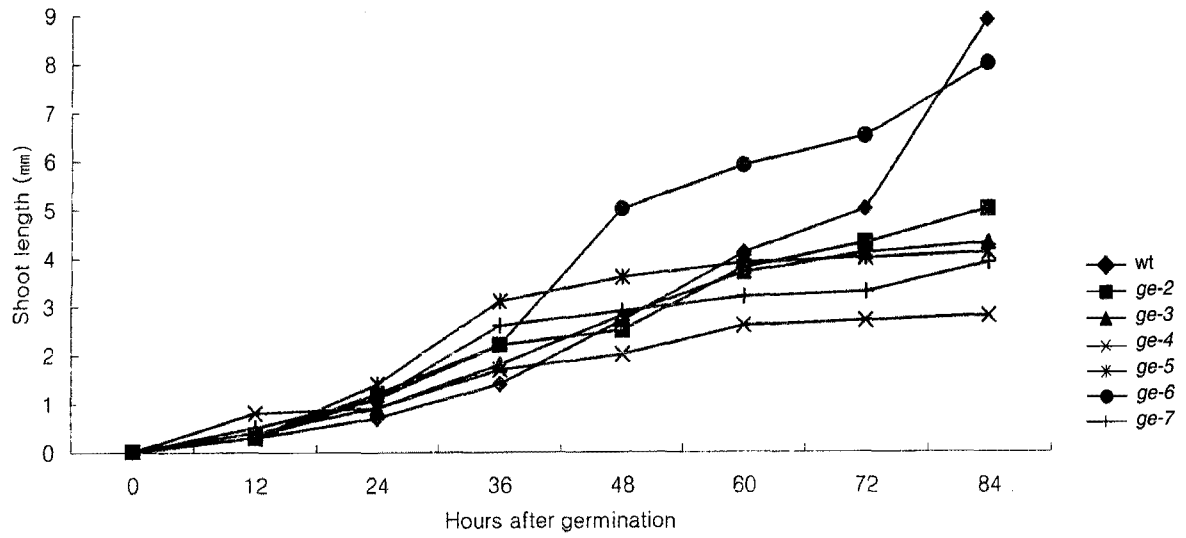

Fig. 3. Growth of the shoot after germination in *giant embryo* mutants.

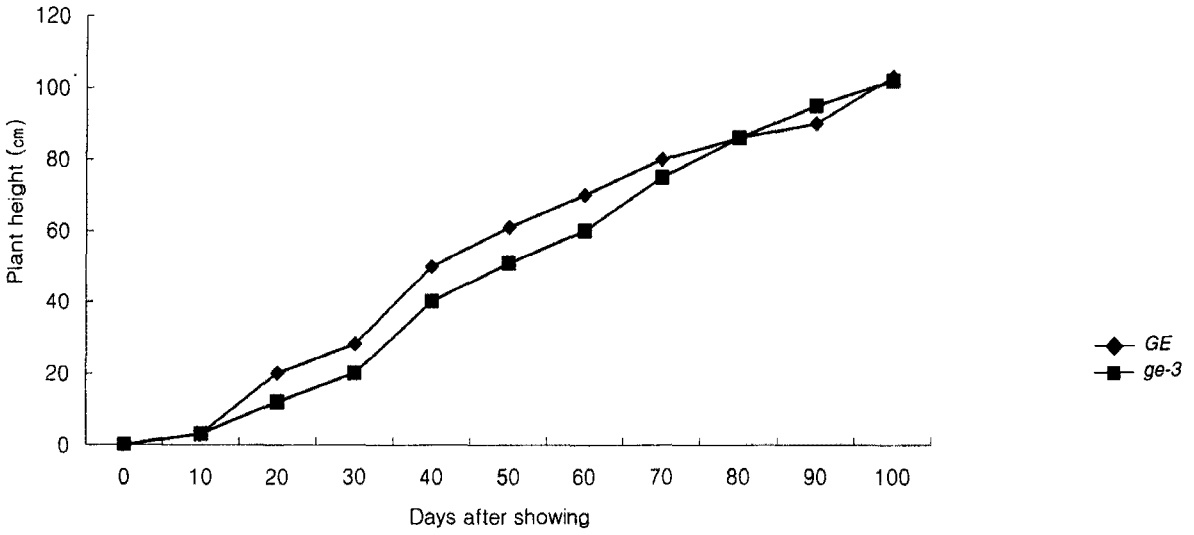

Fig. 4. Plant height of dominant homozygote (*GE*) and recessive homozygote (*ge-3*) in *giant embryo* mutants (odm 44).

and delay of germination among respective giant embryos is different, means that the function depending on allele is different.

Figure 3 shows the growth result of a shoot after the germination. The growth of a shoot after germination among 5 *ge* family lines showed little differences, however, the growth was being delayed in comparison to a wild type. Therefore, Once germinated, there was no big difference in the shoot early stage of growth between alleles as well as with a wild type.

Figure 4 shows the change of the plat height during 100 days after seeding over a recessive homozygote and dominant homozygote of *ge-3*. No outstanding change of plant height between the two was found. Therefore, this gene site is regarded as the gene, which is revealed only in the seed development and the gene that is not revealed after the germination.

Table 2. Phenotype of mature embryos in *reduced embryo* mutations.

Line	Gene	Embryo size (㎛)		Shoot apex size (㎛)		Radicle size (㎛)		No. of cells †
		Length	Thickness	Height	Diameter	Lenght	Diameter	
odm 16	*re1-1*	719±44**	540±82**	46±4**	40±4**	75±10**	120±19**	3450**
odm 48	*re2*	698±86**	350±93**	38±6**	54±2**	101±9**	144±6**	3340**
odm 49	*re1-2*	860±92**	628±52**	43±4**	45±8**	104±18**	137±25**	3400**
odm 62	*re3*	763±68**	515±97**	37±10**	41±6**	84±18**	126±23**	3610**
Wild type T-65		1860±112	996±20	75±12	68±15	299±24	331±22	7810

Size data are presented in ㎛± s.d.

† Counted in medium longitudinal sections.

** Significantly deviated from the wild type at 1% level respectively

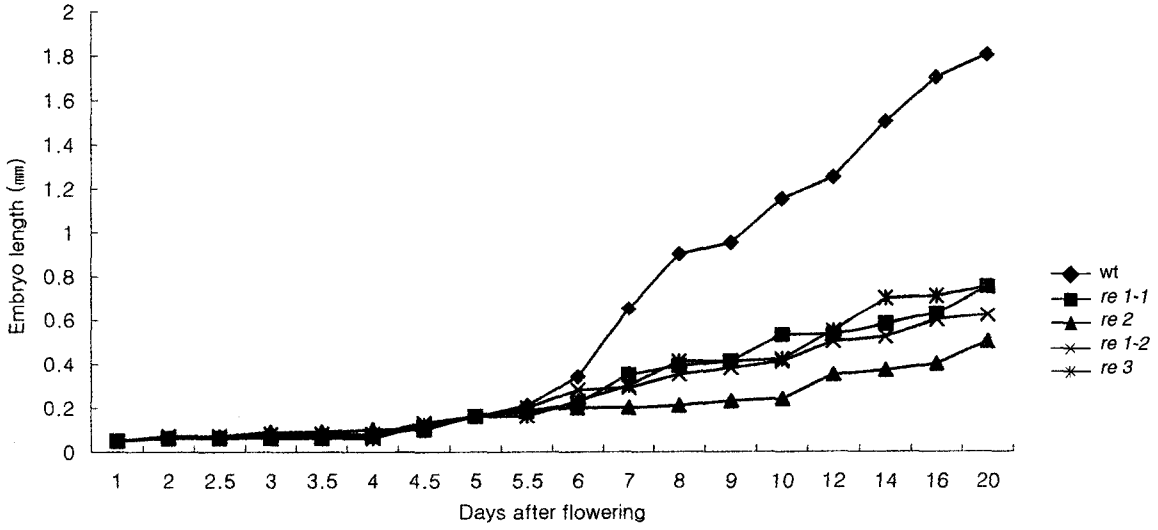

Fig. 5. Growth of the embryo length after flowering in *reduced embryo* mutants.

2. Analysis of re mutation

1) Test of allelism in *re*

The showing type of F_1 has been studied with the preparation of each recessive homozygote and enforcement of the reciprocal cross. F_1 of odm 16 (*re 1*) and odm 49 formed a complete reduced embryo, however, F_1 of other cross combinations showed the same size of embryos as ones of a wild type. Hence, odm 49 is regarded as the mutation by the same gene site as *re 1*. odm 48 and odm 62 are considered the mutations by other gene sites respectively. Therefore, odm 16 *re 1*, odm 49, odm 48 and odm 62 are set to be called *re 1-1*, *re 1-2*, *re 2* and *re 3*, respectively. And their gene sites are set to be called *RE 1*, *RE 2* and *RE 3*. So, three gene sites which form reduced embryos by mutations exist, at least.

2) Analysis of embryogeneis in *re*

The Table 2 shows the particularities of mature embryos of 4 *re* kinds and wild type T-65. Sizes of embryos show no similarity among the *re'* s, and embryo lengths of every *re* decreased by 35~60 % compared to wild types. Shoots and radicules are also

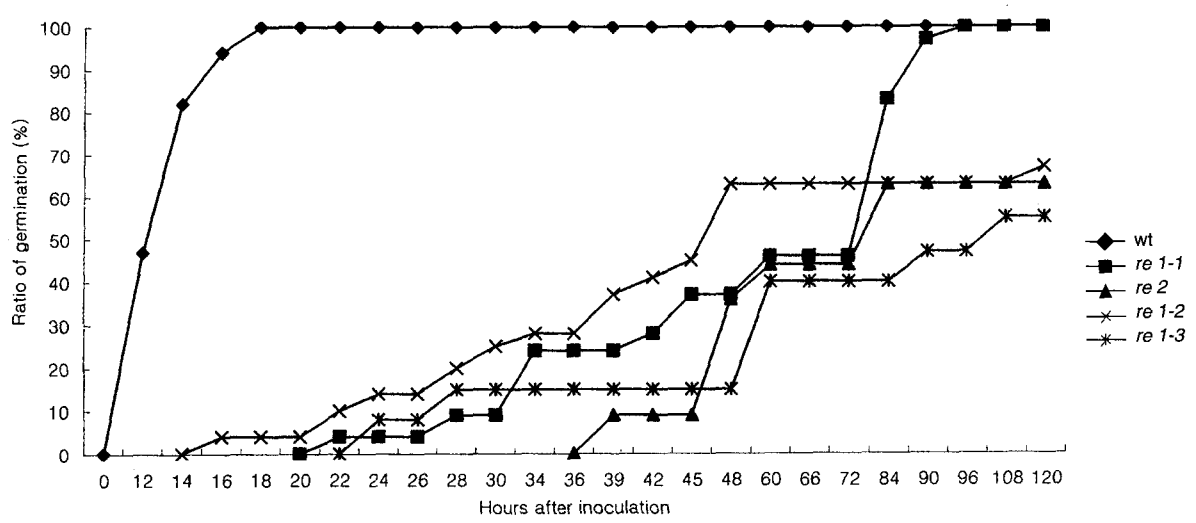

Fig. 6. Ratio of germination in *reduced embryo* mutants.

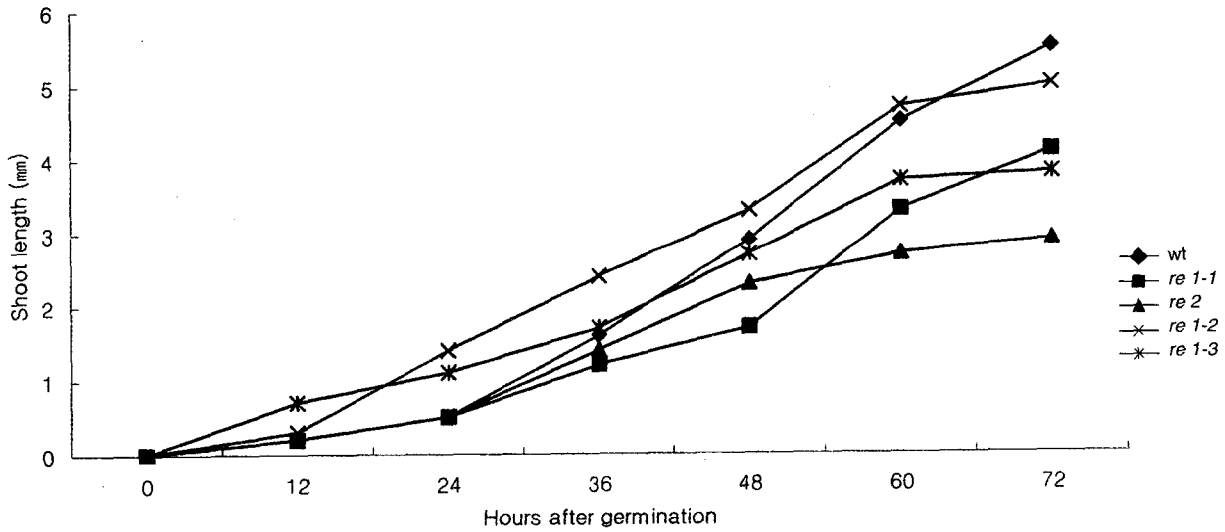

Fig. 7. Growth of the shoot after germination in *reduced embryo* mutants.

reduced by the same ratio, but no abnormal form is found. There is no change in the size of embryo, yet the number of cells in the central section was the half of wild types. Therefore, the three gene sites of *re'* s used in this experiment seem to control the size of the entire organs forming an embryo by the number of cells.

The growth process during embryogeneses is shown in Figure 5. There was few notable differences among *re'* s were found in terms of growth. Compared to T-65, a wild type, the size of embryo length was nearly impossible to see until the fifth day after germination when organogenesis starts. Unlike the size of the wild types embryo length grows fast after the fifth day, the *re'* s size of embryo length increased in a slow pace. However, the morphogenesis of *re'* s was not delayed compared to that of the wild type. The morphogenesis

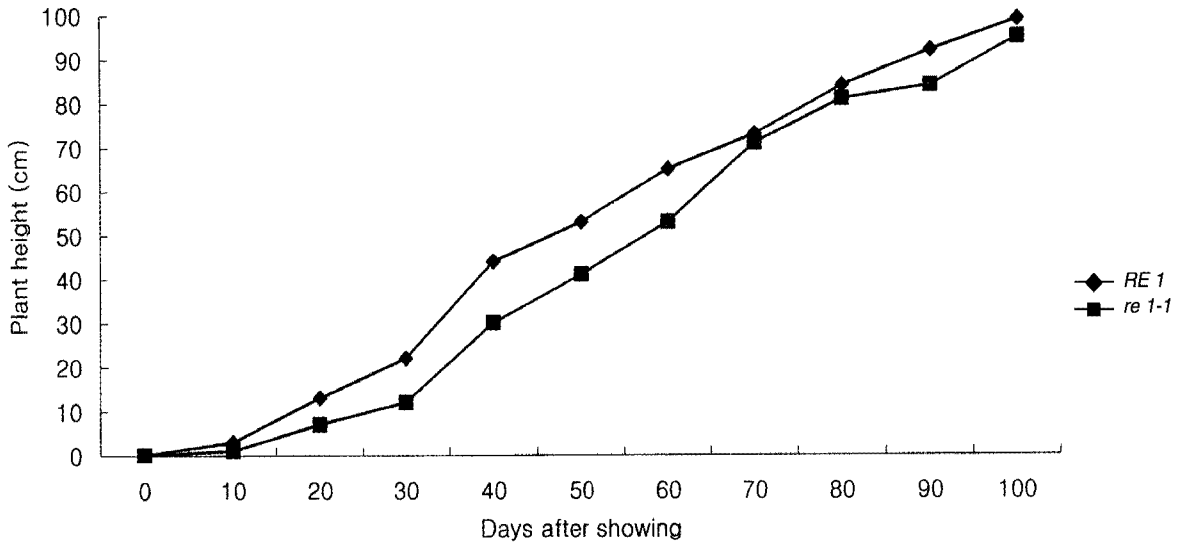

Fig. 8. Plant height of dominant homozygote (*RE*) and recessive homozygote (*re 1-1*) in reduced embryo mutants (odm 44).

of *re'* s tends to have much smaller number of cells than the wild type. Thus, it is thought to be a gene showing problems in the measuring structure of number of cells in relation to morphogenesis.

3) Changes after germination of *re*

The germination ratio of *re'* s showed a relatively high ratio compared to that of *ge'* s, which were more than 50 % (Figure 6) in every kind. Especially, *re 2* showed a germination ratio of 100 %, and other *re* kinds showed germination ratios ranged from 50 % to 70 %. The germination start point of *re 2* was relatively slower than that of other three *re* kinds, yet its changes in germination ratios showed very unique features. Therefore, *RE 2* site has different functions compared to other *re* 1 gene sites. Moreover, the showing types of *re 1-1* and *re 1-2*, allerism genes, showed little difference.

With regard to the growth of shoots, few differences were found among four *re'* s, but the growth tended to be a little slower than that of wild types (Figure 7). As a result of measuring plant heights of *re 1-1* for 100 days after seeding, the height in the beginning of growth was a little shorter than that of wild types, but in the end of growth, its plant height reached almost the same height as wild types (Figure 8).

Similar to the *GE*, therefore, *RE 1*, *RE 2*, and *RE 3* are regarded as gene sites revealed in only seed developments, not revealed after germination. Considering the results of the analyses on *ge'* s and *re'* s, the size of embryo seems to be defined by reciprocal interactions between genes revealed in endosperms and in embryos. The effects of endosperm directly come to scutellums, but genes revealed in embryos are regarded to have influences on various kinds of organs existing throughout an embryo.

3. Analysis of the temperature sensibility in *eml 1*

As results of the analysis on the frequency of occurrence of embryoless seed, 72 granules (27.8 %) among the total seeds of 259 granules were found to be embryoless, 186 granules were wild types, and only 1 granule was endospermless (*enl*). Accordingly, it was confirmed that the *eml 1* was driven from the single factor recessive mutation.

Table 3. Temperature sensitivity of *eml 1* (embryo and endosperm) seeds.

Temperature (℃)	No. of seeds	Embryoless	Reduced embryo	Normal type seed	Giant embryo	Endospermless
30-35	116	106 (91.4)	6	2	0	2
	119	115 (96.6)	2	1	0	1
Average	117.5	110.5 (94.0)	4 (3.4)	1.5 (1.3)	0 (0)	1.5 (1.3)
25-20	91	62 (68.1)	7	16	0	6
	99	48 (48.5)	24	11	0	16
	45	33 (73.3)	8	4	0	0
Average	78.3	47.7 (60.9)	13 (16.6)	10.5 (13.2)	0 (0)	7.3 (9.3)
20-20	79	47 (59.5)	7	5	3	17
	70	12 (17.1)	11	20	20	7
	41	18 (43.9)	2	7	12	2
Average	63.3	25.7 (40.5)	6.7 (10.6)	10.7 (16.9)	11.7 (18.5)	8.7 (13.7)

In the experiment, various types of seeds such as an embryoless, reduced embryo, wild type, giant embryo (endospermless or poor endosperm), etc. were obtained. The frequency of showing type of seeds under the 3-tire temperature conditions is shown in the Table 3. Under the condition of Days / 30 ℃ ~ Nights / 25 ℃, most seeds were shown to be embryoless type. However, a few seeds with embryos were also shown. Among the seeds with embryos, especially reduce embryos (having reduced scutellums) were often found. Under the condition of Days / 25 ℃ ~ Nights / 20 ℃, The frequency of embryoless seeds fell to around 60 %. Among the seeds with embryos, many seeds having wild type embryos and endospermless having giant embryos (enlarged scutellum) were found to have high degree of frequency. When taking into consideration of the developping endosperm, such results can be explained as the size of endosperms is likely to shrink under a low temperature (abortion in an extreme case). Such tendency becomes notable when the temperature is fixed at 20 ℃ during the days and nights, reduced the frequency of embryoless seeds to around 40 % while increased the frequency of endospermless or giant embryo seeds whose endosperm gets smaller while embryo enlarges. Therefore, the *eml 1* is defined to have sensibility against temperature. The endospermgenesis gets active at a high temperature, sometimes embryos disappear due to an over active developping endosperm. On the contrary, embryos enlarge due to the degeneration of endosperm at a low temperature.

Judging from that the various showing types of *eml 1* are mingled within a spike, a possibility that its location within the spike may also have some influences was suggested. Thus, I examined the showing types while recording their locations in a spike, but the tendency that embryoless and endospermless seeds are tend to be located in a certain place of spike was not supported. They are found to be located randomly in a spike. Accordingly, the showing type of *eml 1* is basically controlled by temperature, but it also seems to be adjusted by a minimal difference of conditions such as minerals On the other hand, the result of a case where the temperature control is applied on the third day after flowering also generated an identical showing type of seeds. Therefore, it is proved to have the temperature sensibility at least in the beginning stage during the endosperm or embryo genesis.

DISCUSSION

All *ge*′ s used in this experiment are regarded as the

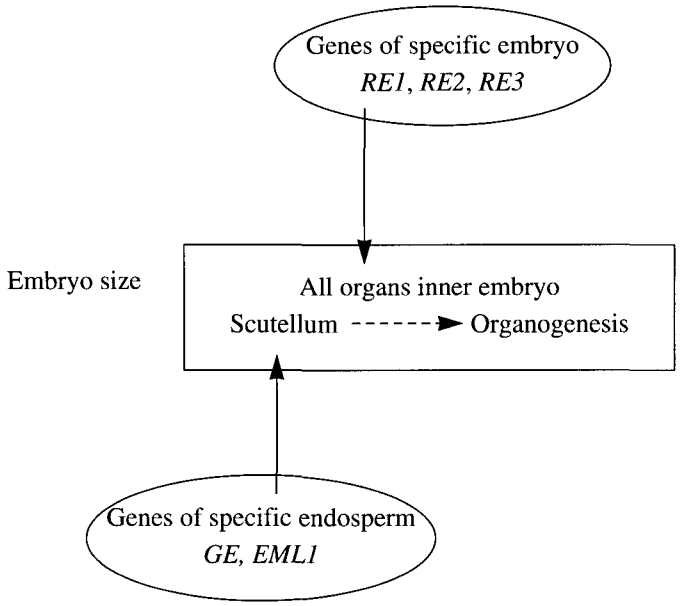

Fig. 9. Schematic presentation of genetic control of embryo size in rice seed.

result of the corpulence of scutellum. This seems to be a mutation of gene that controls the size of scutellum, yet the recent experiment suggests the *ge* might be generated by a mutation of genes in endosperms. In the double mutation between *ge* and *cle* (club-shaped embryo), or *gle* (globular embryo), a large aperture was made, where small club shaped or globular embryos existed. (Hong *et al.* 1996). Such results represent the *ge* is a gene that controls the size of endosperm from which the large aperture is generated. If embryos gene is a wild type, the embryo will grow into a ge that is able to fill the aperture. However, in case of a gene type without growing ability, the embryo will not grow, resulting the aperture unfilled. Thus, the *GE* also suggests a possibility that it might be the gene controlling the size of endosperm.

Likewise, the size of embryo seems to be defined according to the size of endosperm. In case of *ge*, only the size of embryo is controlled while the size of shoot or radicule has little distinction from wild types. The size of shoot or radicule seems be controlled in an individual structure apart from the size of embryo. In this case, only its scutellum has enlarged as well, regardless of the fact that the size of embryo for *eml*′ s also enlarged (Kageyama *et al.* 1991).

During the experiment, it was possible to isolate three gene sites causing *re*′ s. Two antagonistic genes were found from the *RE 1* site. However, they appeared in similar forms, and their genetical mechanism showed no evident difference. Judging from the fact that the entire organs of embryos were reduced in every *re*, the three gene sites seem to control sizes of embryos and all other organs. *re 2* showed a little different form from other *re*, implicating the *RE 2* site has some functionalities differ from that of *RE 1* or *RE 3*.

It has not been proven whether the *RE 1*, *RE 2* and *RE 3* are the gene sites revealed in embryos or in endosperms. However, they are suspected to be the gene sites revealed from embryos, given the facts that the size of a shoot and radicule in case of *ge* did not

change, no small aperture appeared for the double mutation between *re 1-1* and *gle 1*, and they showed an identical form of *gle 1*.

With regard to the question whether the temperature sensibility of *eml 1* is based on the genes revealed in embryos or in endosperms, the *ge'* s obtained so far are regarded as the result of the enlarged embryo driven from reducing endosperm. The giant embryos shown among *eml 1* also have only enlarged embryos like the way of *ge* cases. The embryoless, wild type embryos, and giant embryos do not represent the sizes of embryos only, and they can not be explained as a result of the gene mechanism of a gene revealed in embryos. Moreover, some endospermless are reported now days (Kageyama *et al.* 1991), yet in this case also show that only their scutellums were enlarged and developed into *ge'* s. Therefore, the *eml 1* is also regarded as a gene revealed in endosperms, which actively generates an enormous amount of endosperms at a high temperature while becoming inactive in forming endosperms at a low temperature and degenerated in an extreme case.

The *eml 1* has been viewed generally as an embryoless mutation. However, recently it shows some wired shapes of embryo or changed frequencies of the occurrence of embryoless, which make it hard to explain the functionality of this gene. In addition, we can even consider a possibility that it may be a mutation of a gene having housekeeping functions that is revealed in endosperms taking into consideration that the embryo develops into the beginning stage of *gle*. However, by assuming this as a gene of temperature sensibility controlling the formation of endosperms, partial explanations on phenomena that have been hard to understood were possible in the experiment. Furthermore, the *eml 1* is very important to understand and examine the reciprocal interactions between an endosperm and embryo, since it is possible to control the forms by changing temperatures.

So far, the number linage could be got by embryoless mutations. Among them, the frequency of embryoless seeds is changed depending on a growing year, but growing region as well. Such results of *eml 1* suggest a possibility that those *eml'* s might include a mutation of gene related to the generation of endosperm, in addition, a unique endosperm gene related to many embryogeneses exists.

As a result of the experiment, it is clearly proven that the size of embryo is controlled through at least 2 pathways(Figure 9). First, the size of embryo is controlled by endosperm specified in the analysis of ge and *eml 1*. The analysis explains that a giant embryo is due to the corpulence of scutellum in case of reduced endosperms, and an embryoless is driven from the deterioration of growth in embryo due to an abnormal development of endosperm. The second pathway is specified in the analysis of re, which the size of embryo is defined according to its own embryo genes. Some mutations that reduces the size of embryo are found. In this case, however, the entire organs comprising an embryo will be reduced. On top of that, the generation process occurs in a nearly normal way, which suggests that there might be a structure measuring the size or the number of cells in embryo. If so, under which structure the current size of embryo was defined? As explained above, the size of embryo seems to be defined by the reciprocal interaction between embryo and endosperm. In case of rice plants, it is evident that the selection seems to be made favoring endosperms to be enlarged, and also the size of endosperm is defined to have more room for enlargement. In fact, endosperms are filling the field of embryos in case of reduce embryo seeds due to physiological or genetic reasons. Meanwhile, the size of embryo has little notable distinctiveness compared to the wild types. Thus, the size of embryo has little relation with plant sizes after germination and especially for the size of matured plants, provided that shoots and redicules are completely developed. Added to that the amount may not be mattered. Thus, reducing

the size of embryos while enlarging endosperms as much as the reduced size would be the most practically important matter.

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