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Genetic Analysis of Newly Induced Short-root Mutants in Rice (*Oryza sativa* L.)

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Summary

Short root mutants of rice (*Oryza sativa* L.) were isolated and characterized. In seedlings of two short root mutants, RM1 and RM2, root length was 30 to 50 % of the wild type 'Oochikara', while a significant difference was not observed between the mutants and the wild type in plant height, number of roots and number of root hairs. In the root, final cell length in the mutants was about 60 % of the wild type suggesting that the short root phenotype is mainly based on defective cell elongation. In root length, the F_1 plants between the mutants and between those and the wild type were short and normal, respectively, while the F_2 plants segregated into normal and short, giving a good fit to a 3:1 ratio. The F_2 between the mutants did not segregate normal plants. These results show that the short root is controlled by a recessive gene, which is symbolized as *srt-1*.

Key Words : Mutation, *Oryza sativa,* Short-root, Rice, Root.

Introduction

The structure of the rice plant (*Oryza sativa* L.) is formed by two principal types of organs, root and shoot. These organs differ from one another not only in structure, but also in function. The root of rice is associated with water and nutrient uptake, anchorage of the plant and biosynthesis of various growth substances. The development of root system also affects shoot characteristics and lodging in the late growth stage. Despite the importance of roots, the study of root morphogenesis has not received as much attention as the development of aerial part organs. This is especially true for genetic studies (O'Toole and Bland 1987).

Although there have been many studies of morphological, physiological and biochemical aspects of growth and development in roots grown under natural or environmentally regulated conditions, there have been few genetic studies, especially on root characters which are monogenically controlled. Genetic variation in root morphology has been described in many plant species (O'Toole and Bland 1987). However, most of these examples involve polygenic variation. The isolation of single-gene mutants of roots has been hampered by the difficulty in observing large number of root systems and by the environmental variation in root morphology. However, it is likely that much will be learned from single gene mutants of roots by analogy to the great amount of that has been learned from such mutants in shoots. A few root mutants have been isolated and characterized in rice (Futsuhara and Kitano 1985), tomato (Zobel 1975, 1991, Hochmuth *et al.* 1985), maize (Pilet 1985), barley (Tagiliani *et al.* 1986) and arabidopsis (Aeschbacher *et al.* 1995, Baskin *et al.* 1995, Celenza *et al.* 1995, Cheng *et al.* 1995, Hauser *et al.* 1995, King *et al.* 1995). We wished to isolate root mutants of rice and then carry out genetic analysis of them.

We have found mutants that have short roots compared with wild type but are the same shoot shape. The present report describes their isolation and characterization.

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Materials and Methods

Screening for mutants was performed using M_2 seedlings derived from M_1 seeds of rice (*Oryza sativa* L. cv. Oochikara, japonica type) mutagenized by gamma-ray (200 Gy).

Screening for mutants

M_2 seeds were sown on a net float in a plastic container in a greenhouse at 25 to 30°C with no supplemental lighting. The seedlings were cultured with a nutrient solution, adjusted to pH 5.0, containing 365 μM $(NH_4)_2SO_4$, 91μM K_2SO_4, 547 μM $MgSO_4 \cdot 7H_2O$, 183 μM KNO_3, 365 μM $Ca(NO_3)_2$ and 182 μM KH_2PO_4. The solution was exchanged every seven days. Plants showing features different from roots of the wild type were selected on the 21st day after sowing. The selected seedlings were transplanted to 1/5000 *a* Wagner pots with soil and cultured in a greenhouse to produce M_3 seeds.

In M_3 generation, seedlings were grown in the same solution as applied in the M_2 generation. Morphological characteristics of roots were determined in a seven-day-old seedling comparing each M_3 line with the wild type.

Morphogenesis of mutants

Characterization was conducted using M_3 root morphological mutants that were significantly different from the wild type, fully fertile and in which phenotypic segregants were not observed. Seedlings of mutant lines and the wild type were cultured in the same method as applied in the M_2 generation. Seedling height, root length, number of roots and number of root hairs were

measured for three weeks. To measure cell length, apical 1cm segments were cut from adventitious roots of two-week-old seedlings, and fixed in FAA (50 % Ethyl alcohol : Acetic acid : Formalin = 90 : 5 : 5). The size of cortex cells was determined up to 1cm from the root tip by the procedure of Ichii (1995).

Genetic analysis

The mutant lines were crossed to wild type, and the F_1-F_2 progenies, as well as parentals, were grown in the same method as applied in the M_2 generation. Root length was determined at the 2nd week after sowing.

Agronomic characteristics of mutants

To determine the effects of mutant genes on agronomic characters the mutant lines and their wild type were used as materials. Twenty-day-old seedlings were transplanted on June 12 to 1/5000 *a* Wagner pots with a single plant per pot. The transplanted plants were grown in a nutrient solution, adjusted to pH 5.0, containing 365 μM $(NH_4)_2SO_4$, 91 μM K_2SO_4, 547 μM $MgSO_4 \cdot 7H_2O$, 183 μM KNO_3, 365 μM $Ca(NO_3)_2$ and 182 μM KH_2PO_4 and 15 μM $FeC_6H_5O_7 \cdot xH_2O$. On the 35-40th day after heading, culm length, panicle length, number of panicle, root length and root dry weight were recorded.

Results

Isolation of short root mutants

In order to screen for plants abnormal in morphogenesis and growth of the root system, 100,000 M_2 seedlings were cultured with a nutrient solution. A total of ten plants that showed a significant difference from the wild type were grown in Wagner pots with soil. Root length of these plants was significantly short at the transplanting stage. Among the ten plants, root length of six plants was more than 50% less than those of the wild type. Among the selected plants, three plants withered and another grew poorly and produced little seed. Sufficient seeds for screening in the M_3 generation were obtained from the other six plants. Root length of seedlings in the lines of the M_3 generation was compared to wild type. Three of the six lines had roots that were 50 % shorter than those of the wild type, two of them had slightly but significantly shorter roots than wild type, and the roots of the last line were not different from wild type. For all six lines, plant height was the same as the wild type. The M_3 populations were uniform and did not segregate observable phenotypes. We therefore concluded that the five lines were short root mutants. Among the five lines, the two lines, RM1 and RM2, that had sufficient seeds were used in the following morphological and genetic characterization.

Characterization of the mutants, RM1 and RM2

1) *Growth of seedlings*

Growth of seedlings was compared between mutant lines, RM1 and RM2, and the wild type, Oochikara, during three weeks (Fig. 1). Plant height was slightly smaller in the mutant lines than in the wild type, though the difference between the former and the latter was not significant (Fig. 2). Root length of the mutant lines was 30 to 50% of that of the wild type through the three-week observation (Fig. 3). No difference was observed between the mutant lines and the wild type in number of roots and number of root hairs (Fig. 4 and Table 1). In plant height, root length, number of roots, and number of root hairs, RM1 and RM2 exhibited the same charac-

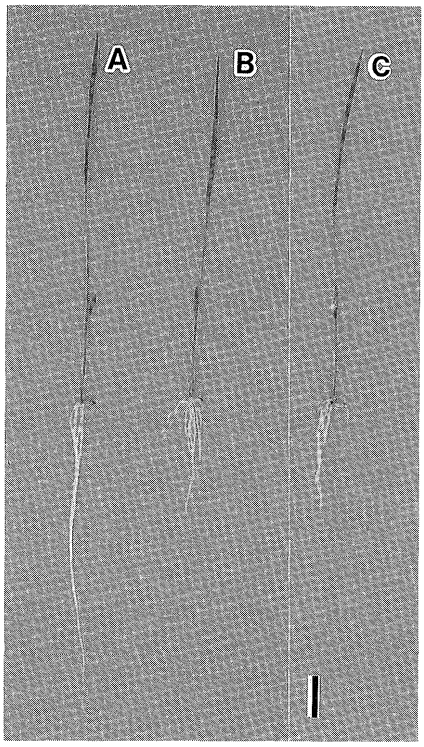
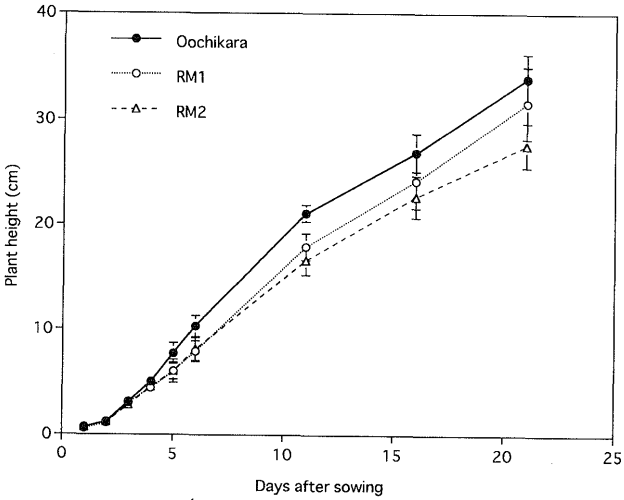
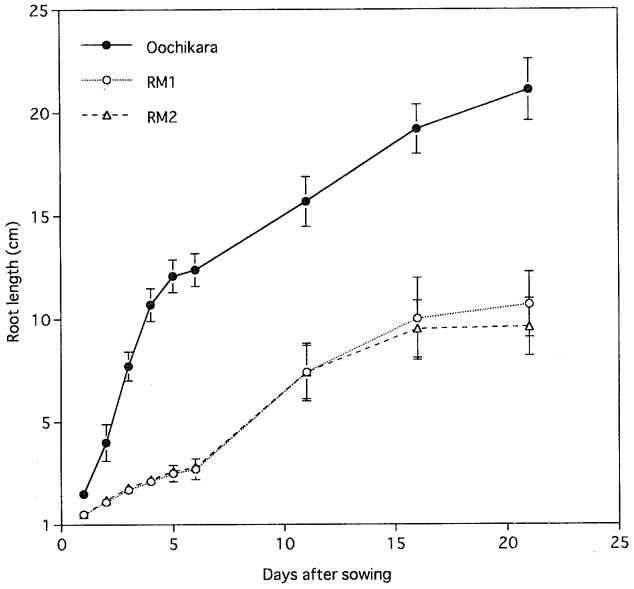


Fig. 1. Two-week-old seedlings of short root mutants and Oochikara.
A: Oochikara, B: RM1, C: RM2, bar: 2cm.

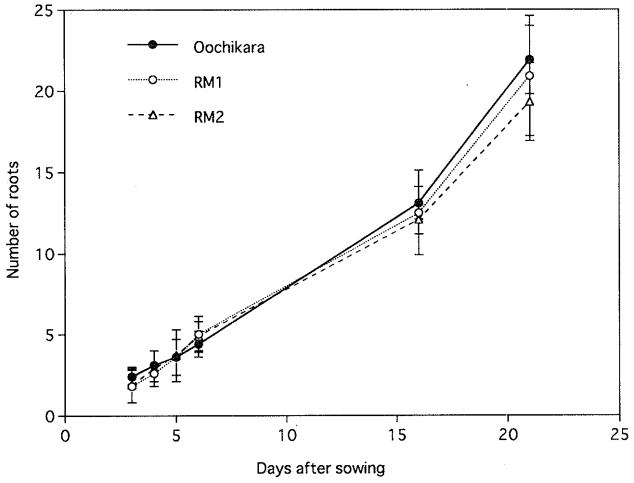


Fia. 2. Comparison of plant height between short root mutants and Oochikara.
Bars indicate standard deviation.

teristics. The two mutant lines cannot be distinguished in seedlings by appearance.



Fia. 3. Comparison of root length between short root mutants and Oochikara.
Bars indicate standard deviation.



Fia. 4. Comparison of number of root between short root mutants and Oochikara.
Bars indicate standard deviation.

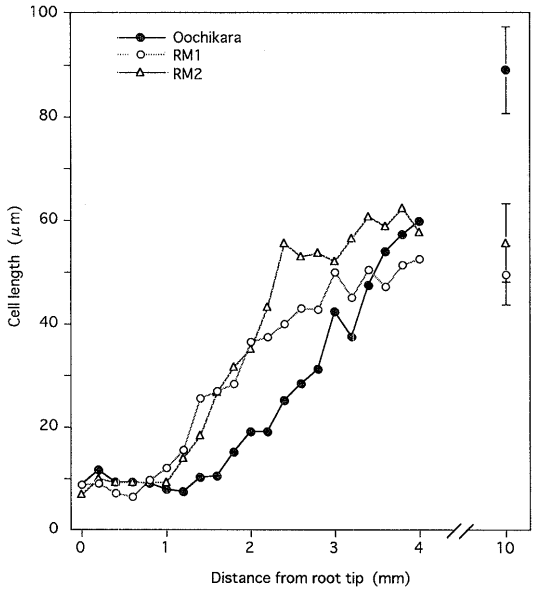
Table 1. Number of root hairs of seedlings in short root mutants and Oochikara

Line	Seminal	Adventitious
Oochikara	525±52[1)]	515± 67[1)]
RM1	510±75	482± 45
RM2	505±62	510±112

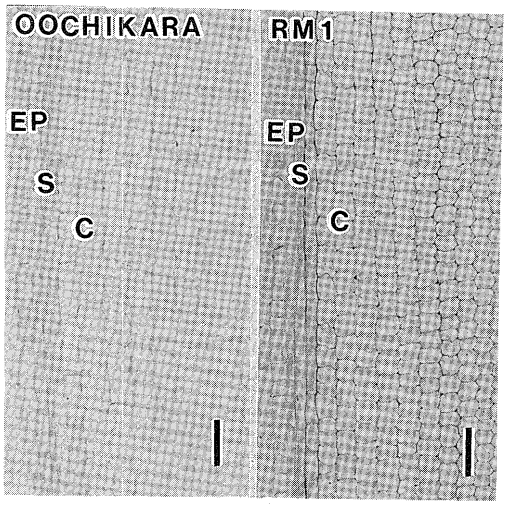
Number of root hairs per sq mm was determined at 1.0cm from the root tip on the 2nd week after sowing. [1)] Standard deviation.

2) *Cell size of roots*

To detect the cause of the short root phenotype, the length of cortex cells was measured in seedlings of the mutant lines and the wild type. Cell length was constant in the first millimeter of the root and then increased logistically with distance from the root tip (Fig. 5). A significant difference in the mutant lines from the wild type was observed in the site at which cell length began to increase. In the wild type, cell length started to increase at 1.5mm from the root tip, while in the mutants this increase occurred at 1mm. Evidently, the meristematic region of roots is shorter in the mutant lines than in the wild type. Cells did not continue elongation



Fia. 5. Change with distance from root tip in the cortex cell length of root of seedlings in short root mutants and Oochikara.
Bars indicate standard deviation.



Fia. 6. Morphology of seminal root cortex cell of seedlings in RM1 and Oochikara.
EP: Epidermis, S: Sclerenchyma, C: Cortex, bar: 100μm.

beyond 4.0mm from the root tip in the mutant lines, but continued in the wild type. Along with the meristem, the elongating region of the mutant lines was shorter than that of the wild type. Finally root cells grew to about 50 to 60 μm long in the mutant line, while to at least 90 μm long in the wild type (Fig. 6). There was a significant difference between the mutant lines and the wild type in size of root cortical cells.

3) *Genetic analysis of short root*

To investigate inheritance of the mutant trait, the mutant lines, RM1 and RM2, were crossed with the wild type, Oochikara, and RM1 also was crossed with RM2. The F_1 from the cross between either mutant and wild type gave plants with wild type root length; whereas the F_1 from the cross between the two mutants gave plants with root length the same as either mutant parent (Table 2). Root length in the F_2 between RM1 and Oochikara and between RM2 and Oochikara segregated into 61 normal and 15 short, and 60 normal and 12 short, respectively, and was consistent with the 3:1 ratio expected for a single recessive mutation. The F_2 between the mutant lines did not segregate normal plants, and all plants had root length similar to the parental lines. These indicate that two mutant lines, RM1 and RM2, were induced by a mutation at a single locus, and that the short root phenotype was transmitted by a single recessive nuclear gene, which we designate *srt-1* for short root.

Table 2. Segregation of root length in the F_1 and F_2 between short root mutants and Oochikara, and between the mutants

Line	Root length		Expected ratio	χ^2	Probability
	Normal	Short			
Oochikara	20	0			
RM1	0	20			
RM2	0	20			
(RM1×Oochikara) F_1	20	0			
(Oochikara×RM1) F_1	20	0			
(RM2×Oochikara) F_1	20	0			
(Oochikara×RM2) F_1	20	0			
(RM1×RM2) F_1	0	20			
(RM1×Oochikara) F_2	61	15	3 : 1	1.12	0.30~0.20
(RM2×Oochikara) F_2	60	12	3 : 1	2.67	0.20~0.10
(RM1×RM2) F_2	0	50			

Root length was observed on the 2nd week after sowing.

Table 3. Agronomic characteristics in short root mutants and Oochikara

	Oochikara	RM1	RM2
Heading date	Aug/10a	Aug/13b	Aug/15b
Culm length (cm)	61.5a	61.0a	62.2a
Panicle length (cm)	18.2a	19.8a	19.9a
Number of panicle	13.3a	14.0a	15.2a
Root length (cm)	50.3a	23.7b	23.3b
Root dry weight (g)	3.5a	1.5b	1.6b

Twenty-day-old seedlings were grown with solution culture following June 12.
Means followed by a common letter within the trait are not significantly different at the 5% level by Duncan's Multiple Range Test.

4) *Agronomic characteristics*

To observe broadly agronomic characteristics of the mutant lines and the wild type, their plants were cultured with a nutrient solution as mentioned above. Root length and root weight of the mutant lines were 40-50 % of those of the wild type (Table 3). Heading date was later in the mutant line than in the wild type. No difference was observed between the mutant lines and the wild type in culm length, panicle length and number of panicle. In every character, RM1 and RM2 exhibited the same characteristics. The two lines cannot be distinguished in mature plants in the manner as seedlings by appearance.

Discussion

In seedlings and mature plants, root length of the mutant lines was 40-50 % of that of the wild type. Cortex cell length of the mutant lines was 60 % of that of the wild type. It was suggested that the short root phenotype in the mutant lines mainly was not brought about by reduction of cell number, but by reduction of cell length. However, we cannot be clear what the cause of inhibition of cell growth is.

The recessive gene, *srt-1,* inhibited growth of seminal and adventitious roots immediately after germination, but did not inhibit plant height of seedlings. The root-growth inhibition of *srt-1* was accompanied by the reduction of root cell length. A recessive gene, *rt,* of rice was found in the progenies of Fukei 71 treated with a chemical mutagen and was characterized by extreme root-growth inhibition (Kitano and Futsuhara 1989). The inhibition of the seedling root growth by *srt-1* gene was less than that of the *rt* gene. The *srt-1* gene did not have an influence the number of root hairs of seminal or adventitious roots, though the *rt* gene inhibited root hair growth. It is suggested from these observations that *srt-1* locus and *rt* are different loci. Hereafter, we ought to make an examination on the relationship between the two genes.

Semidwarfing genes which bring about reduction of growth of plant height and culm length in rice are known well. Although some of them do not influence root growth (Ichii and Okamoto 1990), others do. The *srt-1* gene at seedling stage inhibited root growth but had little influence on shoot growth. The mutant lines, RM1 and RM2, possessing the *srt-1* gene should be a useful material to investigate the genetic regulation of root growth, as well as some physiological aspects in root and shoot growth interaction in rice.

To improve genetically the morphogenesis and function of roots in higher plants, it is important to collect

various mutants associated with roots. Some mutants were isolated in crops such as barley (Tagiliani *et al.* 1986), maize (Pilet 1985, Miller and Moore 1990) and rice (Futsuhara and Kitano 1985), and possessed gross abnormalities in root growth and resistance to plant hormones. However, these mutants have not been studied in the context of crop yield. RM1 and RM2 showed normal performance in aerial part phenotype despite the inhibition of root growth, though they were cultured with a nutrient solution. We hope to extend our analysis of the genetics of root performance to include plant productivity and thus be able to contribute to the genetic improvement of rice plant.

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