***RRL1*, *RRL2* and *CRL2* loci regulating root elongation in rice**

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To analyze the genetic mechanism regulating root elongation, we characterized five recessive mutants in rice (BRX65, BRX117, BRX430, BRX448 and *crl2* mutant). In fifteen-day-old seedlings, the root length of BRX65 was 36 % of that of the wild type root, while the length of the roots of BRX117, BRX430 and BRX448 was about 45 % of that of the wild type root. These types of mutation were designated as *reduced root length* with the gene symbol *rri*. In contrast, the length of the *crl2* mutant root was 114 % of that of the wild type root. Allelism test indicated that *rri* mutations occurred in two complementation groups and that these mutant genes were not allelic to *crl2*. The first *RRL* locus was designated as *RRL1* (BRX65) and the other locus as *RRL2*, and the alleles were designated as *rri2-1* (BRX117), *rri2-2* (BRX430) and *rri2-3* (BRX448). In the *rri1* mutant, only the mature cortical cell length was significantly shorter than that of the wild type, whereas, the values of the cell length, apical meristem size and cell flux of the *rri2-1* mutant root were all clearly lower than those of the wild type root. On the other hand, the values of the mature cortical cell length, apical meristem size and cell flux of the *crl2* mutant root were all clearly higher than those of the wild type. Mature cortical cell length of the *rri1* *crl2* double mutant was intermediate between that of the parental single mutants, while the cell length, meristem size and cell flux of the *rri2-1* *crl2* double mutant were all intermediate between those of the parental single mutants, respectively. These results suggest that there are genes that inhibit or promote the maintenance of root apical meristem and cell elongation, and the opposite effects between these individual genes determine the extent of root elongation in rice.

Key Words: *Oryza sativa*, cell flux, cell length, mutation, root apical meristem, root elongation.

Introduction

A plant root system consists of various component roots such as seminal root, crown root, and their lateral

roots. Root system structure is defined by the development of component roots represented by several parameters, such as length, number, and their configuration in the soil profile. This structure predominantly determines the overall function of the root system but not the physiology of individual component roots (Yamauchi *et al.* 1996). Among all, the root length is one of the most important and thus frequently measured parameters that are related to a plant ability to acquire soil resources (Evans and Barber 1964, Taylor 1982, Teo *et al.* 1995, Kashiwagi *et al.* 2000).

Furthermore, the root system structure and its dynamics are particularly important for crop growth under stressful conditions (Yamauchi *et al.* 1996, Azhiri-Sigari *et al.* 2000, Kamoshita *et al.* 2000). For example, deep rooting has been considered to be a drought-resistance mechanism in a number of plants (Sullivan and Ross 1979, Narayan 1991, Fukai 1995, Nemoto *et al.* 1998, Oyanagi 1998), and a few attempts have been made to use molecular markers as selection tools for incorporating the root traits into improved cultivars (Kamoshita *et al.* 1999, Zheng *et al.* 2000).

On the other hand, the analysis of the physiological basis and genetic control of various developmental traits such as initiation (number) and elongation (length) of roots lags far behind that of aerial plant organs. In this regard, to promote a breeding program related to root traits, information about the genetic regulation throughout root development is essential, and the mutant can be a very useful tool to study such an aspect. Recently, many root mutants have been identified in *Arabidopsis thaliana* that exhibit defects in the commencement of cell division following germination (Cheng *et al.* 1995, Vernoux *et al.* 2000), the maintenance of root meristem (Benfey *et al.* 1993) and orientation (Baskin *et al.* 1992, Benfey *et al.* 1993, Hauser *et al.* 1995) and the extent of cell expansion (Baskin *et al.* 1995, Takahashi *et al.* 1995). In addition, mutants showing a small meristem size and/or short cell length have also been described in rice (Kitano and Futsuhara 1989, Liang and Ichii 1996, Ichii and Ishikawa 1997) and these mutants may enable to identify the genes that regulate the root elongation process.

However, all of these mutations lead to a reduction of the plant root length and thus, it remains to be determined what kinds of genes inhibit the root elongation and how these genes interact with genes which promote the elongation. In this regard, we previously reported the identification of the *crl2* mutant of rice characterized by an increased seminal root length as well as reduced number of crown

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roots (Inukai *et al.* 2001). Recently, we have also identified four rice mutants characterized by a reduced seminal root length (BRX65, BRX117, BRX430 and BRX448). These mutants that have long and short root traits should, therefore, be suitable materials for addressing the problems indicated above. In the current study, we characterized those newly identified four mutants and the *crl2* mutant genetically and phenotypically, and then analyzed the mutations at the histological level to determine which process causes abnormal root growth, cell elongation or cell division. Furthermore, we investigated the morphological characters of three different double mutants segregated in the F₃ progenies from the crosses among BRX65, BRX117 and the *crl2* mutant to examine their genetic interactions.

Materials and Methods

Screening for mutants

Screening for mutants was performed using M₂ progeny of rice (cv. Blue Rose, japonica type) which was subjected to 200 Gy γ -ray irradiation as described in the report of Inukai *et al.* (2001). After these M₂ seedlings were grown in water culture without nutrients for two weeks, we selected seedlings that apparently differed in the root number, length and diameter from the wild type. The reappearance of each mutant characteristic was confirmed in the subsequent two generations.

Genetic analysis

We used the newly identified four rice mutants with a shorter seminal root (BRX65, BRX117, BRX430 and BRX448) and a recessive *crl2* mutant with a longer seminal root and fewer crown roots compared with the wild type (Inukai *et al.* 2001). First, for the examination of the inheritance mode of the respective mutant characters, segregation ratio of the phenotype in each M₃ progeny derived from M₂ heterozygote plants was calculated. Next, to determine whether each of the mutant genes identified in this experiment was an allele of other mutant genes or not, crosses of ten different combinations were performed among these five mutants, and the phenotypes of the F₁, F₂ or F₃ progenies were examined. Since the *crl2* mutant was sterile, heterozygous plants were used for the crosses with other mutants.

Characterization of mutant morphological characters

Seeds of five mutants (BRX65, BRX117, BRX430, BRX448 and *crl2* mutant) and the wild type were surface-sterilized and germinated. These seeds were placed in 0.8% agar medium to raise the seedlings, which were kept in a plant growth chamber maintained at 30 $\pm$ 1°C under a 12 h photoperiod (320 μ mol photons m⁻² s⁻¹) regime. After fifteen days, the seedlings were sampled to measure their plant height, plant age (leaf number), length of seminal roots, diameter of seminal roots, number of crown roots and branching density of lateral roots on the seminal root axis. In addition, the length of the seminal roots of the three

mutants (BRX65, BRX117 and *crl2* mutant) selected from each complementation group and the wild type was measured every day for three weeks.

For the histological observations, the seminal root segments excised from 9-day-old seedlings were fixed in FAA (formalin: acetic acid: 70% ethanol (1:1:18)). These segments were rinsed twice in 0.1 M phosphate buffer (pH 7.0) each for 30 min, dehydrated in a graded acetone series, embedded in a water-soluble methacrylate resin, Technovit 7100 (Kulzur Co., Ltd.), and polymerized at 45°C. They were cut into sections at 5 μ m thick longitudinally and stained with Delafield's hematoxylin. Cell length in the third layer from the outermost layer of the cortex was measured under a light microscope.

Furthermore, to investigate the characteristics of the three different double mutants segregated in the F₃ progenies from the crosses among BRX65, BRX117 and *crl2* mutant, we measured the plant height and seminal root length of these double mutants in fifteen-day-old seedlings. Again, the cortical cell length of these double mutants was measured in the same way as mentioned above.

Estimation of cell flux

We also estimated the cell flux in the three mutants (BRX65, BRX117 and *crl2* mutant), three double mutants among these mutations and the wild type as follows. Cell flux is defined as the number of cells passing a given point per unit time (Silk *et al.* 1989, Thomson and Atwell 1989, Fraser *et al.* 1990). If growth and cell production are steady (time-independent), then the cell flux is uniform throughout the cell file. Therefore, the cell flux can be evaluated at the base of the elongation zone as the ratio of the root elongation rate to the mature cell length (Silk *et al.* 1989).

Results

Identification of mutants, inheritance of mutant characters and allelism test

The M₂ seedlings were screened to identify mutants showing apparently a different root number, length and diameter from the wild type, and we identified 23 mutants that were classified into 8 types according to their root characteristics. In this paper, we selected four mutants with a defect in the elongation of the seminal root (BRX65, BRX117, BRX430 and BRX448). This type of mutation was designated as *reduced root length* with the gene symbol *rrl*. These mutant phenotypes were found to be stable for at least three generations. Segregation of the four *rrl* mutant phenotypes in each M₃ progeny derived from M₂ heterozygous plants fitted to a 3 (wild type): 1 (mutant) ratio (Table 1), indicating that these mutant phenotypes are controlled by a single recessive gene, respectively.

All the F₁ progenies from the crosses among BRX117, BRX430 and BRX448 showed the mutant phenotype, while those from the crosses between BRX65 and each of BRX117, BRX430 and BRX448 showed the wild type phenotype

Table 1. Segregation of phenotype in M₃ progenies derived from selfed M₂ heterozygous plants for the four mutant lines (BRX65, BRX117, BRX430 and BRX448)

Lines	Phenotype		χ^2 (3:1)	P.
	Wild	Mutant		
BRX65	177	67	0.79	0.25-0.50
BRX117	133	38	0.70	0.25-0.50
BRX430	145	49	0.01	0.90-0.95
BRX448	113	32	0.66	0.25-0.50

(Table 2) indicating that the mutations occurred in two complementation groups. The first locus was designated as *RRL1* (BRX65), and the other locus as *RRL2*, and the alleles were designated as *rrl2-1* (BRX117), *rrl2-2* (BRX430) and *rrl2-3* (BRX448). These mutants were also crossed with the *crl2* mutant controlled by a single recessive gene (Inukai *et al.* 2001). Since the *crl2* mutant was sterile, the heterozygous plants were used for crosses between the *crl2* mutant and each of BRX65, BRX117, BRX430 and BRX448. In each F₂ progeny from the crosses among these mutants, wild type and mutant segregation was consistent with the 9:7 ratio (Table 2). The results indicated that *crl2* is not allelic to *rrl1*, *rrl2-1*, *rrl2-2* nor *rrl2-3*.

Characterization of mutant morphological characters

Compared with the wild type, the length of the seminal roots in the *rrl* mutants was significantly shorter (Fig. 1 and Table 3). These mutants also showed other abnormal morphological characters. Both shoot growth and rooting ability

Table 2. Segregation of phenotype in F₁ or F₂ progenies of the crosses among five mutants (BRX65, BRX117, BRX430, BRX448 and *crl2* mutant)

Combinations	Phenotype		χ^2 (9:7)	P.
	Wild	Mutant		
BRX117 × BRX430 (F ₁)	0	20	—	—
BRX117 × BRX448 (F ₁)	0	20	—	—
BRX430 × BRX448 (F ₁)	0	20	—	—
BRX65 × BRX117 (F ₁)	20	0	—	—
BRX65 × BRX430 (F ₁)	20	0	—	—
BRX65 × BRX448 (F ₁)	20	0	—	—
BRX65 × <i>crl2</i> ¹⁾ (F ₂)	128	106	0.23	0.50-0.75
BRX117 × <i>crl2</i> ¹⁾ (F ₂)	150	98	1.81	0.10-0.25
BRX430 × <i>crl2</i> ¹⁾ (F ₂)	145	95	1.69	0.10-0.25
BRX448 × <i>crl2</i> ¹⁾ (F ₂)	132	110	0.29	0.50-0.75

¹⁾: Heterozygous plants were used for the allelism tests, since the homozygous plants were sterile.

of the lateral roots of the *rrl1* mutant were poorer and lower than those of the wild type, respectively (Table 3). The *rrl2-1*, *rrl2-2* and *rrl2-3* mutants showed very similar morphological characters. No significant difference was detected between these three mutants and the wild type seedlings in plant height and plant age (leaf number), whereas the seminal roots and branching density of the lateral roots in these mutants were thinner and higher than those of the wild type, respectively (Table 3). On the other hand, the length of the seminal roots in the *crl2* mutant was significantly longer than that of the wild type (Fig. 1 and Table 3). In addition, the mutant shoot growth was poorer, the seminal root was

Fig. 1. Fifteen-day-old seedlings of the wild type, *rrl1*, *rrl2-1*, *crl2* and three double mutants among these mutations. A: wild type, B: *rrl1*, C: *rrl2-1*, D: *crl2*, E: *rrl1 rrl2-1*, F: *rrl1 crl2*, G: *rrl2-1 crl2*. Bars = 40 mm.

Table 3. Morphological characters of the *rrl1*, *rrl2-1*, *rrl2-2*, *rrl2-3*, *crl2* mutants and wild type in fifteen-day-old seedlings

Character	Wild type	<i>rrl1</i> mutant	<i>rrl2-1</i> mutant	<i>rrl2-2</i> mutant	<i>rrl2-3</i> mutant	<i>crl2</i> mutant
Plant height (cm)	14.0 ± 0.4	11.5 ± 0.2**	14.5 ± 0.6 ^{ns}	13.9 ± 0.6 ^{ns}	13.2 ± 0.8 ^{ns}	9.2 ± 1.0**
Plant age (leaf number)	3.0 ± 0.2	3.5 ± 0.2**	3.2 ± 0.3 ^{ns}	3.2 ± 0.3 ^{ns}	3.1 ± 0.2 ^{ns}	2.5 ± 0.4*
Length of seminal roots (cm)	23.9 ± 1.0	8.7 ± 0.5**	10.6 ± 1.1**	10.7 ± 0.9**	11.1 ± 0.9**	27.2 ± 0.9**
Diameter of seminal roots (μm) ¹⁾	358.8 ± 10.2	373.3 ± 12.1 ^{ns}	243.5 ± 12.1**	236.8 ± 14.5**	240.4 ± 10.3**	603.2 ± 16.0**
Number of crown roots	5.8 ± 0.6	6.0 ± 0.3 ^{ns}	6.3 ± 0.5 ^{ns}	6.5 ± 0.4 ^{ns}	6.5 ± 0.4 ^{ns}	1.0 ± 0.5**
Branching density of lateral roots (cm ⁻¹) ²⁾	11.2 ± 0.5	6.3 ± 0.2**	14.9 ± 0.8**	15.1 ± 1.2**	15.6 ± 0.8**	7.1 ± 0.4**

Data show means ± S. E. (n = 6).

¹⁾ This value was determined in a zone located at 1.0 cm behind the root tip.

²⁾ Number of first order lateral roots on seminal root axis/length of seminal root axis.

ns, *, **: not significant, significant at 5% and 1% levels from the wild type, respectively.

thicker and the branching density of the lateral roots was lower compared with those of the wild type (Table 3).

Because of the similarities in the morphological characters of the *rrl2-1*, *rrl2-2* and *rrl2-3* mutants (Table 3), we used the *rrl2-1* mutant as a representative of this complementation group and then examined the growth of the seminal root after germination in the *rrl1*, *rrl2-1* and *crl2* mutants and the wild type. The roots of the *rrl1* mutant grew more slowly than those of the wild type throughout the observation period and almost stopped growing 12 days after germination (DAG) (Fig. 2). The root growth of the *rrl2-1* mutant became slower at 6 DAG compared with that of the wild type and almost stopped by about 9 DAG (Fig. 2), although the root of this mutant grew as fast as that of the wild type roots until 5 DAG (Fig. 2). On the other hand, the roots of the *crl2* mutant grew more slowly than those of the

wild type until 4 DAG, but subsequently the former surpassed the latter (Fig. 2). After three weeks of growth, the length of the *rrl1* and *rrl2-1* mutant roots was 35% and 41% of the length of the wild type root, respectively, while the length of the *crl2* root was 114% of the length of the wild type root.

Histological observation of *rrl* and *crl* mutants

The seminal root elongation rate was determined from the difference in root length between 3 and 6 DAG when the root length increased linearly with time for all the three mutants and the wild type (Fig. 2).

Compared with the wild type, the mean cortical cell length in the mature part was shorter and the position of the area where cell elongation started was more basipetal in the *rrl1* mutant (Figs 3B, 4A and Table 4). In contrast, no significant difference was detected in the cell fluxes between the *rrl1* mutant and the wild type (Table 4). These observations suggest that the short root trait observed in the *rrl1* mutant was caused by both the delay in the commencement of cell elongation along the root axis and the decrease of the mature cell length and not by the reduction of the cell flux. Similarly, the mean cortical cell length in the mature part of the *rrl2-1* mutant root was significantly shorter than that of the wild type root (Fig. 4A and Table 4). However, the reduction in the cell length of this mutant was not as severe

Fig. 2. Change with time in the seminal root length of the wild type, *rrl1*, *rrl2-1* and *crl2* mutants. The error bars represent the standard errors. Each value represents the mean measurement for 6 seedlings.

Table 4. Cortical cell flux evaluated at the base of the elongation zone of the wild type, *rrl1*, *rrl2-1*, *crl2* and three double mutants among these mutations

	Root elongation rate (mm h ⁻¹)	Mature cell length (μm)	Cell flux (h ⁻¹)
Wild type	0.84	88.3	9.51
<i>rrl1</i>	0.46**	47.5**	9.68 ^{ns}
<i>rrl2-1</i>	0.60**	76.1**	7.88**
<i>crl2</i>	1.12**	102.5*	10.93**
<i>rrl1 rrl2-1</i>	0.45	59.2	7.62
<i>rrl1 crl2</i>	0.70	63.5	10.99
<i>rrl2-1 crl2</i>	0.78	84.6	9.21

Data show means (n = 6).

ns, *, **: not significant, significant at 5% and 1% levels from the wild type, respectively.

Fig. 3. Histological characteristics of seminal roots in the wild type, *rrl1*, *rrl2-1*, *crl2* and three double mutants among these mutations in 9-day-old seedlings. A, B, F, G and H: median longitudinal sections of mature parts. Bars = 100 μ m. C, D, E and I: median longitudinal sections of root apices. Bars = 100 μ m. A and C: wild type, B: *rrl1*, D: *rrl2-1*, E and F: *crl2*, G: *rrl1 rrl2-1*, H: *rrl1 crl2*, I: *rrl2-1 crl2*. cc: central cylinder, c: cortex. Arrows indicate cells that already started elongation and vacuolation.

as that of *rrl1* (Fig. 4A and Table 4). On the other hand, the cells in the proximal region of the *rrl2-1* mutant meristem apparently differentiated; they were already elongated and vacuolated compared with those of the wild type meristem (Figs. 3C and D). Basipetal distribution of the cortical cell length along the root axis showed that the area where the cell did not begin elongation yet, i. e., the meristematic region in this mutant root was clearly smaller than that in the wild type root (Fig. 4A). Cell flux of this mutant was also significantly reduced compared with that of the wild type (Table 4). These results indicate that the short root trait observed in the *rrl2-1* mutant was caused by a reduction in both cell flux and cell length.

The mean cortical cell length in the mature part of the *crl2* mutant root was significantly longer than that of the wild type (Figs. 3F, 4A and Table 4). The value of the meristematic region size and cell flux in this mutant root, in addition, were clearly higher compared with those of the wild type (Figs. 3E, 4A and Table 4). These facts suggest that the long root trait observed in the *crl2* mutant was caused by the increase in both cell length and cell flux.

Double mutant analysis

In the F_2 progeny from the cross between the *rrl1* and *rrl2-1* mutants, four different phenotypes; wild type, parental single mutants and double mutant were segregated and this segregation was consistent with the 9:3:3:1 ratio (data not shown). For further confirmation, the F_2 plants showing

either parental single mutant phenotype were also self-pollinated and the segregation ratio of the phenotypes in the F_3 progeny was investigated. It was shown that the segregation of either parental single mutant to double mutant also agreed with the 3:1 ratio (Table 5). The same observations applied to the crosses between the *crl2* and each of the *rrl1* and *rrl2-1* mutants (Table 5).

There were several differences and similarities in the morphological characters between the three double mutants (*rrl1 rrl2-1*, *rrl1 crl2* and *rrl2-1 crl2*) and the respective parental single mutants as follows. Since the elongation rate was found to be constant from 3 to 6 DAG for these double

Table 5. Segregation of phenotype in F_3 progenies that were produced by the self-pollination of the F_2 plants showing either parental single mutant phenotype from the crosses among the *rrl1*, *rrl2-1* and *crl2* mutants

Combinations	Phenotype		χ^2 (3:1)	P.
	F_2 phenotype ³⁾	double mutant ⁴⁾		
<i>rrl1</i> $\times$ <i>rrl2-1</i> ¹⁾	83	36	1.75	0.10-0.25
<i>rrl1</i> $\times$ <i>crl2</i> ¹⁾	109	24	3.43	0.05-0.10
<i>rrl2-1</i> $\times$ <i>crl2</i> ²⁾	65	21	0.02	0.99 <

¹⁾, ²⁾: F_2 plants showing the *rrl1* and *rrl2-1* phenotypes were self-pollinated to produce F_3 seeds, respectively.

³⁾: Individuals showing the *rrl1* mutant phenotype were counted in crosses of *rrl1* $\times$ *rrl2-1* and *rrl1* $\times$ *crl2*, and individuals showing the *rrl2-1* mutant phenotype in the cross of *rrl2-1* $\times$ *crl2*.

⁴⁾: Individuals showing the non- F_2 phenotype were counted.

Fig. 4. Change with the cell number from the tip of the root apical meristem in the cortical cell length of the wild type, *rrl1*, *rrl2-1*, *crl2* and three double mutants among these mutations in 9-day-old seedlings. ■, ◆, ●, ▲ and + show the mature cell length of the wild type, *rrl1*, *rrl2-1*, *crl2* and respective double mutants, respectively. The error bars represent the standard errors. Each value represents the mean measurement for 6 seedlings.

mutants (data not shown), the cell flux was calculated using the seminal root elongation rate determined from the difference in root length between 3 and 6 DAG.

rrl1 rrl2-1 double mutant: The seminal root length was much shorter than that of both parental single mutant roots (Figs. 1E and 5) and the mature cortical cell length was intermediate between that of the parental single mutants (Figs. 3G, 4B and Table 4). On the other hand, the meristematic region size and cell flux were about the same as those of the *rrl2-1* mutant (Fig. 4B and Table 4) and the plant height was as short as that of the *rrl1* mutant (Fig. 5).

rrl1 crl2 double mutant: The seminal root length and cortical cell length were intermediate between those of the parental single mutants, respectively (Figs. 1F, 3H, 4C and 5). The plant height was shorter than that of both single parental mutants (Fig. 5). On the other hand, the cell flux was similar to that found in the *crl2* mutant (Table 4).

rrl2-1 crl2 double mutant: The seminal root length, meristematic region size, cell flux and cortical cell length were all intermediate between those of the parental single mutants, respectively (Figs. 1G, 3I, 4D, 5 and Table 4). On the other hand, the plant height was as short as that of the *crl2* mutant (Fig. 5).

Discussion

Several researchers have identified and characterized rice mutants with reduced seminal root length (Kitano and Futsuhara 1989, Liang and Ichii 1996, Ichii and Ishikawa 1997, Noda *et al.* 2000). However, since no mutant with a long root trait had been identified, it remained to be determined what kinds of genes inhibit the root elongation and how these genes interact with the genes that promote the elongation. In this study, we examined both types of rice mutants to elucidate the functions of the genes that promote or inhibit the root elongation, and then investigated the interactions between these genes by characterizing the respective double mutants.

Root elongation is regulated by the combined effect of two linked processes, cell division and elongation. Our results showed that the short root trait observed in the *rrl1* mutant was caused by both the delay in the commencement of cell elongation and the decrease in mature cell length and not by the reduction of the cell flux, suggesting that *RRL1* regulates both the commencement and extent of cell elongation.

In contrast, the short root trait observed in the *rrl2-1* mutant was caused by a reduction of both cell flux and cell

Fig. 5. Plant height and seminal root length of the wild type, *rrl1*, *rrl2-1*, *crl2* and three double mutants among these mutations in fifteen-day-old seedlings. The error bars represent the standard errors. Each value represents the mean measurement for 6 seedlings.

length. Cell flux has two different components; the number of dividing cells and their rate of division (Beemster and Baskin 1998). To date, two short root mutants, *rt* and *srt-1*, showing a small meristem size and reduced cell length in the mature parts have been reported in rice and both of these mutants exhibited a lower root elongation rate than that of the respective wild types immediately after germination (Kitano and Futsuhara 1989, Ichii and Ishikawa 1997). However, the *rrl2-1* mutant roots grew as fast as those of the wild type until 5 DAG. If the reduction of the cell flux in the *rrl2-1* mutant resulted from at least the decrease of the dividing cell number due to the acceleration of the transition of meristematic cells from division to elongation, early root elongation could not have decreased sharply in spite of the reduction of the cell flux. Therefore, we propose that *RRL2-1* inhibits the transition of the cells from division to elongation.

This idea is also supported by the morphological characters of the *rrl1 rrl2-1* double mutant. Since the reduction in the mature cortical cell length of the *rrl1* mutant was more severe than that of the *rrl2-1* mutant, the length of the *rrl1 rrl2-1* double mutant should have been shorter than that of

the *rrl1* mutant (additive interaction) or similar to that of the *rrl1* mutant (epistatic interaction). However, the length of the *rrl1 rrl2-1* double mutant was intermediate between that of the parental single mutants. Since the interruption of cell division and commencement of elongation are independent processes (Ivanov 1981), this assumption is not valid, if the reduction of the cell flux in the *rrl2-1* mutant is caused only by the reduction of the cell division rate. As mentioned above, it is considered that *RRL1* promotes the commencement of cell elongation. Therefore, assuming that *RRL2-1* inhibits the transition of meristematic cells from division to elongation, it seems reasonable to consider that the intermediate cell length of the *rrl1 rrl2-1* double mutant resulted from opposite effects between each of the mutation genes.

We also investigated the *crl2* mutant with increased seminal root length and it was found that the long root trait of this mutant was caused by the increase in both cell length and cell flux. Although the root growth of this mutant became faster from 8 DAG compared with the wild type, the length of this mutant was significantly shorter than that of the wild type 3 DAG (significant at 5% level). Therefore, in contrast to *RRL2-1*, one of the *CRL2* functions may promote the transition of meristematic cells from division to elongation.

The root meristem size of the *crl2* mutant was clearly longer than that of the wild type. This type of mutation had not been reported so far, while two mutants accumulating too many cells in the shoot apical meristem had been identified in *Arabidopsis*, *clavata1* (*clv1*) and *clv3* (Clark *et al.* 1993, 1995). In contrast, *shoot apical meristemless1* (*stm-1*) and *stm-2* mutants have been also identified, that fail to organize and maintain a shoot apical meristem (Barton and Poething 1993, Clark *et al.* 1996). In the *stm clv* double mutants, the meristem was often produced and maintained suggesting that *STM* and *CLV* exert opposite effects and are actually the regulators involved in the balance between cell proliferation and differentiation of the meristem (Clark *et al.* 1996). In the *rrl2-1* *crl2* double mutant, both root apical meristem size and cell flux were intermediate between those of the parental single mutants. These observations suggest that *RRL2-1* and *CRL2* also exert opposite effects and are required for meristem homeostasis and that similar genetic mechanisms regulate each of shoot and root apical meristems.

As mentioned above, the cortical cell length in the mature parts of the *rrl1* and *rrl2-1* mutants was shorter, while that of the *crl2* mutant was longer compared with the wild type. In both *rrl1* *crl2* and *rrl2-1* *crl2* double mutants, the cortical cell length was intermediate between that of the respective parental single mutants. These results suggest that *CRL2* counteractively acts on *RRL1* and *RRL2-1* in the regulation of cell elongation.

Although the genetic mechanism regulating root elongation is almost unknown, this study revealed that there are genes that counteractively act on the maintenance of the root apical meristem and cell elongation. In order to promote a breeding program related to root traits, information about

the genetic regulation throughout root development is essential and further phenotypic characterization and evaluation of the hormonal regulation of the *rrl* and *crl* mutants may enable to determine how root elongation is regulated by these individual genes.

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