

The rice RCN1/OsABCG5 mutation is associated with root development in response to nutrient shortage

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Abstract: Plants constantly sense changes in their environment. When nutrient elements are scarce, plants often allocate a greater proportion of their biomass to the root system. In the present study, we characterized the early root development of rice *reduced culm number* (*rcn*) mutants under standard growth conditions. Short lateral root length was consistent in five *rcn* mutants, and the remaining root traits varied between the *rcn* mutants. Subsequently, we characterized the effects of *rcn1* mutation in response to nutrient shortage. Root development, involving the elongation of seminal, crown, and lateral roots and branching of the lateral roots, was promoted in wild type in response to nutrient shortage. However, in *rcn1* mutants, crown root elongation was constant and lateral root branching was reduced in response to nutrient shortage. The present results indicate that RCN1/OsABCG5 is associated with root development in response to nutrient shortage.

Keywords: Nutrient shortage, RCN1/OsABCG5, reduced culm number, rice, root architecture

Introduction

Plants constantly sense changes in their environment and when nutrient elements are scarce, plants often allocate a greater proportion of their biomass to the root system. It is known that deficiencies in essential macronutrients (nitrogen, phosphorus, potassium, and magnesium) result in an accumulation of carbohydrates in leaves and roots, which modifies the root-shoot biomass ratio (Reviewed by Hermans et al.

2006). To date, ion transport mechanisms and the biochemical pathways affected by mineral deficiencies have been well elucidated (Epstein et al. 2005); however, the molecular and physiological events responsible for sensing and signaling mineral resource limitations and their ultimate effects on plant development and biomass allocation are not well understood.

The rice *reduced culm number 1* (*rcn1-2*) mutant, reported as a low tillering mutant (Takamure et al. 1989, Yasuno et al. 2007, 2009), delayed flowering by more than 40 days, and reduced shoot and root dry weights in comparison with wild type under nutrient-limiting conditions. This suggests that *rcn1-2* mutation reduces plant development for sensing nutrient shortage and/or reduces nutrient uptake from the soil under low-nutrient conditions (Ariyaratna et al. 2011). We determined that RCN1 encodes a novel protein in the ATP-binding cassette protein G (ABCG) subfamily, also known as the white-brown complex (WBC) subfamily, OsABCG5 (OsWBC5) (Yasuno et al. 2009). The molecular function of RCN1/OsABCG5 remains to be elucidated. The present study was conducted to determine if RCN1/OsABCG5 is essential for root development in response to nutrient shortage. In addition to *rcn1*, ten other genes with mutations leading to reduced culm number have been reported (Takamure et al. 1989, Takamure and Kinoshita 1993, Takamure 1994, Tang et al. 2001, Li et al. 2003, Jiang et al. 2006, Yasuno et al. 2007, Ariyaratne et al. 2009); however the root phenotypes of *rcn* mutants have not been described yet. In the present study we conducted two experiments. In the first experiment, we characterized the early root development of *rcn1* mutant in comparison with the four other independent reduced culm number

mutants (*rcn2*, *rcn4*, *rcn5*, and *rcn6*) in rice from the *japonica* background under normal conditions. In the second experiment, we examined whether *rcn1* mutation affects early root development in response to nutrient shortage.

Materials and Methods

Experiment 1

We examined the rice (*Oryza sativa* L. ssp. *japonica*) cultivar Shiokari and five rice mutants originating from Shiokari; S-97-61 (*rcn1-2*), N-174 (*rcn2*), N-187 (*rcn4-2*), N-185 (*rcn5*), and N-186 (*rcn6*). These five *rcn* mutations are at independent loci, based on an allelism test (Takamure et al. 1989, Takamure and Kinoshita 1993, Takamure 1994). All seeds were surface sterilized and incubated in the dark at 30°C for 2 days to allow germination. Germinated seeds were transferred to plastic boxes (26 × 21 × 0.5 cm) containing Murashige and Skoog (MS) medium (Murashige and Skoog 1962) (without sugar, pH 5.8, 1.3% agar) at ×1 (1-MS) concentration and were grown under a regime of 16 h of light (350 μmol/m²s) at 28°C and 8 h of darkness at 24°C. The lower parts of the plastic boxes were covered with black paper to keep the roots continuously in the dark. The following observations were recorded daily from Day 5 to Day 12: the number of leaves (leaf stage), shoot length, lengths of the seminal and crown roots, the number of crown roots, and the number and length of lateral roots on the seminal root. The density of the lateral roots on the seminal root axis was determined by dividing the number of lateral roots by the length of the seminal root for each plant. Lateral root lengths were measured with a ruler using 0.5 mm increments.

Experiment 2

Shiokari and *rcn1-2* seeds were surface sterilized and incubated in the dark at 30°C for 2 days to allow germination. Germinated seeds were transferred to plastic boxes (26 × 21 × 0.5 cm) containing MS medium (Murashige and Skoog 1962) (without sugar, pH 5.8, 1.3% agar) at ×1 (1-MS), ×0.5 (0.5-MS), and ×0 (0-MS) concentrations and were grown as mentioned in Experiment 1. At 10 days after germination we recorded the same traits as Experiment 1.

Statistical analysis

For the analysis of root traits in Shiokari and the five *rcn* mutants, one-way analysis of variance (ANOVA) was performed for each trait in Experiment 1. Least significant difference (LSD) was used for comparison



Fig. 1. Root morphology of wild-type Shiokari and the five *rcn* mutants 12 days after planting. Plants were grown on 1-MS agar medium (without sugar, pH 5.8, 1.3% agar). Scale bar represents 5 cm.

among the mean values of the six strains. Two-way ANOVA was performed to analyze the effect of genotype and nutrient treatments on root traits in Experiment 2. Means were separated by the LSD test if the *F*-test indicated a significant difference ($P < 0.05$). All statistical analysis was conducted using PASW[®] Statistics 18 (SPSS, Inc.).

Results

Root architecture of the five *rcn* mutants

Seedlings were grown on 1-MS agar medium to compare root morphology of the different *rcn* mutants. The root architecture of 12-day-old *rcn* seedlings and the wild-type Shiokari is shown in Fig. 1. Shoot growth was delayed in all *rcn* mutants by 1 or 2 days compared to that in the wild type (Table 1). To eliminate the effect of leaf stage on root development, we measured root architecture when the seedlings were at the 3-leaf stage (Table 1). *rcn1-2* seedlings had significantly shorter lateral roots and a larger lateral root density in comparison with Shiokari. *rcn2* seedlings had a significantly longer seminal root, larger number of lateral roots and shorter total crown and lateral root length in comparison with Shiokari. *rcn4-2* seedlings had significantly shorter shoots, seminal and lateral roots, fewer number of lateral roots and lower lateral root density in comparison with Shiokari. *rcn5* seedlings had significantly shorter seminal roots, total crown and lateral roots, higher lateral root density and a fewer number of crown roots in comparison with Shiokari. *rcn6* seedlings had significantly shorter total crown and lateral root length and a fewer number of crown roots.

Among all the characteristics tested in the five *rcn* mutants, the reduction in lateral root length was the most evident. To characterize the effect of *rcn* genes on lateral root elongation, the kinetics of lateral root

Table 1. Morphological analysis of seedlings of wild-type Shiokari, *rcn1-2*, *rcn2*, *rcn4*, *rcn5*, and *rcn6* at the 3-leaf stage.

Genotype	Days to 3rd leaf stage	Shoot length (cm)	Seminal root length (cm)	Crown root number	Total crown root's length (cm)	Lateral root number	Lateral root length (mm)	Lateral root density (cm ⁻¹)
Shiokari	9.0±0.0 d	13.6±0.6 ab	10.4±0.8 b	5.0±1.1 a	18.1±5.0 a	113.2±18.3 b	5.1±0.7 a	10.9±1.9 b
<i>rcn1-2</i>	10.5±0.5 b	12.5±1.2 bc	9.8±1.0 bc	5.0±1.1 a	15.9±4.1 a	131.2±26.5 ab	2.6±0.6 c	13.3±2.2 a
<i>rcn2</i>	10.7±0.5 b	12.3±1.4 c	11.6±0.8 a	3.8±1.7 ab	9.2±4.6 c	149.5±29.1 a	3.6±0.8 b	12.9±2.2 ab
<i>rcn4-2</i>	10.2±0.9 bc	10.9±0.9 d	6.7±0.1 d	4.7±1.5 a	13.4±4.9 ab	50.0±14.3 c	2.1±0.3 c	7.5±2.2 c
<i>rcn5</i>	11.2±0.9 a	14.1±0.9 a	9.1±1.0 c	3.0±0.7 b	10.9±1.6 bc	136.0±11.5 ab	2.7±0.9 c	15.0±0.6 a
<i>rcn6</i>	9.8±1.0 c	12.7±1.3 bc	10.6±0.1 ab	2.8±1.0 b	9.7±2.1 bc	139.3±22.4 ab	4.0±1.0 b	13.2±2.0 ab

To evaluate morphological characteristics, the seedlings were grown on 1-MS agar medium (without sugar, pH 5.8, 1.3% agar). Data are the average for six plants (± SD).

Different letters indicate significant differences ($P < 0.05$) between genotypes.

elongation were compared (Fig. 2). The lateral roots of Shiokari and *rcn6*; *rcn1-2*, *rcn2*, and *rcn5*; and *rcn4-2* seedlings began elongating at 5, 6, and 7 days after germination (DAG), respectively. The lateral root elongation of *rcn1-2*, *rcn5*, and *rcn6*; Shiokari and *rcn2*; and *rcn4-2* seedlings almost ceased at 7, 8, and 12 DAG, respectively. Based on the kinetics of lateral root elongation, the five *rcn* mutants were divided into three groups. In the first group, the elongation period was shortened, as represented by *rcn6* seedlings, in which the elongation rate was similar to Shiokari at between 5 and 6 DAGs and elongation ceased at 7 DAG. In the second group, the onset was delayed and the elongation period was

shortened, as represented by *rcn1-2*, *rcn2*, and *rcn5*. In the third group, the onset was delayed until 7 DAG and the elongation rate was reduced, as represented by *rcn4-2* seedlings.

Response to nutrient shortage in *rcn1-2* mutant

We tested the possibility that *rcn1-2* affects root development in response to nutrient shortage using 10-day-old seedlings. The two-way ANOVA test indicated significant interaction between genotype and treatment in seminal root length, crown root number, length of the longest crown root, total crown root length, lateral root number and lateral root density (Table 2). In Shiokari seedlings, no differences were detected between 1-MS and 0.5-MS medium in all root traits tested (Fig. 3). When 0-MS media was used as the nutrient shortage condition on Shiokari seedlings, it affected shoot elongation and the development of three root-types; seminal, crown, lateral roots. Shiokari and *rcn1-2* seedlings showed reduced shoot length on 0-MS medium in comparison with 1-MS or 0.5-MS (Fig. 3A). Shiokari seedlings on 0-MS medium significantly promoted the elongation of seminal, the longest crown and total crown length compared with 1-MS by factors of 1.8, 2.3, and 1.6 times, respectively. For *rcn1-2*, the significant difference between 0- and 1-MS was detected in seminal root length. In *rcn1-2* seedlings, the elongation of seminal root length in 0-MS medium was 1.5 times larger than that in 1-MS medium, and the longest crown, total crown and lateral root lengths in 0-MS medium tended to be greater than those in 1-MS medium. Crown root number in Shiokari seedlings was significantly reduced by 34% on 0-MS medium compared to 1-MS, whereas the crown root number in *rcn1-2* on 0.5-MS medium was significantly increased compared to that on 1-MS medium (Fig. 3C). On 0-MS medium, Shiokari seedlings

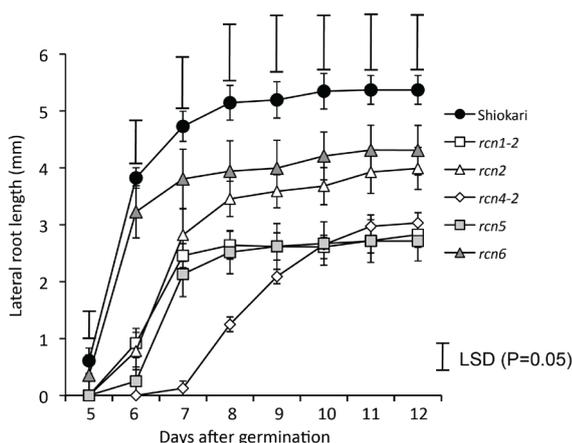


Fig. 2. Kinetic comparison of lateral root elongation between wild-type Shiokari and the five *rcn* mutants. The length of lateral roots emerging from the region between 2 cm and 4 cm from the base of the seminal root is represented. Plants were grown on 1-MS agar medium (without sugar, pH 5.8, 1.3% agar). Each value represents the average for six plants (± SE).

Table 2. Two-way ANOVA statistics of the effect of *rcn1-2* genotype on shoot length, seminal root length, crown root number, length of the longest crown root, total crown root length, lateral root number on seminal roots, lateral root density on seminal roots, and lateral root length on seminal roots of 10-day-old seedlings sorted by MS medium concentration.

Traits	Variation	df	MS	F	P
Shoot length	Genotype	1	5160.0	19.6	<0.001
	Treatment	2	3034.2	11.5	<0.001
	Genotype × Treatment	2	78.4	0.3	ns
	Error	30	263.9		
Seminal root length	Genotype	1	9834.0	17.8	<0.001
	Treatment	2	18098.1	32.8	<0.001
	Genotype × Treatment	2	3788.1	6.9	<0.01
	Error	30	551.9		
Crown root number	Genotype	1	4.7	2.7	ns
	Treatment	2	13.0	7.5	<0.01
	Genotype × Treatment	2	12.7	7.3	<0.01
	Error	30	1.7		
Length of the longest crown root	Genotype	1	26136.1	105.4	<0.001
	Treatment	2	11166.6	45.0	<0.001
	Genotype × Treatment	2	9373.7	37.8	<0.001
	Error	30	247.9		
Total crown root length	Genotype	1	326041.0	42.9	<0.001
	Treatment	2	75472.7	9.9	<0.001
	Genotype × Treatment	2	61062.8	8.0	<0.01
	Error	30	7605.5		
Lateral root number	Genotype	1	21904.0	40.4	<0.001
	Treatment	2	2149.8	4.0	<0.05
	Genotype × Treatment	2	17557.3	32.3	<0.001
	Error	30	542.9		
Lateral root density	Genotype	1	0.0	0.0	ns
	Treatment	2	90.3	9.7	<0.001
	Genotype × Treatment	2	57.1	6.1	<0.01
	Error	30	9.3		
Lateral root length	Genotype	1	66.0	49.9	<0.001
	Treatment	2	8.7	6.6	<0.01
	Genotype × Treatment	2	3.9	3.0	ns
	Error	30	1.3		

showed a 1.6 times increase in lateral root number with a constant density, whereas *rcn1-2* showed a reduction in both lateral root number and density by 36% and 60%, respectively, compared to 1-MS (Fig. 3F, 3G). As a result, the lateral root number and density of *rcn1-2* seedlings were significantly lower

than those of Shiokari seedlings on 0-MS medium.

The developmental characteristics in 10-day-old plants of the lateral roots along the seminal root grown on 1-, 0.5-, and 0-MS media were examined in detail. The relationship between the number and length of lateral roots for each 2-cm segment of the

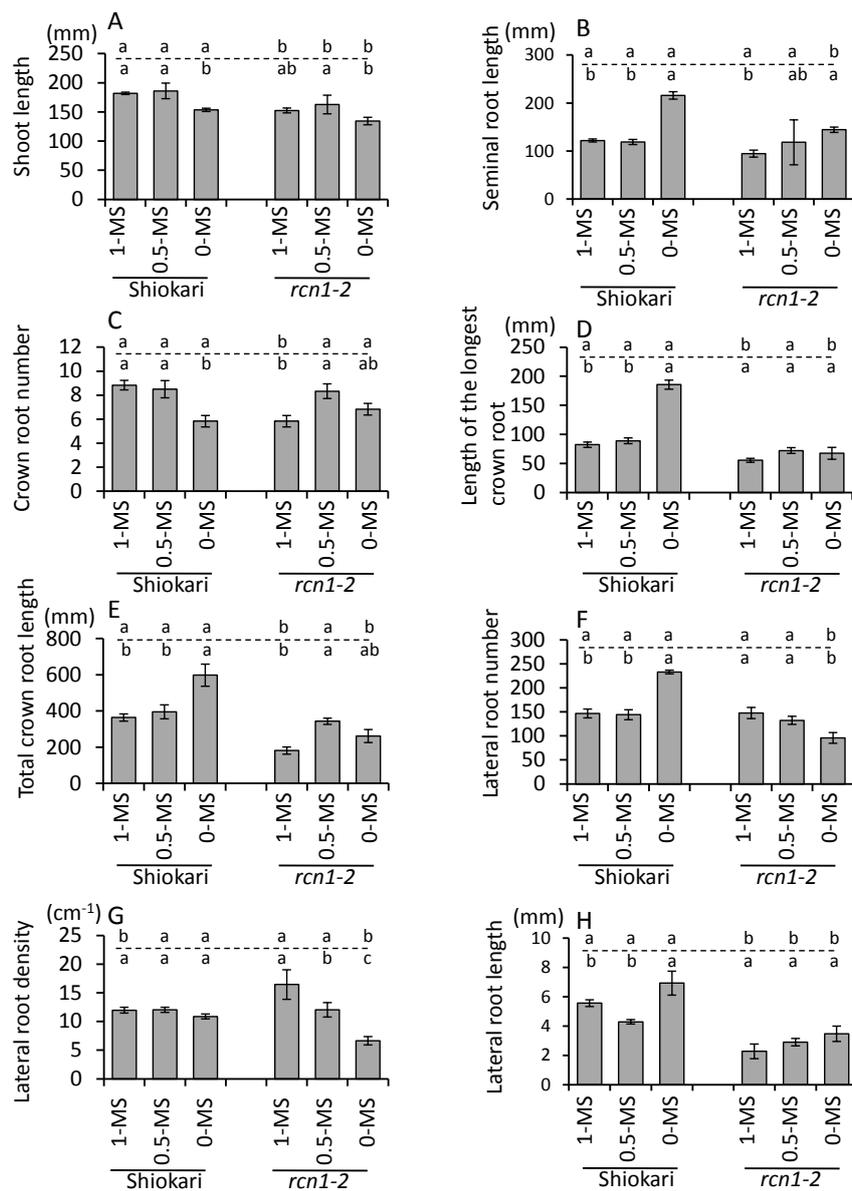


Fig. 3. Shoot length (A), seminal root length (B), crown root number (C), length of the longest crown root (D), total crown root length (E), lateral root number on seminal roots (F), lateral root density on seminal roots (G), and lateral root length on seminal roots (H) of 10-day-old seedlings. Each value is the average for six plants (\pm SE). For each strain, different letters under the dotted line indicate significant differences ($P < 0.05$) between nutrient conditions. For each nutrient condition, different letters on the dotted line indicate significant differences ($P < 0.05$) between strains.

seminal root axis is shown in Fig. 4. Each genotype has a similar number of lateral roots in each segment on 1- and 0.5-MS media (Fig. 4A, 4B, 4C, 4D). In Shiokari seedlings, the lateral root number per segment remained constant on 0-MS medium, with a maximum number of 25–30 roots at the basal part of seminal roots, between the base and 16 cm. In the same region of *rcn1-2* seedlings, the lateral root number was reduced to less than 20 roots. Combining the effects of shortened seminal roots and reduced lateral root branching per segment resulted in a

reduction in the total lateral root number of *rcn1-2* seedlings on 0-MS medium. Both the minimum and maximum length of the lateral roots per seminal root segments in both genotypes increased on 0-MS medium in comparison with 0.5-MS and 1-MS medium. On 0-MS medium, the lateral root elongation in Shiokari increased in the section of the seminal root between 6 cm and 16 cm. In *rcn1-2*, there were few differences in lateral root length among the MS treatments.

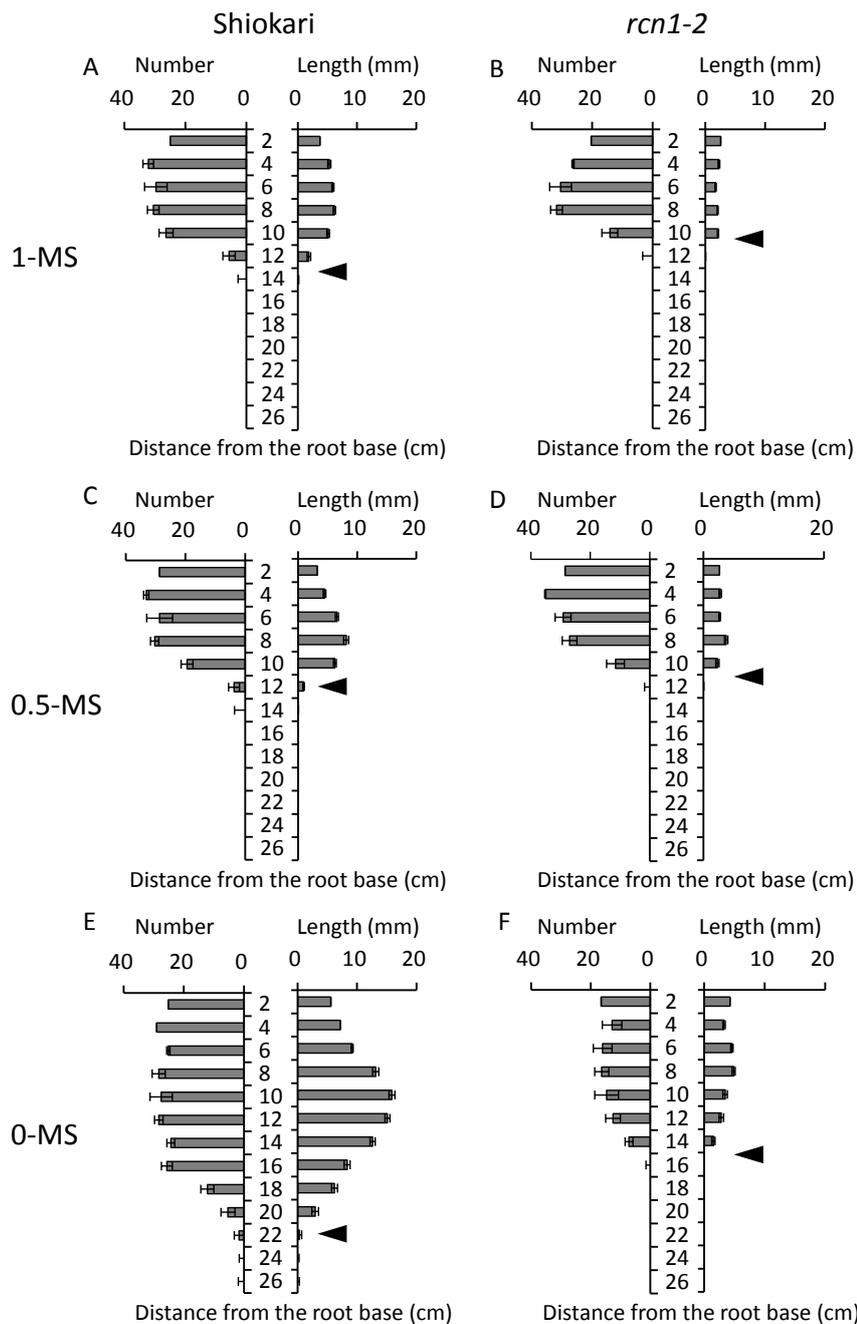


Fig. 4. Lateral root number (left) and length (right) in each 2-cm segment of the seminal root of 10-day-old Shiokari (A, C, E) and *rcn1-2* (B, D, F) grown on 1-, 0.5- and 0-MS medium (without sugar, pH 5.8, 1.3% agar). Each value is the average for six plants (\pm SE). Black triangles represent the positions of the root tip.

Discussion

The present study showed the variable effects of the *rcn* gene on early root development, independent of shoot growth and node number. Among these root characteristics, the lateral roots of the five *rcn* mutants were consistently shorter than those of the wild-type Shiokari (Fig. 2, Table 1). Lateral roots

play important roles in providing an absorptive area for nutrients and water, as well as anchoring the plant in the soil (Banoc et al. 2000, Ma et al. 2001, Bailey et al. 2002, Kamoshita et al. 2004). Our study suggests that shoot branching during growth is influenced by the same genetic system that affects root branching. However, we could not eliminate the possibility that nutrient limitation due to limited root

development also reduced shoot branching in *rcn* mutants.

It is clear that plants have evolved sophisticated pathways for sensing and responding to changes in various external nutrients as well as their own internal nutrient status. Shortage of nutrients in soil rapidly slows plant shoot growth, whereas root growth is often less inhibited, thereby increasing the relative surface area available for ion absorption (Chapin 1990). Nitrogen and phosphorus deficiencies result in an increase in the root-shoot biomass ratio (Hermans et al. 2006). The present study showed that nutrient shortage reduced shoot length and promoted elongation of seminal, crown, and lateral roots as well as the emergence of lateral roots in Shiokari seedlings (Fig. 3). Under nutrient limiting conditions, the crown root number was reduced, whereas total crown root length increased; showing that promoting the elongation of fewer crown roots resulted in an increase of total crown root length in Shiokari. In Shiokari under nutrient limiting conditions, lateral root density was maintained, seminal root elongation was promoted, and lateral root length increased, which in turn resulted in an increase in total lateral root length. In contrast, the degree of elongation of all three root types tended to be lower and the emergence of lateral roots were significantly reduced in *rcn1-2* mutants under nutrient shortage conditions. The present evidence suggests that the RCN1/OsABCG5 mutation is associated with sensing nutrient shortage or the morphogenesis in root development in response to nutrient shortage. The reduction in root development in response to nutrient shortage may inhibit root functions in taking up water and nutrients from the soil, sensing abiotic and biotic stresses, and serving as an anchor that supports shoots. In a future study, we need to clarify the effect that this poor root development in *rcn1-2* on its root functions under nutrient shortage conditions.

Nutrient deficiency was reported to stimulate abscisic acid (ABA) biosynthesis in roots and intensify the root-to-shoot ABA signal (Chapin 1990, Peuke et al. 1994, 2002, Saab et al. 1990, Jeschke et al. 1997, Jeschke and Hartung 2000, Schraut et al. 2005). Accumulation of ABA leads to a higher root-shoot biomass ratio in nutrient-deficient plants (Saab et al. 1990, Vystoskaya et al. 2008). Vystoskaya et al. (2008) reported that ABA accumulates in the root apices of nutrient-deficient plants and is involved in maintaining root growth. In addition to ABA, cytokinin-mediated signaling seems to control plant development under nitrogen deficiency, and coordinated changes in cytokinin, auxin, and ethylene concentrations may reprogram development under phosphorus deficiency (Hammond et al. 2004, Ticconi and Abel 2004, Franco-Zorrilla et al. 2004).

RCN1/OsABCG5 mRNA accumulates not only in leaf primordia of main and axillary shoots and in the vascular cells and leaf epidermis of older leaves, but also in the crown root primordia, endodermis, pericycle, and stele in the roots (Yasuno et al. 2009). In future studies, we need to clarify which element and phytohormone is associated with RCN1/OsABCG5 function in roots. Finally, identification of the remaining *rcn* genes and clarification of their functions at the molecular level could explain whether *rcn* genes control the growth of both lateral shoots and roots.

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