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Analysis of the *rrl3* Mutants Reveals the Importance of Arginine Biosynthesis in the Maintenance of Root Apical Meristem in Rice

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Abstract

We characterized *reduced root length3(rrl3)* mutantsof rice that exhibit a short-root phenotype under conditions producing mechanical impediments to growth, such as aerated water culture medium. The mutants were not able to maintain the quiescent center (QC) identity and produced disorganized root apical meristem (RAM) under aeration because of impaired cell division. A map-based cloning approach showed that *RRL3* encodes carbamoyl phosphate synthetase (CPS) which is thought to be required for the conversion of ornithine into citrulline during arginine biosynthesis. The *RRL3* gene is expressed highly at the root tip area that includes the root cap and division zone. The *RRL3* gene expression level was greatly affected by aeration treatment, indicating that the spatiotemporal expression of the *RRL3* gene with respect to the aeration is important for the maintenance of RAM. Furthermore, the application of citrulline and arginine could rescue the root phenotype, which implied that arginine biosynthesis was impaired in the *rrl3-1* mutant. These results suggest that the *RRL3* regulated arginine biosynthesis is important for the maintenance of RAM organization in the presence of mechanical impediments.

Keywords: root apical meristem, carbamoyl phosphate synthetase, arginine biosynthesis, mechanical impediments, rice, mutant

1. Introduction

Roots are important to plants for a wide variety of processes, including nutrient and water uptake, anchoring and mechanical support, and storage functions, and they act as the major interface between the plant and various biotic and abiotic factors in the soil environment (Gewin, 2010; Herder, Van Isterdael, Beekman, &Smet, 2010; Smith &Smet, 2012). The root length is one of the most important and thus one of the most frequently measured parameters related to the plant ability of acquiring soil resources (Kashiwagi, Iwama, & Hasegawa, 2000; Teo, Beyrouty, Norman, &Gbur, 1995). Root length is determined by the number of proliferating cells and their final size (Beemster& Baskin, 1998). In the root apical meristem (RAM), cells undergo repeated rounds of cell division in the division zone, subsequently exiting the division zone to enter the elongation zone where they undergo significant gain in size followed by differentiation. Root meristem contains a distinct central region of mitotically inactive cells termed as the quiescent center (QC) (Clowes, 1956). Stem cells within the meristem are maintained in a specialized cell environment, called the stem cell niche (Scheres, 2007), and the identities of those stem cells are regulated by the signals that originate from the QC (van den Berg, Willemsen, Hendriks, Weisbeek, &Scheres, 1997). Mutations that disrupt the functions of the stem cells or those of the QC often lead

to an impaired root phenotype in Arabidopsis (Benfey et al., 1993; Di Laurenzio et al., 1996). To date, many studies on rice root mutants have been published that have enhanced our understanding about the emergence and development of rice roots under non-stress conditions (Liang &Ichii, 1996; Ichii& Ishikawa, 1997; Inukai, Miwa, Nagato, Kitano, & Yamauchi, 2001; Inukai, Miwa, Nagato, Kitano, & Yamauchi, 2003; Scarpella, Rueb, & Meijer, 2003; Yao, Taketa, &Ichii, 2003; Debi et al., 2003; Yao, Mushika, Taketa, &Ichii, 2004; Jiang et al., 2005; Inukai et al., 2005, Kitomi, Ogawa, Kitano, &Inukai, 2008; Kitomiet al., 2011; Kitomi, Inahashi, Takehisa, Sato, &Inukai, 2012; Inukai et al., 2012). Especially, the *WUS*-type homeodomain transcription factor*QHB*, an ortholog of *WOX5*, is suggested to have a role in maintenance of the RAM in rice (Kamiya et al., 2003). Besides, the analysis of the *Osiaa23* mutant reveals the importance of *OsIAA23*-derived auxin signaling for the postembryonic maintenance of QC in rice (Jun et al., 2011). However, the genetic information about RAM maintenance in rice is still limited (Li et al., 2006; Suzaki, Yoshida, & Hirano, 2008).

The structure of the root system and its dynamics are of particular importance in crop growth under stressful conditions. For example, mechanical stress, which shifts its strength as the soil water content decrease, is often a major limitation to root elongation in aerable soils and is important to consider in breeding programmes for drought-resistant crops (Yamauchi, Pardales, &Kono, 1996; Azhiri-Sigari, Yamauch, Kamoshita, & Wade, 2000). Therefore, it is important to understand the genetic regulation of such a response to stimuli. In a previous study, we reported that the *rrl3* mutant showed a short-root phenotype under conditions producing mechanical impediments to growth, such as aerated water culture medium (Inukai et al., 2003). Under mechanical stress condition, the cell flux in the growing region of *rrl3-1* mutant reduced significantly while the mature cell length is not different from the wild type (Inukai et al., 2003).

In this study, we report the functional mode of the novel gene *RRL3*, which encodes a member of carbamoyl phosphate synthetase (CPS), possessing a large subunit ATP-binding domain. CPS is thought to catalyze the conversion of glutamine and bicarbonate into carbamoyl phosphate and glutamate, and the resulting carbamoyl phosphate is utilized in the synthesis of arginine and pyrimidine nucleotides (Holden, Thooden, &Raushel, 1999). Arginine treatment could complement the short-root phenotype, which suggests that an impaired arginine biosynthesis occurs in the *rrl3* mutant. Based on the mutant phenotype and the expression pattern of the *RRL3* gene, we concluded that spatiotemporal expression of this gene is important in arginine biosynthesis for RAM maintenance under conditions that present mechanical obstructions to the growth of the rice plant.

2. Materials and Method

2.1Plant Growth Conditions

Seedlings of the wild types (Blue Rose and Taichung 65), *rrl3-1*, and HK8117 rice mutants were grown in nutrient-free water in a growth chamber at 29 ℃ exposed to continuous light, with or without aeration. For the allelism test, F_1 plants derived from the crosses between *rrl3-1* and HK8117 mutants were grown under identical conditions with aeration. For the citrulline, ornithine, and arginine treatment experiments, Blue Rose and *rrl3-1* plants were grown under identical conditions supplemented with 0.0, 0.05, 0.1, and 0.5 mMcitrulline, ornithine, or arginine.

2.2 Histological Analysis

BrdU (5-bromo-2′-deoxyuridine) staining was performed according to the procedure described by Ogawa, Kitamichi, Toyofuku, & Kawashima (2006). Briefly, 4-day-old plants were grown in a hydroponic system without aeration. Then, some plants were transferred to another water culture system containing 10 μM bromodeoxyuridine (BrdU) and 1 μM 5-fluorodeoxyuridine; after 7 hr, approximately 5-mm-long segments of seminal root tip were sampled for 0 days. Then, the hydroponically grown plants were aerated for 2 days and 4 days, transferred to the BrdU and 5-fluorodeoxyuridine solution, treated for 7 hr, and sampled at each time interval. Immediately after sampling, the samples were fixed with 4% (w/v) paraformaldehyde in PBS buffer (pH 7.4), serially dehydrated with graded ethanol, and embedded in Technovit 7100 (Okenshoji Co. Ltd.). We obtained 2-μm-thick longitudinal sections by using a microtome, and the sections were dried at 37 ℃. The sections were then rehydrated with 10 mM phosphate buffer, stained with Meyer Heamatoxylin (Sakura Finetek Japan Co., Ltd.) for 1 hr, washed with water for 5 min, dried at 37 ℃, and mounted with Eukitt. Finally, the stained samples were observed under a light microscope (Olympus) and digitally imaged.

To visualize the nuclei in the RAM cells, root tip samples were stained using 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) at a concentration of 0.5 μg/mL in 0.1% (v/v) Triton X-1000 for 10 min, and then washed twice with water. DAPI-stained nuclei were observed under a stereo microscope and digitally imaged.

2.3 Map-based Cloning and Expression Analysis

To map the *RRL3* gene, linkage analysis was performed using F_2 plants derived from crosses between the *rrl3-1* mutant (japonica variety) and Kasalath (indica variety). A BLAST search was performed in the rice DNA database in the Rice Genome Research Program (rapdb.dna.affrc.go.jp) and TIGR database (htt://www.tigr.org).

For RNA extraction, the root tips were sampled zone wise; for instance, zone 1 (0–1 mm), zone 2 (1–2 mm), zone 3 (2–3 mm), and zone 4 (5 mm) from the root tip of the plants grown under non-aerated conditions at 4 days after germination (DAG). For analyzing the expression pattern in relation to the aeration, sampling was performed on 5-mm root tips before starting the aeration at 1 hr, 3 hr, 6 hr, 9 hr, 12 hr, and 24 hr at 4 DAG. TRIzol reagent (Invitrogen) was used for RNA extraction, and the extracted RNA was purified using RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Quantitative real-time PCR was performed using One Step SYBR PrimeScript RT-PCR Kit II (Perfect Real Time) (TaKaRa) and StepOnePlus Real-Time PCR (Applied Biosystems). The expression levels of the *RRL3* gene were normalized against those of an internal control, *eEF-1α*.

3. Results

3.1Inheritance of Mutant Phenotype and Allelism Tests

We have shown previously that a recessive mutant, *reduced root length3-1* (*rrl3-1*), of the rice plant has short seminal, crown, and lateral roots; in that study, we had focused on the effects of mechanical stimulus on cell multiplication in the root meristematic zone (Inukai et al., 2003). We screened another mutant line, HK8117, which exhibited a similar phenotype of the *rrl3-1* mutant. Segregation ratio of the HK8117 phenotype in the M_3 progeny derived from an M_2 heterozygous plant fit the 3 (wild type):1 (mutant) ratio (Table 1), indicating that the mutant phenotype is also controlled by a single recessive gene. We crossed the 2 mutants, *rrl3-1* and HK 8117, with each other. All F_1 progenies from the crosses of *rrl3-1* × HK8117 mutant and HK8117 × *rrl3-1* had the mutant phenotype (Table 1). These results indicate that the mutant gene of HK8117 was an allele of *rrl3-1*, and therefore, we named the mutant gene as *rrl3-2*.

Table 1. Segregation of short root phenotype in M_3 progenies derived from selfed M_2 heterozygous plants for the HK8117 and in F_1 progenies of the crosses between *rrl3-1* and HK8117

Phenotype in M_3 Progenies		χ^2 (3: 1)	P.
Normal	Short root		
126	44	0.07	0.79
Combination		Phenotype in F_1 progenies	
(♀ × ♂)		Normal	Short root
rrl3-1 × HK8117		0	15
HK8117 × *rrl3-1*		0	12

3.2 Characterization of the rrl3 Mutants

The rrl3-1 and rrl3-2 mutants showed dimorphic characteristics for root elongation under environmental stimuli. In the water culture without aeration, the seminal root lengths of the rrl3-1 and rrl3-2 mutants were about 80% and 90% of those of the wild-type, Blue Rose and Taichung 65, respectively (Fig. 1a, b). In the aerated water culture, wild-type Blue Rose and Taichung 65 seminal root length did not vary, but the rrl3-1 and rrl3-2 mutants showed significantly shorter root lengths (Fig. 1b).

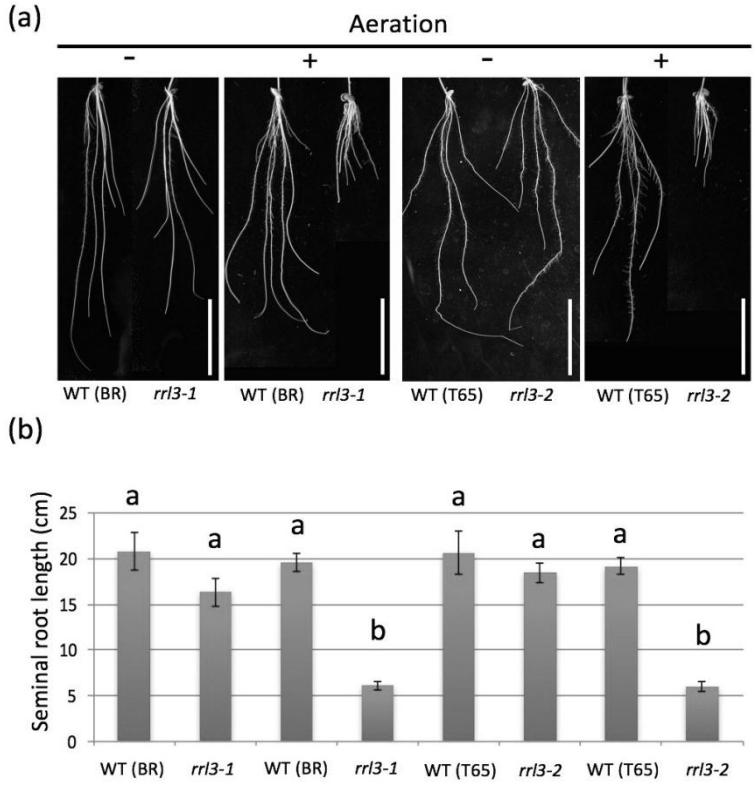

Figure 1. Phenotype of the *rrl3* mutants

Note: (a): Root phenotype of wild type, Blue rose (BR), and *rrl3-1* mutant (left group); wild-type, Taichung65 (T65), and *rrl3-2* mutant (right group); without aeration (−) and with aeration (+) at 10 days after germination (DAG). Scale bar = 5 cm. (b): Seminal root lengths corresponding to those in (a). Different letters indicate significant differences among the genotypes ($P < 0.05$) by Tukey's test.

3.3 The Short-Root Phenotype of rrl3 Mutants was a Result of the Defect in the Root Apical Meristem Maintenance

To elucidate the mechanisms by which the *rrl3* mutations affect the root elongation process, we checked the RAM phenotype by using DAPI and BrdU-labeling with respect to aeration. Before aeration, the RAM of the wild type and the *rrl3-1* mutant did not differ (Fig. 2a, h). The wild-type QC rarely divides (Fig. 2c-e). In contrast, the *rrl3-1* mutant showed the QC divided periclinally at 2 days after aeration (Fig 2j-l). With the progress of aeration, the *rrl3-1* mutant developed the meristem in which the cell size was already larger than that of the wild type (Fig. 2f, m).

We studied the cell division activity in the RAM of seminal roots of the *rrl3-1* mutant as detected by BrdU staining. The BrdU-labeled cells in the RAM of wild type and *rrl3-1* did not show any difference without aeration (Fig. 2b, i). On the other hand, 4 days after aeration, there were very few dividing cells in the division zone of the *rrl3-1* mutant compared to that of the wild type (Fig. 2g, n). Taken together, these results suggest that the *rrl3* mutant was not able to maintain the QC identity because of increased cell division resulting in a disorganized RAM; consequently, the RAM produced fewer cells and led to the short-root phenotype.

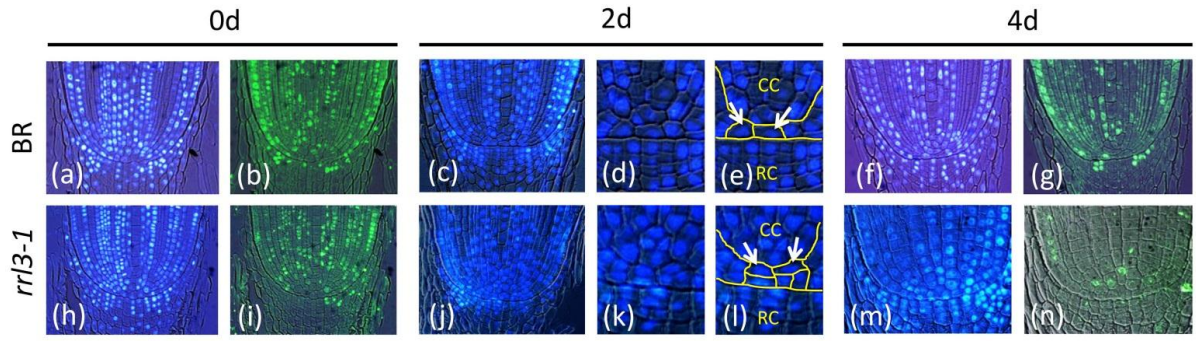

Figure 2. Changes in the RAM phenotype in the *rrl3-1* mutant grown under aeration

Note: (a) - (g): wild-type seminal roots and (h) - (n): *rrl3-1* mutant seminal roots. (a) and (h) DAPI-stained cells, (b) and (i) BrdU-stained cells in the RAM of wild type and *rrl3-1*, respectively, before aeration. (c) and (j) DAPI-stained cells in wild type and *rrl3-1*, respectively, at 2 days after aeration; (d) and (k) magnified image of the QC region of c and j, respectively. (e) and (l) showing the central cells of QC by white arrows; the *rrl3-1* mutant had dividing central cells. CC and RC indicates the central cylinder and root cap cells, respectively. (f) and (m) DAPI-stained and (g) and (n) BrdU-stained cells in the RAM of wild type and *rrl3-1* mutant, respectively, at 4 days after aeration. Scale bar = 100 μm.

3.4 Isolation of the Causative Gene in the rrl3 Mutants

A map-based cloning approach was employed to isolate the causative gene, which showed that the locus was located on the long arm of chromosome 1, approximately 90.0 cM around the molecular marker RM11602 (Fig. 3). This region includes the *RRL3* gene LOC_Os01g38970 (Os01g0570700) on the BAC clone AP003334. *RRL3* encodes a member of the CPS large subunit, CarB, which is assumed to be required for the conversion of ornithine into citrulline in the arginine biosynthesis pathway in plants. Comparison of the nucleotide sequences of the mutants *rrl3-1* and *rrl3-2* with those of the wild types showed a single-nucleotide substitution, G to A, which resulted in a single amino acid substitution, glycine (Gly) to serine (Ser) in *rrl3-1*, and C to T, which resulted in a single amino acid substitution, alanine (Ala) to valine (Val) in *rrl3-2* (Fig. 3). These results indicate that LOC_Os01g38970 is the causative gene in *rrl3* mutants.

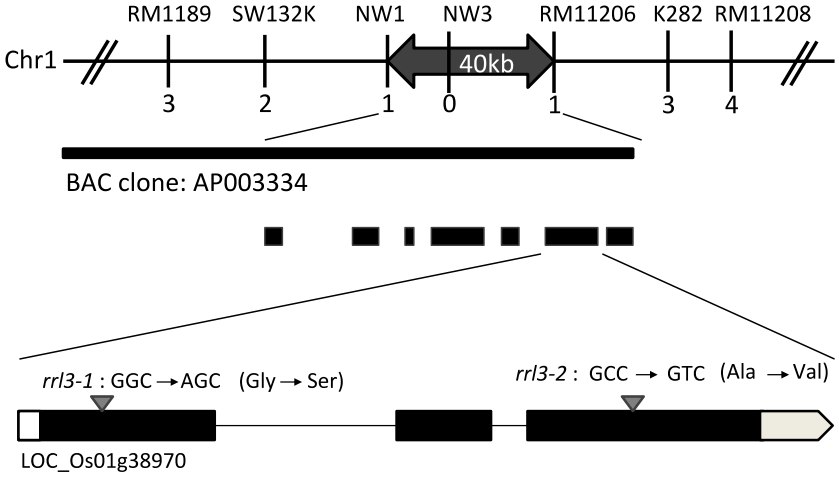

Figure 3. Map-based cloning and structure of the *RRL3* gene

Note: High-resolution linkage and physical map of the *RRL3* locus. The vertical bars represent molecular markers and the numbers of recombinant plants indicated below the linkage map. The open reading frame (ORF) of *RRL3* was 3519 bp; *rrl3-1* had a G/A substitution at 481 bp in the first exon that resulted in a single amino acid substitution of Gly to Ser, and *rrl3-2* had a C/T substitution at 2,789 bp in the third exon that resulted in a single amino acid substitution of Ala to Val. The black boxes and horizontal lines represent the exons and introns, respectively; the arrowheads indicate the mutation sites.

3.5 Expression Analysis of the RRL3 Gene

First, we checked the expression levels of *RRL3* in the 5-mm root tip segments and found that the gene is expressed in the root tip region (Fig. 4a). Then, we checked the expression pattern of the *RRL3* gene according to the different zones from the root tip of the plants grown under non-aerated conditions. Zone-wise expression patterns (Fig. 4a) revealed that the *RRL3* expression was higher in zone-1 (1 mm from the tip), which includes the root cap and division zone, than the other regions such as zone-2 (1–2 mm), which is the early elongation zone, and zone-3 (2–3 mm) i.e., the late elongation zone.

The *rrl3-1* and *rrl3-2* mutations are influenced by the environmental stimuli that are dependent on aeration, that is why we employed a time-course-based expression analysis of the *RRL3* gene in relation to the aeration. The result revealed that after starting aeration at 1 hr, the *RRL3* expression level declined, followed by a gradual increase at 3 hr; it reached the highest peak at 6 hr after aeration. After reaching the peak, the expression levels gradually decreased and returned to the normal, non-aerated levels after 2 days (Fig. 4b).

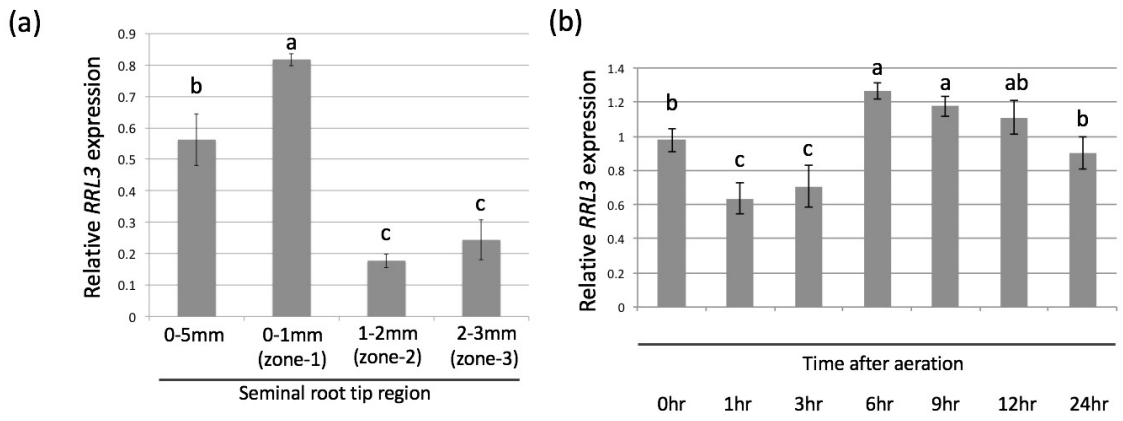

Figure 4. Expression patterns of *RRL3* gene in the wild type

Note: (a): Zone-wise expression pattern of the *RRL3*, the zone-1 (1 mm from the tip) includes the root cap and division zone; zone-2 (1–2 mm) includes the early elongation zone; and zone-3 (2–3 mm) includes the late elongation zone. (b): Time-course of the *RRL3* gene expression; 0 hr indicates before aeration; and 1 hr, 3 hr, 6 hr, 9 hr, 12 hr, and 24 hr after aeration. Different letters indicate significant differences among the genotypes ($P < 0.05$) by Tukey's test.

3.6 Application of Exogenous Arginine Biosynthesis Intermediates and Arginine Modified the Phenotype of the rrl3-1 and rrl3-2 Mutants

CPS is thought to catalyze the conversion of glutamine and bicarbonate into carbamoyl phosphate (CP) and glutamate (Holden et al., 1999). CP participates in the biosynthesis of arginine and in the *de novo* biosynthesis of pyrimidines (Zrenner, Stitt, Sonnewald, &Boldt, 2006). Ornithine and CP are the substrates of ornithine transcarbamylase (OTC), which catalyzes the synthesis of citrulline via the arginine biosynthesis pathway (Fig. 5a) (Slocum, 2005). The mutations in the *RRL3-1* and *RRL3-2* occurred in the CPS large subunit that led to the possibility of impaired arginine biosynthesis.

To examine whether the short-root phenotype of the *rrl3-1* mutant occurs because of the changes in the activity of the CPS, we grew the *rrl3-1* mutants under hydroponic condition supplemented with intermediates of the arginine biosynthesis pathway (ornithine and citrulline) as well as arginine. When we used ornithine, the root lengths of both the *rrl3-1* mutant and the wild type gradually decreased with an increasing concentration of ornithine, indicating that too high concentration of ornitine negatively affects root growth (Fig. 5b, c). In contrast, treatments with citrulline or arginine increased the root length and could complement the *rrl3-1* mutant phenotype about the wild type level at 0.1 mM. Above this concentration, the root growth of the mutant as well as that of the wild type declined probably due to also too high concentrations of them for both root growth (Fig. 5b, c). Taken together, these results indicate that the short-root phenotype of the *rrl3-1* mutants was because of a block in the conversion of ornithine to citrulline in the arginine biosynthesis pathway, resulting in the inhibition of arginine biosynthesis.

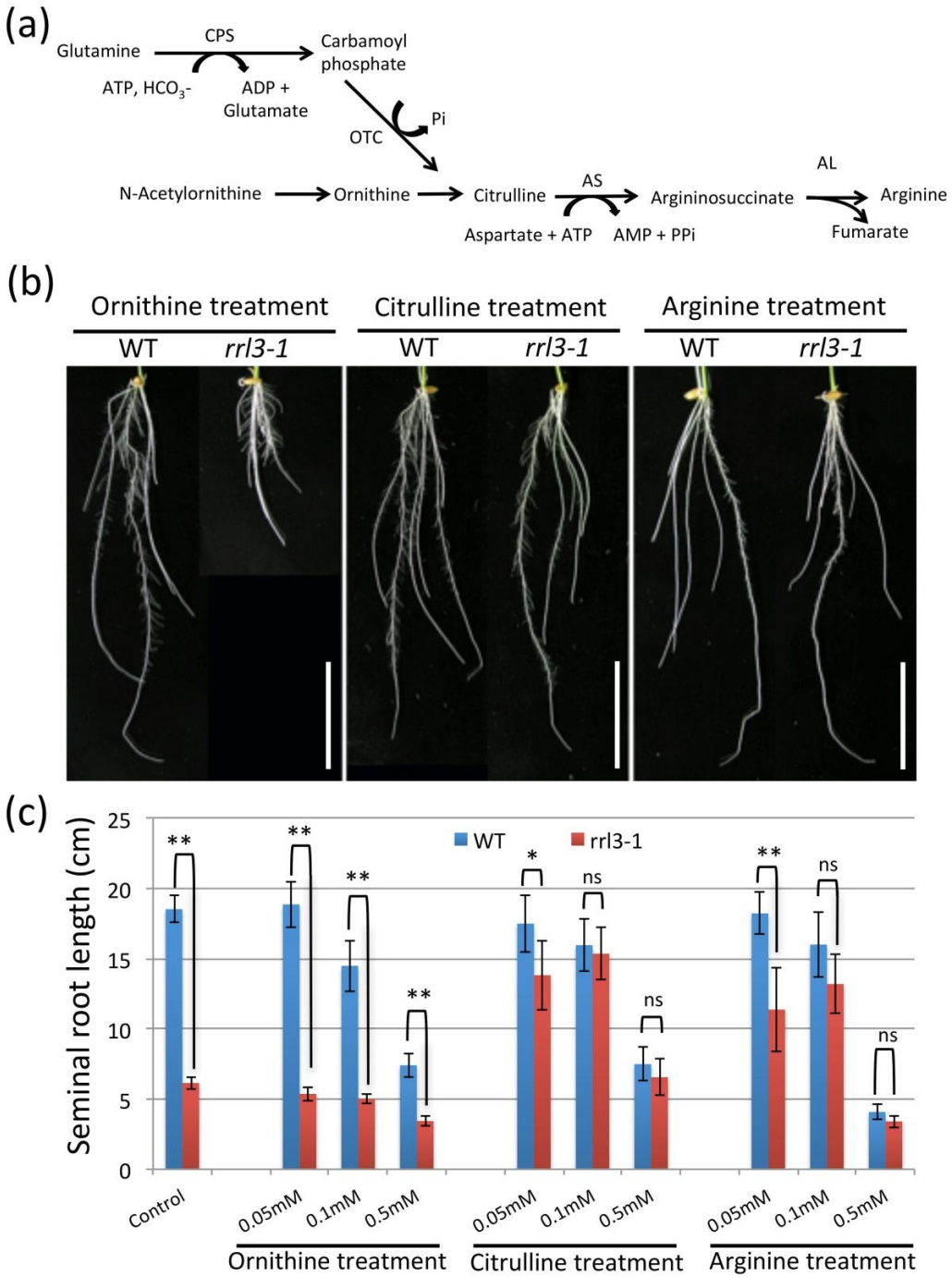

Figure 5. Effects of arginine and intermediates of arginine biosynthesis pathway in *rrl3-1* mutant

(a): Synthesis of arginine from the ornithine intermediate in plants. CPS, carbamoyl phosphate synthetase; OTC, ornithine transcarbamylase; AS, argininosuccinatesynthetase; AL, argininosuccinatelyase. (b): Root phenotype of 10-day-old plants treated with 0.1 mM ornithine (left group), citrulline (middle group), and arginine (right group); in each group, the left plant is the wild type, and the right plant is the *rrl3-1* mutant. Scale bar = 5 cm. (c): Seminal root length of the control and the ornithine-, citrulline-, and arginine-treated plants; ns, **, and * indicate not significant, significant at 1% level, and significant at 5% level, respectively.

4. Discussion

In this study, we showed that *rrl3-1* and *rrl3-2* are allelic mutants of the *RRL3* gene. *RRL3* encodes CPS large subunit, CarB, which is thought to catalyze the conversion of glutamine and bicarbonate into carbamoyl

phosphate and glutamate. The CarB protein has 2 catalytic domains (N-terminus ATP-binding, residues 104–500; C-terminus ATP-binding, residues 655–1063) that are assumed to participate in 2 different ATP-dependent reactions, which include the phosphorylation of bicarbonate (104–500) and carbamate (655–1063) (Slocum 2005). In the *RRL3-1* mutant, the amino acid substitution takes place in the N-terminal ATP-binding domain (161 residue), and in *RRL3-2*, the amino acid substitution takes place in the C-terminal ATP-binding domain (930 residue). These amino acids have been shown to be conserved in Arabidopsis CPS (Molla-Morales *et al.* 2011). This suggests that *rrl3-1* and *rrl3-2* are loss-of-function mutants of the *RRL3* gene.

In plants, a single CPS (EC 6.3.5.5) provides common carbamoyl phosphate intermediates for the synthesis of both arginine and pyrimidines, which are coordinately regulated (Slocum, 2005).As *RRL3* is present as a single copy in rice, the phenotype of mutant alleles cannot be masked by redundant homologs. The lack of known mutants in the arginine biosynthesis pathway suggests that arginine is essential for plant metabolism, and that the null alleles of a gene in this pathway are lethal at the gametophytic or embryonic levels (Molla-Morales et al., 2011). Arabidopsis *ven3* mutant resulted from the mutation in the conserved domains of CPS subunit, CarB, exhibiting differential pigmentation of veinal and interveinal tissues (Molla-Morales et al., 2011). In contrast, a T-DNA insertional mutation in the 3′-untranslated region (UTR) of the ornithine transcarbamylase (*OTC*) gene shows increased sensitivity to exogenous ornithine, but could not produce any visible phenotype (Quesada, Ponce, &Micol, 1999). Although another mutant of *Arabidopsis* with a T-DNA insertion in the promoter of *CarB* (*VEN3*) reduces the expression levels, its effect on arginine biosynthesis is negligible and it does not produce any visible phenotype (Potel et al., 2009). These 2 T-DNA insertional mutants had a mutation in the non-coding regions; therefore, these 2 mutants still retain the wild-type OTC and CarB proteins, although at reduced levels. Therefore, it is conceivable that the catalytic activities of the *rrl3-1* and *rrl3-2* genes reduced but can still maintain sufficient arginine levels for survival under normal conditions, but not in the presence of mechanical impediments to growth.

Arginine is an important constituent of proteins, and is thought to serve, under some circumstances, as a store of nitrogen for the biosynthesis of secondary products such as polyamines (Shargool, Jain, & McKay, 1988) and other amino acids during seed development (de Ruiter&Kolloffel, 1982). It has been reported that defects in overall protein biosynthesis, such as in mutants of ribosomal protein genes, caused a significant reduction in cell numbers (Horiguchi et al., 2010). In Arabidopsis *cue1* mutants, disruption of the aromatic amino acid biosynthesis causes an imbalance in the levels of other amino acids (Streatfield et al., 1999; Voll et al., 2003). Arabidopsis *ven3-2* mutant showed altered amino acids, which suggests that protein synthesis does not proceed efficiently in this mutant (Molla-Morales et al., 2011). Therefore, the mutation in the *RRL3* gene reduced the arginine levels in the RAM, causing a limited supply of other amino acids for the biosynthesis of proteins for the growing tissues in the *rrl3* mutants.

Root growth in *Arabidopsis* is impaired in the *tup5* mutant under conditions of normal light, but not in the dark. The free arginine content in the *tup5* mutant was lowered to 31% of that of the wild type under long-day conditions, but not in the dark; in addition, the free arginine content decreased and the RAM was almost completely utilized in the dark, reflecting that under normal light conditions, plants need higher levels of arginine to maintain the RAM in *Arabidopsis* (Frémont, Riefler, Stolz, &Schmülling, 2013). Consistent with these findings, we believe that rice plants may need to produce increased levels of arginine under mechanical stimuli that impede normal growth, which shows why the *rrl3* mutants could not maintain their RAM in presence of mechanical impediments. The spatiotemporal/differential expression pattern of *RRL3* with and without aeration is also consistent with the phenotype observed in the *rrl3* mutants in presence of similar environmental stimuli.

The *Osiaa23* mutant cannot maintain its QC identity because of abnormal transverse division in the QC cells. The analysis of the QC of *Osiaa23* mutants has shown that the postembryonic maintenance of the QC depends on *OsIAA23*-mediated auxin signaling (Jun et al., 2011). In contrast to *OsIAA23*, the *RRL3* gene maintains the QC through the regulation of arginine biosynthesis. Our previous research has shown that the *RRL3* gene regulates cell production in the root under a mechanically impeded condition, and does not regulate the sensitivities to ethylene, IAA, and ABA (Inukai et al., 2003). In addition, examination of the RiceXPro database reveals that the *RRL3* gene is not induced by auxin (http://ricexpro.dna.affrc.go.jp). Our data emphasize the fact that in addition to the traditional analyses of transcriptional regulation, phytohormones, and signal transduction, a complete understanding of the mechanisms regulating the meristem and initial cell function in plants must also incorporate models describing the role of essential metabolites such as arginine. Hence, we can conclude that *RRL3* is a novel gene that is involved in the production of structural units such as arginine, and ultimately, proteins that maintain the QC and RAM in the rice plant.

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