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Enhanced Root System Development Responses of a Newly Identified Mutation Gene Promoting Lateral Root Development to Various Nitrogen Conditions in Rice

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Abstract. Lateral roots (LRs), which largely constitute the root system, allow the entire root system to expand to a larger area to efficiently capture water and nutrients from the soil. Thus, the optimization of LRs should be considered for the genetic improvement of root system architecture to most notably impact plant acquisition of soil resources for productivity. In this study, we newly identified a rice mutant, 11NB10, which has a high number of thick, long, and highly branched LRs (L-type LRs) with promoted parental root growth. We evaluated the root performance of this mutant under various nitrogen (N) regimes, including 30, 60, and 120 mg N corresponding to low, standard, and high N conditions, respectively. The results showed that under low N conditions, the 11NB10 mutant had a larger root system based on its total root length, which increased further with increasing N levels, compared to its wild-type, Nipponbare. This promoted root system growth could be attributed to the development of highly branched L-type LRs, which in turn might contributed to higher leaf area and shoot dry matter production. These findings suggest that the 11NB10 mutation gene promotes a highly developed root system under low N conditions, and its root performance could be further improved by enhancing LR development through N application. Thus, the 11NB10 mutant is a promising line for the breeding programs targeting root system architecture in rice.
Key words: rice, lateral root, nitrogen, root system architecture, mutant

Introduction

Rice (*Oryza sativa* L.) is consumed as a staple cereal crop by half of the world's population[1)] and is cultivated on over 150 million ha of land globally. However, the recent climatic conditions pose serious risks to rice production, thereby threatening food security[2)]. Irrigated rice fields, where water-saving technology (alternating wet and dry conditions) is practiced, and less favorable environments, such as rainfed lowland or upland ecologies, are more vulnerable to variable weather events, leading to constrained rice production in these areas[3, 4)]. In such uncertain situations, optimizing root system architecture has been suggested as a rational strategy to improve nutrient and water acquisition[5)] in plants and, thus, to achieve food security.

The root system architecture in rice is determined by the overall growth and angle of its various components, including the seminal, crown, and lateral roots (LRs)[6, 7)]. Being the root component that constitutes approximately 90% of the root system, the LRs predominantly determine the overall function of the root system[8)] in a large variety

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of environmental and soil nutrient conditions to optimize plant growth, adaptation, and productivity[9, 10]. In rainfed lowland fields in developing countries, wherein a hardpan is located approximately 20 cm below the soil surface[11, 12], the roots are almost entirely restricted to water and nutrients found above the hardpan. It was reported that in such cases, the branching and elongation of LRs contributed greatly to the total root length, which consequently produced greater total dry matter, especially under mild drought stress[13]. Further evidence was provided that LR production contributed to plant yield under soil moisture fluctuation regimes[14]. Additionally, the development of a deeper root system and LR growth promotion at 30–45 cm soil depths as a response to periods of progressive drought stress alleviated the effects of water deficit on grain yield[15], and similar root phenotype at the same soil depth was likewise reported to maintain shoot dry matter production under progressive drought stress[16]. These root characteristics are associated with increased root water uptake and, to some extent, nutrient uptake. Furthermore, total root length development, which is contributed by LR branching and elongation, could be enhanced by standard and high nitrogen (N) applications. This, in turn, could lead to increased shoot growth under drought conditions[17–19]. These results indicated that LR growth could be induced by controlling the level of N in soils. In this regard, we identified a rice mutant, 11NB10, that exhibits a high number of thick, long, and highly branched LRs (L-type LRs) with normal and promoted parental root growth at the seedling and higher growth stages.

In Philippines, the rainfed systems (upland and lowland) cultivated for rice occupy 1.51 M ha of the 4.8 M ha total rice production areas[20]. In 2018, these production areas produced 3.12 mt yield ha^{-1}, which is significantly lower than their irrigated counterpart that produced 4.37 mt yield ha^{-1}. It is anticipated, therefore, that any improvement in these production areas might contribute to the overall yield increase performance of the country. As 11NB10 mutant line showed promoted root system traits, we further used this material to evaluate the utilization of its root traits under various N regimes and their overall effect on shoot development under Philippine conditions.

Materials and Methods

Plant Materials

The rice mutant, 11NB10, and its wild-type, Nipponbare, which differ in root system development, were utilized in the study. The 11NB10 mutant is an *N*-methyl-*N*-nitrosourea (MNU)-induced mutant, in which the root system development is more promoted compared to Nipponbare.

Pot experiment

The 11NB10 mutant and Nipponbare were evaluated in a pot experiment conducted in a glasshouse located on the campus of Nagoya University, Nagoya, Japan (35°6′42″ N, 137° 4′57″ E) from July to August 2017. The seeds of the 11NB10 mutant and Nipponbare were pre-germinated using tap water mixed with fungicide (benomyl benlate, 0.15% w/v) and incubated in a growth chamber maintained at 28°C under continuous light. The pre-germination of the 11NB10 mutant occurred 2 days earlier than the wild-type plants sowed at the same time (wild-type: 3 days; mutant: 5 days). Three pre-germinated seeds from each line were sown in a 1/5,000 Wagner pot (16 cm diameter, 20 cm height) filled with 4.0 kg air-dried sandy loam soil. The soil in each pot was pre-mixed with fertilizer at the rate of 60-60-60 kg ha^{-1} NPK. The seedlings were later thinned to one seedling per pot at 10 days after sowing (DAS) with water level maintained at 2 cm above the soil surface until the termination of the experiment.

Rootbox experiment

Time and Location

This experiment was conducted in a screenhouse at the PhilRice Central Experiment Station (PhilRice-CES), Science City of Muñoz, Nueva Ecija, Philippines (15°40′ N, 120° 53′E, 57.6 m altitude) between January 15, 2019 to February 22, 2019.

N Treatments and Plant Sampling

Two plants each of the 11NB10 mutant and Nipponbare were targeted to be grown at both sides of a polyvinyl chloride (PVC) rootbox (25 cm × 2 cm × 40 cm, L × W × H)[21]. Firstly, six pre-germinated seeds, i.e., three seeds each of the 11NB10 mutant and Nipponbare, were sown at approximately 5 cm away from both ends of the PVC rootbox (Fig. 3a). The seedlings were later thinned into one seedling each of the PVC rootbox sides after 5 DAS.

As described previously[13, 18, 22], three different levels of N fertilizer (urea: 46% N), including 30 (low), 60 (standard), and 120 mg (high), each thoroughly mixed with 80 mg phosphorus (single superphosphate: 17.5% P_2O_5) and 70 mg potassium (KCl: 60% K_2O), were added into 2.5 kg air-dried soil per rootbox prior to seed sowing.

Physiological Measurements

Leaf photosynthesis of the second youngest fully-expanded leaf was measured using a portable photosynthesis system (Li-6800, LiCOR Inc., Lincoln, Nebraska, USA) starting at 1000 h. The relative chlorophyll content was measured using a chlorophyll meter (Soil and Plant Analysis Development, SPAD-502, Minolta) and expressed as

SPAD value. All measurements were performed at 36 DAS, two days before the termination of the experiment.

Shoot and Root Growth Measurements

Sampling was performed at 38 DAS. The shoots were cut at the stem base and oven-dried at 70°C for 2 days prior to the recording of the dry weight. The roots were sampled using a pinboard and transparent perforated plastic sheet as described previously[21, 23]. The extracted root systems embedded in plastic sheets were stained with 0.25% Coomassie Brilliant Blue R 250 aqueous solution for at least 24 h. This staining procedure was indispensable for taking high-resolution digital scans of the entire root system, including the fine LRs, using an A3-size scanner (EPSON 10000G) at 300 dpi. Subsequently, the total number of crown roots was manually counted, and the root samples were stored in 95% ethyl alcohol for further measurements. For root length measurements, the roots were cut, spread on transparent glass trays with minimal overlapping and scanned at 600 dpi with a pixel threshold value of 175 using an A4-size scanner (EPSON 4990). The scanned images were analyzed for root length using the WinRhizo software (Régent Instruments, Québec, Canada) and categorized according to LR diameter. Previously, LRs have been classified into two types according to diameter and length, the S-type and L-type LRs. The L-type LRs are thick, long, and branched into the higher-order LRs, whereas the S-type LRs are slender, short, and non-branching[24]. The LRs with diameter sizes of $0 < 0.08$ and $0.08 \leq 0.30$ mm were classified as thin and thick, respectively[25].

Results

11NB10 mutant selection and performance under soil conditions

The 11NB10 mutant was observed to have a promoted root system compared to its wild-type, Nipponbare, under tap water conditions at the seedling stage (Fig. 1a-b). The mutant had remarkably longer parental roots and higher number of long and thick LRs (L-type LRs), but the same LR density and number of crown roots as that of the wild type (Fig. 1c-f); thus, it was selected as plant material in our study. These root development differences were consistently observed under soil conditions using pot set-up at the maximum tillering stage (Fig. 2a-b). The whole root system of the 11NB10 mutant appeared to have longer parental roots and highly branched LRs despite the observed

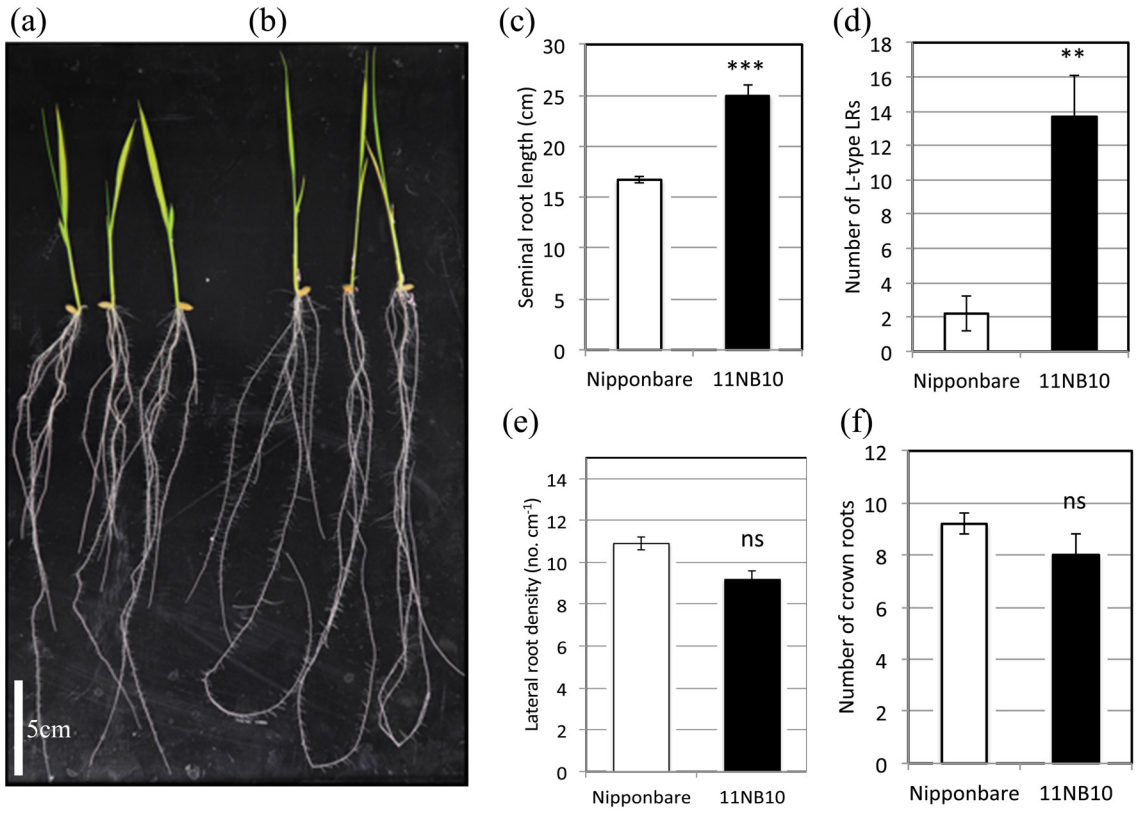

Fig. 1. Root morphological features of 11NB10 mutant at seedling stage.
Shoot and root system of Nipponbare (a) and 11NB10 mutant (b) and their roots traits (c–f) at 15 days after germination grown under tap water conditions. Values represent means ± SE (n=10). ** and *** indicate statistically significant at $P<0.01$ and $P<0.001$ in the means between genotypes as revealed by two-tailed Student's T-test, respectively (ns, non-significant).

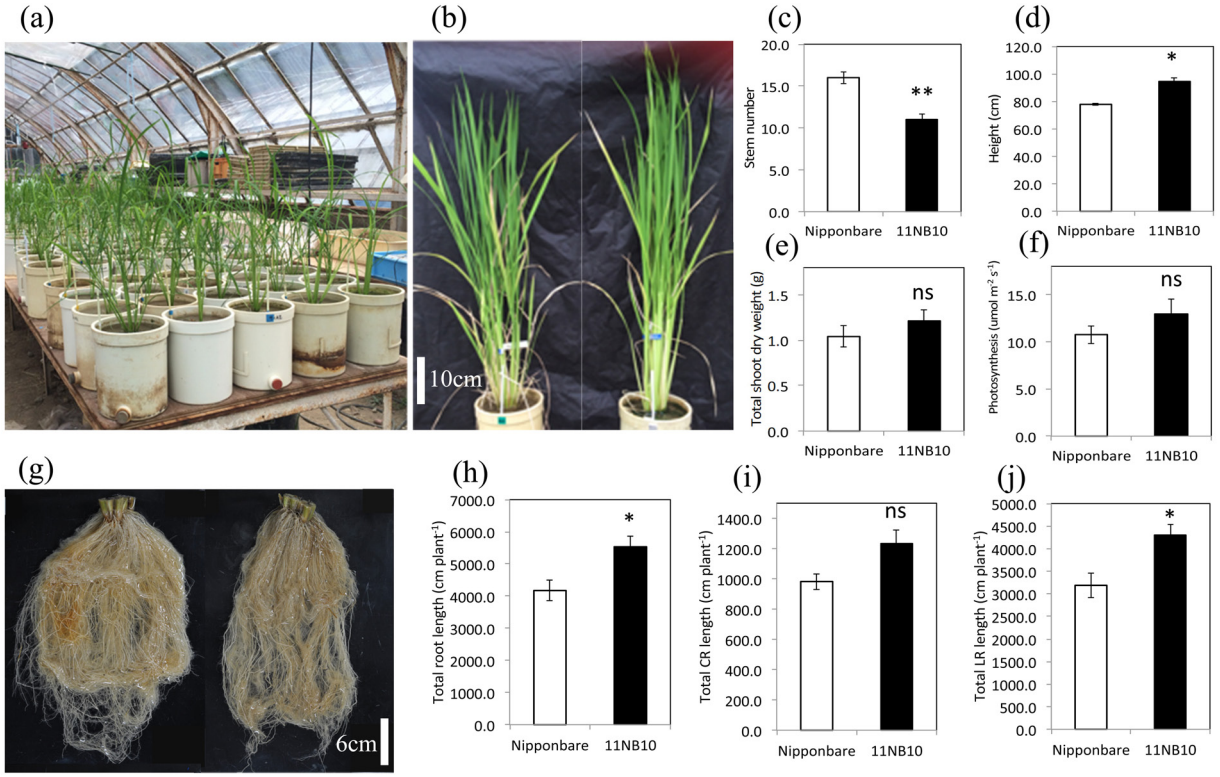

Fig. 2. Shoot and root system profiles of 11NB10 mutant plants under soil conditions at maximum tillering stage. Pot experiment set-up in a glasshouse (a) to evaluate shoot phenotypes (b), shoot traits (c–f), entire root system formation (g) and root traits (h–j) of Nipponbare (left) and 11NB10 mutant (right) grown in soil supplied with 60-60-60 kg ha^{-1} NPK under irrigated conditions at maximum tillering (65 days after sowing). Values presented for total shoot dry weight, total root length, total CR and LR length were ratio of the whole plant traits to the stem number for direct comparison of these traits. CR, crown root; LR, lateral root. Values represent means ± SE (n=4). * and ** indicate statistically significant at $P<0.05$ and $P<0.01$ in the means between genotypes as revealed by two-tailed Student's T-test, respectively (ns, non-significant).

lower number of parental roots from the base (Fig. 2g), which was due to the reduced stem number as shown in Fig. 2b. Because of these differences in stem number (Fig. 2c), the shoot and root traits were directly compared by obtaining the ratio of the trait to the stem number, and we found that the shoot dry weight of the 11NB10 mutant was not negatively affected by the mutation (Fig. 2e). In addition, the 11NB10 mutant had taller plant height and comparable net photosynthesis to the wild-type Nipponbare (Fig. 2d, f), revealing that the mutation negatively affected the stem number only of the shoot part. Notably, the total root length and total LR length per stem number of the 11NB10 mutant were significantly higher than the wild type (Fig. 2h, j), while their crown root lengths were similar (Fig. 2i), confirming that the gene mutation promoted root system development through enhanced LR development despite negatively affecting the stem number of the shoot part.

Shoot and root system developmental responses of the 11NB10 mutant under various N conditions

The 11NB10 mutant performance was further evaluated under various N conditions (low, standard, and high N) following the same treatment method as described previously[13, 18, 22]. N is the mineral nutrient required in the greatest amount, and its availability is a major factor limiting growth, development, and yield[26]. To precisely evaluate the root system development differences of the 11NB10 mutant and Nipponbare, the rootbox-pinboard method was utilized to collect the whole root system with minimal damage and preserve the resulting root architecture (Fig. 4)[21–23]. Furthermore, because of the differences in the stem number between the 11NB10 mutant and wild-type, the tiller outgrowth was cut to maintain the growth of the main stem (Fig. 3b) during the entire duration of plant growth, making the direct comparison of the shoot and root traits feasible.

The net photosynthesis and SPAD value of the 11NB10 mutant and Nipponbare did not differ (Fig. 3c-d), whereas the plant height and leaf area were significantly higher in the 11NB10 mutant than in Nipponbare (Fig. 3e-f) under different N conditions. On the other hand, the shoot dry weight of the 11NB10 mutant was slightly higher than Nipponbare at low and standard N levels and was signifi-

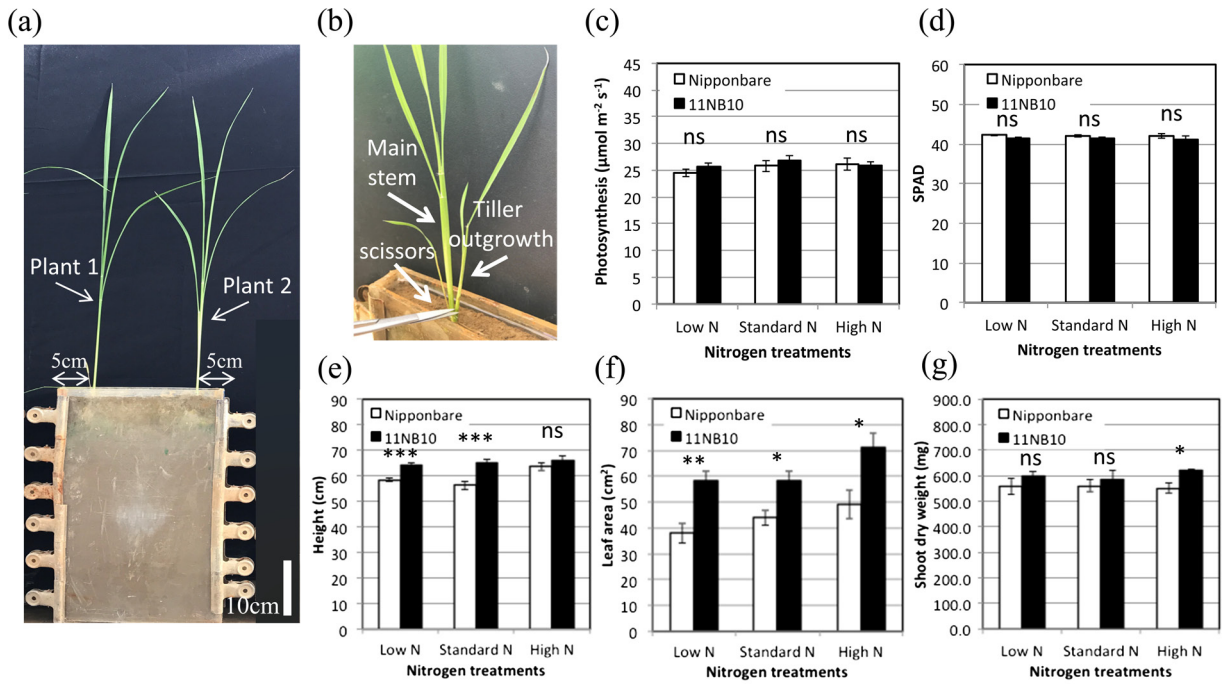

Fig. 3. Shoot phenotypes of Nipponbare and 11NB10 mutant plants with main stem only under different nitrogen (N) conditions. Plants grown in a root box at a screenhouse (a), the cutting of tiller outgrowth to maintain the main stem only for direct comparison of traits between Nipponbare and 11NB10 mutant (b) and their physiological (c–d) and shoot traits (e–g) at 38 days after sowing. Values represent means ± SE (n=5). *, ** and *** indicate statistically significant at $P<0.05$, $P<0.01$ and $P<0.001$ in the means between genotypes within each N treatment as revealed by two-tailed Student's T-test, respectively (ns, non-significant).

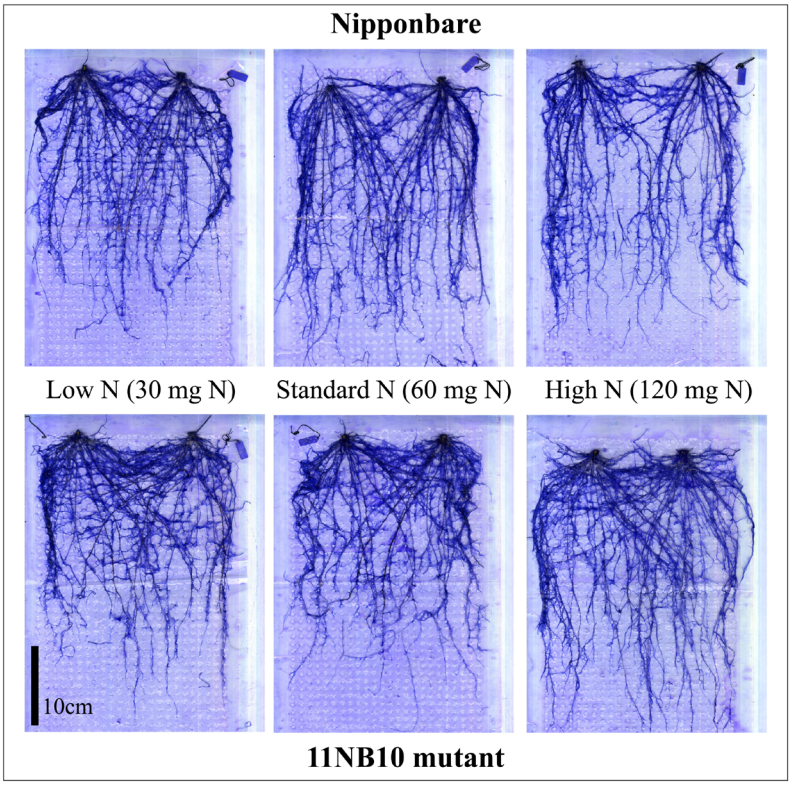

Fig. 4. Root system profiles of Nipponbare and 11NB10 mutant plants at different nitrogen (N) conditions. Root system profiles of Nipponbare and 11NB10 mutant grown under different N conditions (low, standard, high N) for 38 days. The intact whole root system was extracted from a rootbox using a pinboard[20, 16]. Prior to taking digitized photographs in a scanner, the root system were stained with 0.25% Coomassie Brilliant Blue R solutions for at least 24 h.

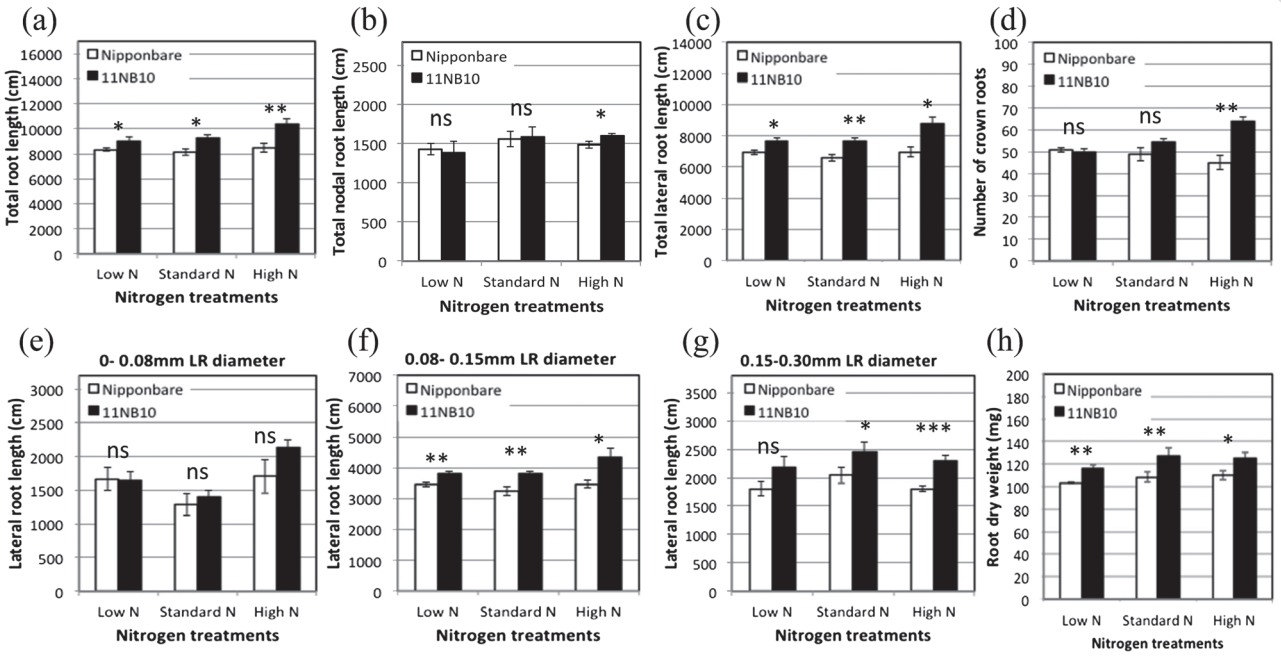

Fig. 5. Root system development of Nipponbare and 11NB10 mutant plants grown under different Nitrogen conditions. Root lengths (a–c), number of crown roots (d), lateral root length according to diameter sizes (e–g) and root dry weight (h) of Nipponbare and 11NB10 mutant grown under different N conditions (low, standard, high N) for 38 days. Values represent means ± SE (n=5). *, ** and *** indicate statistically significant at $P<0.05$, $P<0.01$ and $P<0.001$ in the means between genotypes within each N treatment as revealed by two-tailed Student's T-test, respectively (ns, non-significant).

cantly higher under high N level.

The root system profiles of Nipponbare and 11NB10 mutant grown under different N conditions are shown in Figures 4 and 5. Under low and standard N conditions, the total root length of the 11NB10 mutant was significantly greater than that of Nipponbare, and it increased under high N level (Fig. 4 and 5a). The total root length of the 11NB10 mutant was significantly higher than that of Nipponbare by 8.3% and 22.5% at low and high N conditions, respectively. Between the crown root lengths and LR lengths that comprised the total root length, the lengths of the LRs were more promoted than those of the crown roots and further enhanced under higher N conditions (Fig. 4 and 5b-c). The LR length of 11NB10 mutant was significantly higher than that of Nipponbare by 10.6% and 25.7% at low and high N conditions, respectively, suggesting that the enhanced root system development of the 11NB10 mutant was due to greater LR development and its response to N level. Furthermore, the LR lengths were categorized into different diameter sizes: thin, middle, and thick corresponding to the diameters sizes of $0 < D \leq 0.08$ mm, 0.08 mm $< D \leq 0.15$ mm, and 0.15 mm $< D \leq 0.30$ mm, respectively. The 11NB10 mutant showed greater LR lengths because of the LRs with thick diameter sizes, which are capable of higher order of branching (Fig. 5e-g) and, thus, a highly branched root system. In addition, the mutant had significantly higher root dry weight than Nipponbare at all levels of N (Fig. 5h), while its crown root number was higher only at high N level (Fig. 5d).

Discussions

The ability of rice plants to promote root system development, especially LR development, is a favorable trait to efficiently acquire water and nutrients from a larger soil area for productivity, given the highly dynamic soil environments due to the changing climatic conditions persisting in agricultural areas[9]. This root characteristic was associated with the 11NB10 mutant that showed highly branched root system under various N conditions (from low to increasing N levels), resulting in higher shoot dry matter production than Nipponbare. Remarkably, the root system of the 11NB10 mutant was further enhanced under high N level based on its total root length, which was greatly contributed by LRs with the thick diameter and branching (L-type LRs). Promoted LRs are linked to the enhanced uptake of N as well as other nutrients in different crop species[27–29], implying that these acquired nutrients will be available for growth and development of the shoot part. Increasing N levels did not improve leaf chlorophyll content and photosynthesis in the 11NB10 mutant, but caused a slight increase in shoot dry weight, which could be attributed to the higher number of main-stem leaves (data not shown) that contributed to greater leaf area. It

was reported that N is always distributed mostly to the new growing organs, regardless of the growth stages of the rice plant[30]. Thus, the absorbed N might be preferentially used in the expansion of developing leaves rather than in increasing the chlorophyll content. Moreover, N can stimulate leaf synthesis and growth through the synthesis of proteins involved in cell growth, cell division, and cell wall and cytoskeleton synthesis[31], thus leading to an increase in the leaf area and hence in shoot dry weight.

The findings presented in this report might have implications for genetic improvement and breeding programs in rice that can be utilized under different rainfed lowland and upland fields. The availability of this mutation gene promoting LR development is important for the improvement of root system architecture, and such mutation gene can be used more effectively by understanding proper N levels to further improve LR growth. The root performance of this mutation gene further provides information on natural resource and crop management practices[32]. Unlike in irrigated rice production system, wherein water is available in the fields, the rainfed lowland rice production system is highly drought-prone and dependent on rainfall. Thus, timely N application (i.e. after sufficient rainfall) should be considered to further improve the development of L-type LRs for a more vigorous root system, which will then be useful for overcoming stressful conditions, such as drought and soil moisture fluctuations, as demonstrated in several studies[9]. Further work is required for evaluating the root performance of the 11NB10 mutant under different water regimes (different drought intensities, soil moisture fluctuations, upland conditions) and to further elucidate the importance of promoted root response under N fertilizer and then to water stress conditions.

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