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NITROGEN APPLICATION LEVELS AFFECT ROOT SYSTEM DEVELOPMENT OF RICE UNDER WATER DEFICIT CONDITIONS

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ABSTRACT

This study was conducted to evaluate the effects of nitrogen (N) application on root system development, expressed as total root length, nodal root number, nodal root length and lateral root length, in addition to water use and dry matter production under different soil moisture conditions. The rice plants of Nipponbare, a Japanese rice cultivar, were grown in a PVC rootbox (25 cm × 2 cm × 40 cm, L × W × H) under a vinyl house. There were three moisture treatments: continuously waterlogged (CWL) condition and two water deficit (WD) conditions as 25% w/w and 20% w/w. The N fertilizer (urea: 46%N) was applied at three different levels: 30, 60 and 120 mg N rootbox^{-1}. Each level of N fertilizer was thoroughly mixed with 80 mg phosphorus, 70 mg potassium and 2.5 kg air-dried soil per rootbox prior to seed sowing. The obtained results showed that increasing the N level from 30 to 60 mg N rootbox^{-1} increased root system development (as total root length, nodal root number, nodal root length, and lateral root length), water use, and shoot dry weight of Nippobare under WD conditions (at both 25 and 20% w/w of soil moisture content (SMC)), but there was no significant difference in the above traits between the 60 to 120 mg N rootbox^{-1} treatments. Under CWL conditions, however, the above root traits increased significantly after increasing the N application from 30 to 120 mg N rootbox^{-1}, which led to an increase in water use, and eventually a significant increase in dry matter production. Furthermore, increasing the N application levels increased water use through increased total root length, which led to the positive and notably significant correlation between the total root length and water use under both CWL and WD conditions.

Keywords: Dry matter production, nitrogen, PVC rootbox, rice, root system development, water deficit.

Lượng đạm bón ảnh hưởng đến sự phát triển của bộ rễ lúa trong điều kiện thiếu nước

TÓM TẮT

Nghiên cứu này được tiến hành nhằm đánh giá ảnh hưởng của lượng đạm bón đến sự phát triển bộ rễ được thể hiện qua tổng số chiều dài rễ, số rễ mọc từ mắt thân, chiều dài rễ mọc từ mắt và chiều dài rễ bên; lượng nước cây sử dụng và khả năng tích luỹ chất khô trong điều kiện độ ẩm đất khác nhau. Lúa được trồng trong hộp nhựa quan sát được sự phát triển của bộ rễ trong điều kiện nhà có mái che. Thí nghiệm gồm ba mức độ ẩm đất: tưới nước ngập (CWL), điều kiện thiếu nước (WD) ở 25 và 20% w/w độ ẩm đất. Thí nghiệp áp dụng 3 mức phân đạm bón: 30, 60 và 120 mg N cho mỗi hộp. Phân đạm được trộn đều với 80 mg P_2O_5 và 70 mg K_2O và 2.5 kg đất khô trước khi gieo hạt. Kết quả thí nghiệm cho thấy, trong điều kiện thiếu nước (ở cả 25 và 20% w/w độ ẩm đất), khi tăng lượng đạm bón từ 30 lên 60 mg N đã làm tăng các chỉ tiêu về rễ, lượng nước cây sử dụng và khối lượng chất khô của cây, nhưng không có sự khác nhau về các chỉ tiêu trên khi tăng lượng đạm bón từ 60 lên 120 mg N. Tuy nhiên, trong điều kiện tưới ngập, các chỉ tiêu về rễ tăng dẫn đến cây hút được nhiều nước hơn, kết quả cây tích luỹ chất khô cao hơn khi tăng lượng đạm bón từ 30 lên 120 mg N. Hơn nữa, trong cả điều kiện tưới ngập và thiếu nước, tổng số chiều dài rễ có mối quan hệ thuận và chặt ở mức có ý nghĩa với lượng nước cây sử dụng.

Từ khoá: Bộ rễ, cây lúa, hộp quan sát rễ, khối lượng chất khô, phân đạm, thiếu nước.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the foremost staple food for more than 50% of the world's population. It is estimated that by the year 2025, the world's farmers need to be producing about 60% more rice than at present to meet the food demands of the expected world population at that time (Fageria, 2007). However, the major constraint to rice production, especially in upland and rainfed lowlands, is a water deficit. MacLean *et al.* (2002) and Wade *et al.* (1999) reported that about one third of the world's rice production area is in rainfed lowlands and mostly prone to drought.

The rice root system of an individual plant consists of different components of roots that differ in morphology, anatomy, physiological functions (Yamauchi *et al.*, 1996) and genetic regulations for development (Wang *et al.*, 2005). It plays an important role in adaptation to drought conditions (Yamauchi *et al.*, 1996) through the development of root systems as a result of functional roles of root plasticity (Kano-Nakata *et al.*, 2011 and 2013 and Tran *et al.*, 2014). These adaptations are associated with increased water extractions (Kato *et al.*, 2007), and increased nutrient uptake (Suralta, 2010).

The relatively low yield of rainfed lowland rice may be partly attributed to drought stress (Widawsky and O'Toole, 1990) and nutrient stresses, especially nitrogen (N) deficiency (Khunthasuvon *et al.*, 1998). However, fertilizer application is assumed to mitigate the negative effects of water deficit by improving foliar nutrient concentrations, root growth, plant height, leaf area, photosynthesis, and water use efficiency (Alsafar and Al-Hassan, 2009). Moreover, under both mild drought stress and well-watered conditions, total root length increased with an increase in N application leading to an increase of shoot dry weight (Suralta, 2010; Tran Thi Thiem and Yamauchi Akira, 2015). A report of Suralta (2010) also indicated that N application increased root dry weight, number and length of nodal roots, and linear frequency of lateral roots of rice genotypes grown under continuously waterlogged and drought soil conditions.

In the present study, we used the root boxpin board method to collect the whole root system with minimum impairment to its structure under regulated soil moisture conditions and calculated precisely the water use. Therefore, the research objectives were to determine if the root system development of rice grown under water deficit conditions would be effected by N application levels, and to determine if the total root length would contribute to increased water uptake.

(a) (b)

Picture 1. Rice plants were grown under continuously waterlogged conditions (a) and water deficit conditions (b) for 38 DAS

2. MATERIALS AND METHODS

2.1. Materials and experimental design

Nipponbare, a Japanese standard japonica cultivar, was used in this study. The seeds were supplied by the Rice Genome Research Center of the National Institute of Agrobiological Sciences, Japan: http://www.rgrc. dna.affrc.go. jp/ine NKCSSL 54.html). The experiments were conducted in a vinyl house at the experimental field of Nagoya University, Japan (136°56′6″ E, 35° 9′ 5″ N) during the summer season of 2012.

The seeds were soaked in water mixed with fungicide (benomyl (benlate), 0.15% w/v) and incubated in a seed germinator maintained at 28°C for 72 h prior to sowing. Three pre-germinated seeds were grown in a PVC rootbox (25 cm × 2 cm × 40 cm, L × W × H) filled with 2.5 kg of air-dried sandy loam soil following the method of Kano-Nakata *et al.* (2012) (Picture 1). The seedlings were thinned to one seedling per box at 3 days after sowing (DAS).

For soil moisture treatments, continuously waterlogged (CWL) condition served as the control; and two water deficit (WD) conditions as 25% w/w and 20% w/w were used following the methods of Kano-Nakata *et al.* (2011). In CWL, water level was maintained at 2 cm above the soil surface from 5 DAS until the end of the experiment at 38 DAS. In WD conditions, each rootbox was weighed daily and the amount of water lost was replenished and recorded as evapotranspiration. Four rootboxes without plants were also prepared to measure the amount of water lost through evaporation. The whole plant transpiration was estimated as the difference in water loss between rootboxes with and without plants. The water use was calculated as the accumulated daily whole plant transpiration from 7 DAS up to the termination of the experiment (38 DAS).

The standard N fertilizer level was applied following the results of Suralta *et al.* (2010) and Kano-Nakata *et al.* (2011). The N fertilizer (urea: 46% N) was applied at three different levels: 30, 60 and 120 mg N rootbox^{-1}. Each N application treatment was thoroughly mixed with 80 mg phosphorus (single superphosphate: 17.5% P_2O_5), 70 mg potassium (KCl: 60% K_2O), and 2.5 kg air-dried soil per rootbox prior to seed sowing.

2.2. Data collection

Sampling was done at 38 DAS. Four rootboxes (1 rootbox = 1 replication) were harvested for each treatment combination. The shoots were cut at the stem base and oven-dried at 70°C for 3 days prior to the recording of dry weight. The roots were sampled using a pinboard and transparent perforated plastic sheet following the methods of Kano-Nakata *et al.* (2011).

The collected roots embedded between the plastic sheets were washed free of soil in running water. Cleaned root samples were stored in FAA (formalin: acetic acid: 70% ethanol in 1:1:18 ratio by volume) solution for preservation and further measurements of various root components. The length of nodal roots was measured using a ruler and the total number of nodal roots at the base of each stem was manually counted. For total root length measurements, root samples were spread on the transparent sheets with minimal overlapping. Digital images were then taken using an Epson scanner (ES2200) at 300 dpi resolution. The total length of each root sample was analyzed using Win RHIZO software v. 2007d (Regent Instruments, Quebec, Canada).

2.3. Data analysis

The experiments were arranged in in split-plot design with four replications. The difference in average values among nitrogen treatments was tested by the least significant difference (LSD) at a 5% level of significance using CropStat version 7.2 (IRRI, 2009). The relationships between total root length and water use were determined using correlation analysis.

3. RESULTS AND DISCUSSION

3.1. Response of shoot growth of Nipponbare grown under various levels of water deficit and N application levels

Figure 2 shows the shoot dry weight of the plant grown under CWL and WD (25 and 20%

w/w of SMC) conditions as affected by N application levels. The shoot growth was significantly reduced under WD in comparison with CWL (data not shown), but an increase of N application levels significantly increased the shoot dry weight under both CWL and WD conditions.

Under CWL conditions, at 120 mg N rootbox^{-1}, shoot dry weight was significantly higher by 170.3 mg plant^{-1} and 373.5 mg plant^{-1} in comparison with the 60 mg N rootbox^{-1} and at 30 mg N rootbox^{-1} treatments, respectively. Similarly, the shoot dry weight was significant higher at 60 mg N rootbox^{-1} than it was at 30 mg N rootbox^{-1} by 203.2 mg plant^{-1}. Under WD conditions at both 25 and 20% w/w of soil moisture content (SMC), the shoot dry weight was higher at 60 mg N rootbox^{-1} than it was at 120 mg N rootbox^{-1} by 69.0 mg plant^{-1} and 68.3 mg plant^{-1} at 25 and 20% w/w of SMC, respectively but there were no significant differences. The shoot dry weight was the lowest at 30 mg N rootbox^{-1} (932.5 mg plant^{-1} and 884.3 mg plant^{-1} at 25 and 20% w/w of SMC, respectively). These results confirmed our previous study conducted in the field that the dry matter production increased significantly with an increase of N application under mild drought stress and well-watered conditions (Tran Thi Thiem and Yamauchi Akira, 2015). Furthermore, increasing N application levels significantly increased the total dry matter accumulation under water stress timing (Castillo *et al.*, 2006) and under mild water stress (Belder *et al.*, 2005).

3.2. Response of water use of Nipponbare grown under various levels of water deficit and N application levels

Utilization of the root box-pinboard method helped us calculated precisely the water uptake of rice (Kano-Nakata *et al.*, 2011). Effect of nitrogen application levels on the water use of Nipponbare grown under different soil moisture conditions is presented in Figure 2. The results showed that under WD (at both 20 and 25% w/w) conditions, low N application level (30 mg N rootbox^{-1}) showed a significantly lower water use than at high N application levels (60 and 120 mg N rootbox^{-1}), but the water use was not significantly different under applications of 60 mg N rootbox^{-1} and 120 mg N rootbox^{-1}. However, under CWL, the water use increased significantly with increases of N application from 30 to 120 mg N rootbox^{-1}. These experimental results confirmed previous findings that increasing N application levels significantly increased root water uptake ability through increases of stomatal conductance (Tran Thi Thiem and Yamauchi Akira, 2015). In addition, Suralta (2010) also provided evidence that there were significant differences in water uptake of rice between treatments without N and with N under both CWL and WD conditions.

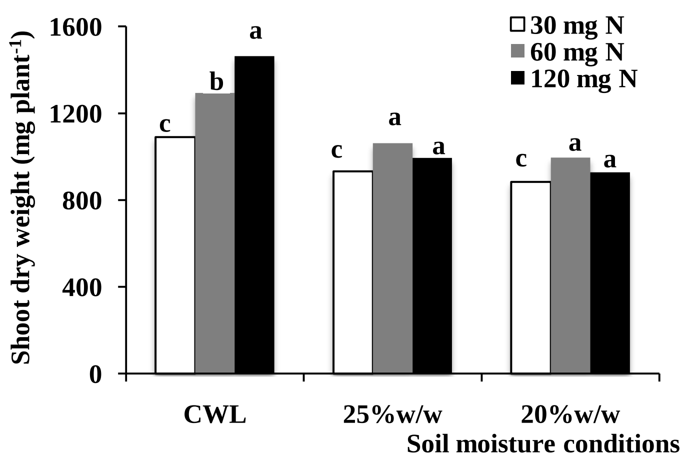

Fig. 2. Effect of nitrogen application level at 30 (□), 60 (■) and 120 mg N rootbox^{-1} (■) on shoot dry weight of Nipponbare under different soil moisture conditions

Note: Values followed by the same letter in a column within each treatment are not significantly different at the 5 % level

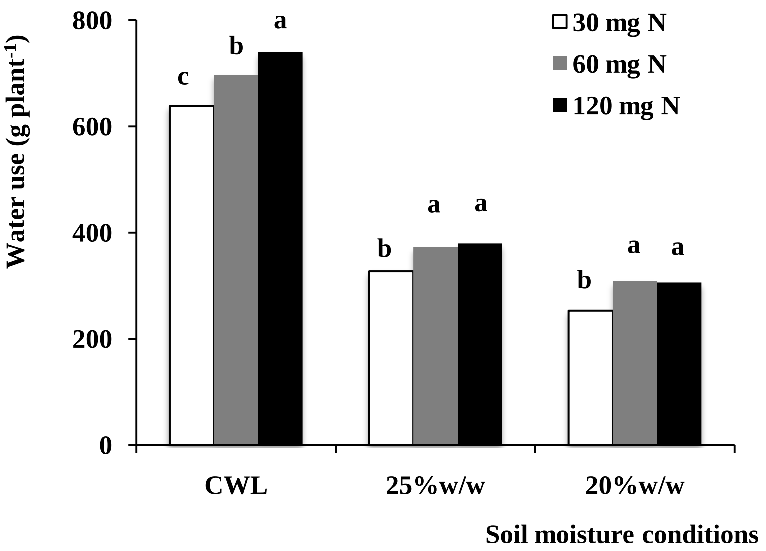

Fig. 3. Effect of nitrogen application level at 30 (□), 60 (■) and 120 mg N rootbox^{-1} (■) on water use of Nipponbare under different soil moisture conditions

Note: Values followed by the same letter in a column within each treatment are not significantly different at the 5 % level

3.3. Response of root system development of Nipponbare grown under various levels of water deficit and N application levels

In rice, the whole root system consists of different component roots such as seminal root, nodal roots, and lateral roots of various branching orders. Among these root components, the lateral roots comprise more than 90% of the total root system in terms of root number (Yamauchi *et al.*, 1987). In this study, the effects of N application under CWL and WD (25 and 20% w/w of SMC) conditions on root system development expressed as the total root length, number of nodal roots, total nodal root length, and total lateral root are shown in Table 1. The ANOVA analysis showed significant effects of SMC and N factors on all the above root traits. Therefore, there were also significant two-way interactions between SMC and N on all the examined traits. The results were consistent with the work of Kano-Nakata *et al.* (2011) in that there was a significant effect of soil moisture content on total root length. In addition, Suralta (2010) reported that the significant effects of N application levels on the root system development were observed under water deficit conditions. Furthermore, the significant interaction effects of N application and SMC on total root length were found in our previous study (Tran *et al.*, 2014).

Under CWL conditions, all root traits increased significantly with increasing N application levels. The total root length at 120 mg N rootbox^{-1} was significantly higher by 849.4 cm plant^{-1} and 1009.4 cm plant^{-1} than it was at 60 mg N rootbox^{-1} and at 30 mg N rootbox^{-1}, respectively. The total root length at 120 mg N rootbox^{-1} was greater than it was at 60 mg N rootbox^{-1}. This was due to the combination of significant increases of nodal root number, nodal root length, and lateral root length as 23 no. plant^{-1}, 251.1 cm plant^{-1}, and 586.3 cm plant^{-1}, respectively. Similarly, the nodal root length and lateral root length at 60 mg N rootbox^{-1} were significantly superior to 30 mg N rootbox^{-1} as 99.5 and 60.5 cm plant^{-1}, respectively. The total root length at 60 mg N rootbox^{-1} was significantly longer than that at 30 mg N rootbox^{-1} by 189.1 and 242.2 cm plant^{-1} under WD conditions both at 25 and 20% w/w of SMC, respectively. But differences in the total root length were not found between 60 mg N rootbox^{-1} and 120 mg N rootbox^{-1}.

The total root length was greater at high N levels than that at low N level owing to the higher number of nodal roots, nodal root length, and lateral root length. Our data supported the findings of Suralta (2010) who showed that increases of N application levels significantly increased root dry weight, nodal root length, and lateral root length. Furthermore, under a line source sprinkler system creating a soil moisture gradient, we also indicated that total root length increased with increases of N application (Tran Thi Thiem and Yamauchi Akira, 2015).

3.4. Relationship between total root length and water use

Increases of N application levels significantly increased the total root length (Table 1), which increased water uptake (Fig. 3) and thus led to the positive and significant correlation between the total root length and water use under both CWL and WD conditions (Figs. 4a, b, c). O'Toole (1982) emphasized the importance of root related traits such as water uptake for improvement of rice growth under drought stress. The ability of a plant to survive under water drought stress was to maintain water use for growth and production (Gowda *et al.*, 2011). The water uptake of plants increases through improving root length density (Benier *et al.*, 2009). Furthermore, the root system development showed close correlations with water use, and the curvilinear regression was highly significant (Kano-Nakata *et al.*, 2011).

4. CONCLUSION

In conclusion, we confirmed the results of the previous study conducted in the field that an appropriate nitrogen application enhanced the total root length in rice which contributed to increases in their dry matter production (Tran Thi Thiem and Yamauchi Akira, 2015). The present study further showed that a suitable nitrogen application significantly increased the root system with an increase of total root length resulting from an increase of nodal root production, and promoting lateral root elongation, which led to enhanced water uptake, and eventually contributed higher dry matter production.

Table 1. Effect of nitrogen application levels on root system development of Nipponbare under different soil moisture conditions

Soil moisture conditions	Nitrogen (mg N rootbox^{-1})	TRL (cm plant^{-1})	NRN (no. plant^{-1})	NRL (cm plant^{-1})	LRL (cm plant^{-1})
CWL	30	4778.4^{c}	84.5^{b}	1339.9^{c}	3438.5^{c}
	60	4938.4^{b}	86.0^{b}	1439.4^{b}	3499.0^{b}
	120	5787.8^{a}	109.0^{a}	1720.5^{a}	4085.3^{a}
25% w/w	30	3979.0^{b}	47.3^{b}	942.5^{b}	3036.5^{b}
	60	4168.1^{a}	49.7^{ab}	967.1^{ab}	3201.0^{a}
	120	4195.5^{a}	53.8^{a}	994.0^{a}	3201.5^{a}
20% w/w	30	3474.3^{b}	43.0^{b}	849.8^{b}	2624.5^{b}
	60	3716.5^{a}	48.3^{a}	954.7^{a}	2761.8^{a}
	120	3621.6^{a}	49.5^{a}	857.1^{b}	2764.5^{a}
SMC		**	**	**	**
N		**	**	**	**
SMC&N		**	**	**	**

Note: TRL: total root length; NRN: number of nodal roots; NRL: nodal root length; LRL: lateral root length;
Values followed by the same letter in a column within each soil moisture treatment are not significantly different at the 5 % level

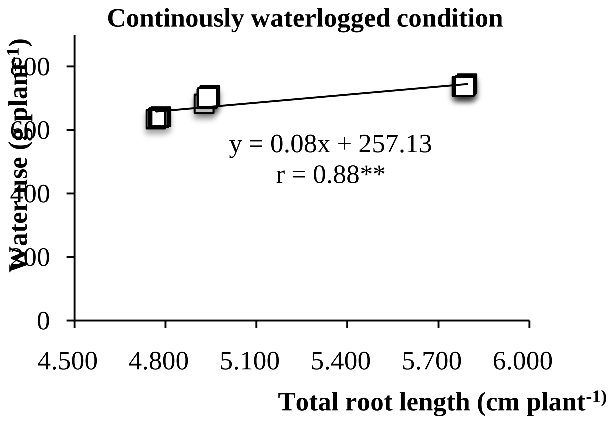

Fig. 4a. Relationship between total root length and water use of Nipponbare at 30 (□), 60 (▒) and 120 mg N rootbox^{-1} (■) under continuously waterlogged conditions

*Note: ** indicates significance at the 1% level.*

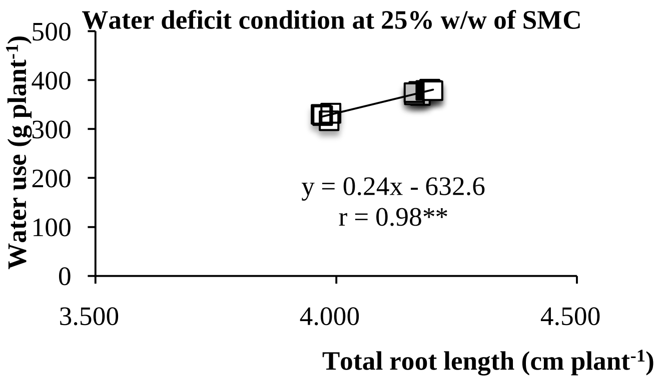

Fig. 4b. Relationship between total root length and water use of Nipponbare at 30 (□), 60 (▒) and 120 mg N rootbox^{-1} (■) under water deficit condition at 25% w/w of soil moisture conditions

*Note: ** indicates significance at the 1% level*

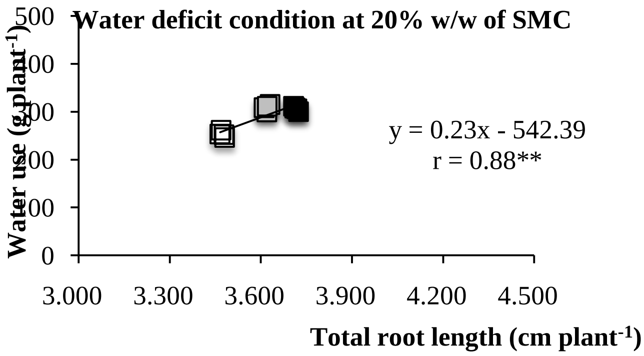

Fig. 4c. Relationship between total root length and water use of Nipponbare at 30 (□), 60 (▒)and 120 mg N rootbox-1 (■) under water deficit condition at 20% w/w of soil moisture conditions

*Note: ** indicates significance at the 1% level*

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