J. Sci. & Devel. 2015, Vol. 13, No. 8: 1388-1396
Tạp chí Khoa học và Phát triển 2015, tập 13, số 8: 1388-1396
www.vnua.edu.vn

EFFECT OF NITROGEN APPLICATION LEVELS ON GROWTH OF RICE UNDER DROUGHT STRESS CONDITIONS

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Received date: 10.08.2015 Accepted date: 29.11.2015

ABSTRACT

The aim of this study was to investigate effects of nitrogen (N) application on root system development, photosynthetic rate and dry matter production under different drought stress conditions. The experiments were conducted in a line source sprinkler system creating a soil moisture gradient under a rain-out shelter. Three N fertilizer treatments were applied: 60, 120 and 180 kg N ha^{-1} . Nitrogen fertilizer was mixed well with phosphorus and potassium fertilizer at the rate of 50 kg (P and K) ha^{-1} and applied as basal dressing at 8 days after transplanting. The obtained results showed that increase of N level from 60 to 120 kg N ha^{-1} increased total root length and shoot dry weight of Nipponbare under severe drought stress conditions (<17% w/w of soil moisture content (SMC)) but did not increased those traits as increased N application from 120 to 180 kg N ha^{-1}. However, under mild drought stress (17-25% w/w of SMC) and well-watered (>25% w/w of SMC) conditions, total root length increased with increased N application leading to increase stomatal conductance and photosynthetic rate, and eventually increased shoot dry weight. In addition, only under well-watered conditions the relationship between the total root length and shoot dry weight was positive and notably significant at 120 and 180 kg N ha^{-1} levels but not at 60 kg N ha^{-1} level. The experiment suggested that the nitrogen application levels of 120 and 180 kg N ha^{-1} increased the dry matter production due to the increased total root length under well-watered conditions.

Keywords: Drought, dry matter production, nitrogen, rice, root.

Ảnh hưởng của mức đạm bón đến sinh trưởng của cây lúa trong các điều kiện hạn khác nhau

TÓM TẮT

Nghiên cứu này nhằm mục đích đánh giá ảnh hưởng của lượng đạm bón đến sự phát triển bộ rễ, quang hợp và tích luỹ chất khô của cây lúa trong các điều kiện hạn khác nhau. Thí nghiệm được tiến hành trong nhà lưới dưới hệ thống tưới phun nước theo hàng tạo ra độ ẩm đất khác nhau. Ba mức phân đạm: 60, 120 và 180 kg N ha^{-1} được trộn đều với 50 kg P_2O và 50 kg K_2O ha^{-1} và bón sau cấy 8 ngày. Kết quả thí nghiệm cho thấy, tổng chiều dài rễ và khối lượng chất khô tăng khi tăng lượng đạm ở mức bón 60 lên 120 kg N ha^{-1}, nhưng khi tăng ở mức bón 120 lên 180 kg N ha^{-1} không làm tăng các chỉ tiêu trên trong điều kiện hạn nặng (<17% w/w độ ẩm đất). Tuy nhiên, trong điều kiện hạn nhẹ (17-25% w/w độ ẩm đất) cũng như tưới nước đủ ẩm (>25% w/w độ ẩm đất), tổng chiều dài rễ tăng, cây hút nước nhiều hơn, quang hợp tốt hơn, dẫn đến cây tích luỹ chất khô cao hơn khi tăng lượng đạm bón từ 60 đến 180 N ha^{-1}. Ngoài ra, chỉ trong điều kiện tưới nước đủ ẩm, tổng chiều dài rễ có mối quan hệ thuận và chặt ở mức ý nghĩa với khối lượng chất khô khi bón đạm ở mức bón 120 và 180 kg N ha^{-1}, nhưng khi bón ở mức 60 kg N ha^{-1} khối lượng chất khô không có mối quan hệ chặt ở mức ý nghĩa với tổng chiều dài rễ. Như vậy, trong điều kiện tưới nước đủ ẩm, việc bón đạm ở mức 120 và 180 kg N ha^{-1} đều làm tăng khả năng tích luỹ chất khô do tổng chiều dài rễ tăng.

Từ khoá: Cây lúa, hạn, khối lượng chất khô, phân đạm, rễ.

1. INTRODUCTION

Worldwide, there are about 79 million ha of irrigated lowland rice, which provide 75% of the world's rice production (Maclean et al., 2002). Approximately 56% of the world's irrigated areas of all crops is in Asia. However, the availability of water for agriculture, particularly for rice production, is threatened in many regions of the world not only by limitations in water resources but also by increases in urban and industrial demands (Wopereis et al., 1994). Tuong and Bouman (2003) estimated that by 2025, 2 million ha of Asia's irrigated dry-season rice and 13 million ha of its irrigated wet-season rice may experience "physical water scarcity", with most of the approximately 22 million ha of irrigated dry-season rice in South and Southeast Asia possibly suffering "economic water scarcity".

Nitrogen is the most limiting nutrient in irrigated rice systems, determining in large part the yield potential of rice in these areas (Cassman et al., 1998). Increasing N availability to the plant increased yield in drought-prone rainfed rice (Boling et al., 2004). However, excess dosage of chemical fertilizers, such as N fertilizer, can contribute to environmental pollutions. In addition, an ever-increasing rise of oil price in recent years is also raising the expenditures for fertilization, along with other costs such as mechanization and transport, and thereby increasing the total cost of rice production. Thus, there is growing need for the development of novel technologies and production systems that maintain or increase rice productivity with less water and N fertilizer.

The root system structure and its response to various soil conditions have been studied intensively, including various soil moisture conditions (Kano et al., 2011) and fluctuating soil moisture conditions (Niones et al., 2012) in rice. The developmental responses of the root system to those stress conditions, due to greater contributions from seminal, nodal and adventitious roots elongation or lateral root development, or both, have been suggested to play significant roles in plant adaptation to the respective conditions (Yamauchi et al., 1996). In addition, it was previously shown that root dry weight, total root length, root volume and root surface area increased with increased N application (Fan et al., 2010; Gharakand et al., 2012). Roots were thinner and root hairs developed more with an increase in N application (Fageria, 2010), and these morphological changes in the root system enabled plants to absorb more nutrients and water compared to thicker roots with less fine root hairs.

This study therefore aimed to examine if root system development and its contribution to dry matter production would be affected by the levels of N application under different soil moisture contents.

2. MATERIALS AND METHODS

2.1. Plant materials

Nipponbare is a Japanese standard japonica cultivar. 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/ineNKCSSL54.html).

2.2. Experimental design, treatments and cultural management practices

The experiment was conducted at the experimental farm of Nagoya University, Nagoya, Japan (35°6′42″N, 137°4′57″E). The experimental soil was sandy loam with field capacity at 32.2% w/w (gravimetric). The experiments were conducted in two watertight experimental beds with a line source sprinkler system under a rain-out shelter. The field was kept watertight by an underlying polyvinyl chloride (PVC) sheet laid at an average soil depth of 25 cm below the soil surface and a manually operated drainage system. The drainage system could be opened to get rid of excess water or closed to prevent draining of water and nutrients. Water mists came out from the nozzles on the PVC pipe installed in the center of the field, and an automated system regulated the timing and the intensity of the irrigation. This system enabled us to maintain a soil moisture gradient perpendicular to the sprinkler line and thus assess the effect of different soil moisture contents on plant growth.

The seeds were soaked in water containing benomyl fungicide (0.15% w/v) for 24 hours, next they were washed in running water, and then incubated in a seed germinator at a constant temperature of 28°C for 48 h. The seeds were sown in black plastic trays with soil under well-watered conditions. Twenty-one day old seedlings were transplanted perpendicular to the PVC pipe, so that they were exposed to and grown under various intensities of drought. Plants were replicated for three rows with a spacing of 20 cm between plants within each line and 40 cm between lines perpendicular to the sprinkler. Each row of the line source sprinkler system contained 8 plants with 3 replications for each N level treatment that received different amounts of water from the PVC pipe during stress treatment. Thus, the nearest plant was 20 cm away from the sprinkler and the farthest one was 160 cm. After transplanting, the whole experimental field was continuously flooded for 7 days to allow recovery from transplanting shock before the water stress treatment was imposed.

At the start of the water stress treatment, the sprinkler was set to release mists of water up to a distance of 80 cm to create a moisture gradient across the field. Therefore, the plants near the sprinkler received more water than those at a farther distance, that is, the amount of water was reduced with increasing distance from the sprinkler. The soil moisture contents (SMC) at five points with 30 cm distance between points on each side of the PVC pipe (total of ten points) were monitored with soil moisture sensors (EC-5 Decagon, Utah, USA) so as to adjust the amount of irrigation to maintain the moisture gradient. The SMCs at 12 cm soil depth were likewise monitored using a time domain reflectometry probe (TDR; Tektronix Inc., Wilsonville, OR, USA). Two stainless steel rods (12 cm in length) were inserted into the soil at a depth of 10 cm allowing a 2 cm protruding above the soil surface where TDR probes were attached to obtain SMC readings. The two steel rods, which were 3 cm apart, were placed in the middle of two plants from each row. The TDR values (%v/v), which were measured for all plants, were then converted to soil moisture values (% w/w) measured using the gravimetric method (%w/w = %v/v x soil bulky density x 0.544). The SMC values from wettest to driest of the soil perpendicular to the sprinkler pipe ranged from 29.3 to 2.8% w/w.

Three N fertilizer treatments applied at 60, 120 and 180 kg N ha^{-1} wwere mixed well with phosphorus and potassium fertilizer at the rate of 50 kg (P and K) ha^{-1} and applied as basal dressing at 8 days after transplanting (DAT). The sources of fertilizer were urea (46% N) for N, solophos (18% P_2O_5) for P and muriate of potash (60% K_2O) for K.

2.3. Measurements

Stomatal conductance and photosynthetic rate were measured using a portable photosynthesis analyzer (LI-6400, Li-COR Inc., USA) on the abaxial side of the flag leaf on the main stem between 10:00-12:00 h at heading stage (65-66 DAT) under the following conditions: leaf temperature, 30°C; CO_2 concentration, 380 μ L L^{-1}; relative humidity, 65 - 75%; quantum flux density, 1200 μmol m^{-2} s^{-1}. We measured 3 plants (3 replications) for each treatment.

At 83 DAT, the plants were harvested. The shoots were cut from the base and oven-dried at 70°C for 72 h. The panicles were separated and weighed separately from the rest of the shoots. The root system was extracted using a monolith stainless cylinder (Kang et al., 1994) with 15 cm diameter and 25 cm height. The collected roots were washed free of soil in running water. The 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. The total number of nodal roots at the base was manually counted. For total root length measurements, the root samples were spread on transparent sheets without overlapping. Digital images were then taken using an Epson scanner (ES2200) at 300 dpi resolution. The total length of root samples were measured using Win RHIZO software v. 2007d (Regent Instruments, Quebec, Canada).

2.4. Statistical analysis

The experiments were arranged in a randomized complete block design (RCBD) with three 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 root traits and shoot traits were determined using correlation analysis.

3. RESULTS AND DISCUSSION

In this study, we used a line source sprinkler system to evaluate the effects of N application on the shoot and root growth of Nipponbare. This system was quite effective for that purpose because it can create soil moisture gradient from very wet to dry conditions (Henry, 2013). Therefore, the SMC were divided into the following three ranges: well-watered (> 25 % w/w of SMC), mild drought (17-25 % w/w of SMC) and severe drought stress (<17 % w/w of SMC) (Kano at el., 2011; Tran et al., 2014). The results are presented in the succeeding sections.

Figure 1 shows the shoot dry weight of Nipponbare at different SMC ranges as affected by N levels. Under severe drought stress conditions (<17% w/w of SMC), low N level application (60 kg N ha^{-1}) showed a significantly lower shoot dry weight than that at high N levels (120 and 180 kg N N ha^{-1}), but the shoot dry weight was not significantly different between at 120 kg N N ha^{-1} and 180 kg N ha^{-1}. However, under mild drought stress (17-25% w/w of SMC) and well-watered conditions (>25% w/w of SMC) 180 kg N ha^{-1} significantly increased the shoot dry weight in comparison with that at 120 kg N ha^{-1} by 4.1 g plant^{-1} and 3.5 g plant^{-1} under mild drought stress and well-watered conditions, respectively. Similarly, the shoot dry weight was significant higher at120 kg N ha^{-1} than at 60 kg N ha^{-1} by 10.7 g plant^{-1} under mild drought stress and by 12.7 g plant^{-1} well-watered conditions. The above

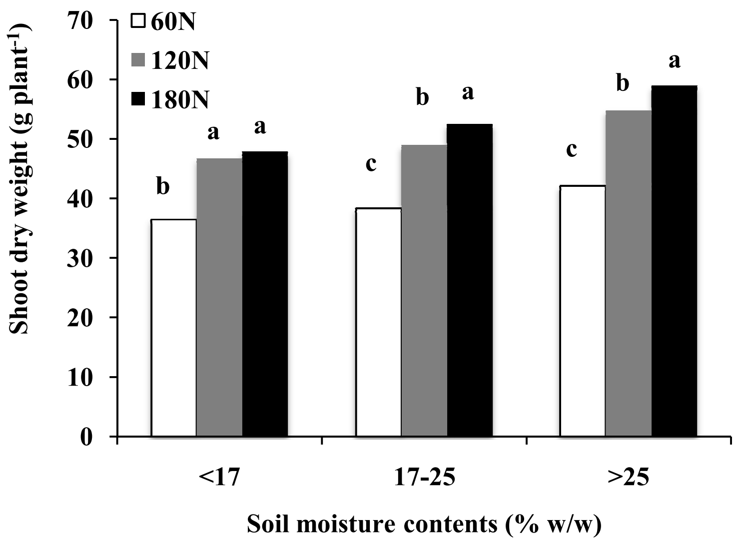


Fig. 1. Effect of nitrogen level application 60 (□),120 (■) and 180 kg N ha^{-1} (■) on shoot dry weight of Nipponbare under different soil moisture content

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

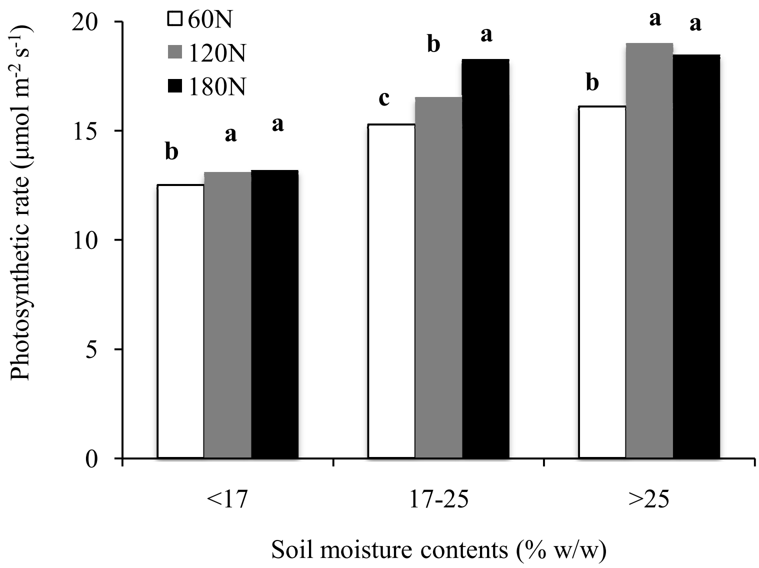


Fig. 2. Effect of nitrogen level application 60 (□),120 (■) and 180 kg N ha^{-1} (■) on photosynthetic rate of Nipponbare under different soil moisture content

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

results supported the previous findings that total dry matter increased with increased N application under mild water stress in water saving system (Belder et al., 2005) or under different irrigation methods (Allahyar, 2011).

Figure 2 shows the effects N level treatments on the photosynthetic rate of Nipponbare under various SMC conditions. The results showed that photosynthetic rate of Nipponbare was significantly lower at low N level application (60 kg N ha^{-1}) than at high N levels application (120 and 180 kg N ha^{-1}) under any of SMC conditions. Suralta (2010) also reported that nitrogen application increased the photosynthetic rate of rice under both continuously waterlogged and drought condition.

Roots play important roles by exhibiting various adapted reponses specific to the prevailing nitrogen application (Suralta, 2010) and soil moisture conditions (Yamauchi et al., 1996). In this study, the root systems' developmental responses to N levels under various SMC conditions based on the total root length are presented in Fig. 3. The total root length significantly differ among N levels application under mild drought stress (17-25% w/w of SMC) or well-watered (>25% w/w of SMC) conditions. Under both mild drought stress and well-watered conditions, the total root length was highest at 180 kg N ha^{-1} (186.0 and 213.3 m plant^{-1} under mild drought stress and well-watered conditions, respectively), the lowest total root length was observed at 60 kg N ha^{-1} (163.3 and 171.7 m plant^{-1} under mild drought stress and well-watered conditions, respectively). Similarly, under severe drought stress conditions, the total root length showed significantly higher at high N levels application (137.4 and 144.5 m plant^{-1} at 120 and 180 kg N ha^{-1}, respectively) than at low N level application (128.2 m plant^{-1} at 60 kg N ha^{-1}). However, the observed differences in the total root length between low N level (60 kg N ha^{-1}) application and high N levels (120 and 180 kg N ha^{-1}) application were more pronounced under well-watered conditions (14.4-41.6m) than under mild drought stress (9.3-26.5 m) and severe drought stress conditions (9.2-16.3m). This implied that when water stress was the limiting factor for growth, increasing N application did not increase appreciably the root system development of Nipponbare.

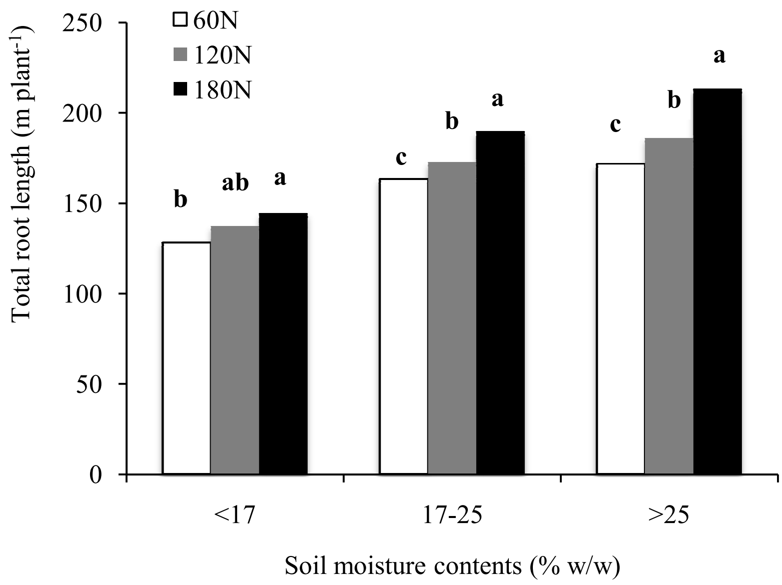


Fig. 3. Effect of nitrogen level application 60 (□),120 (■) and 180 kg N ha^{-1} (■) on total root length of Nipponbare under different soil moisture content

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

Lathovilayvong et al. (1997) also reported that in the rainfed lowland rice ecosystem, nutrient status of soils is often poor and response to applied nutrients is often modest. Therefore, little fertilizer is applied in these systems (Khunthasuvon et al., 1998). However, Suralta (2010) suggested that plastic root system development, in response to drought through greater promotion of L-type lateral root, can enhance not only the uptake of water but also of N if fertilizer N is applied during the onset of progressive drought stress in rice.

Since it was difficult to directly measure water uptake rate of roots in our experiment, we measured stomatal conductance instead, which roughly reflect root water uptake ability (Kano et al., 2011). The results showed that Nipponbare had significantly higher stomatal conductance at 120 and 180 kg N ha^{-1} than at 60 kg N ha^{-1} under severe drought stress conditions (<17% w/w of SMC). There was significant difference in stomatal conductance among N levels application under mild drought stress (17-25% w/w of SMC) and well-watered conditions (>25% w/w of SMC); increasing N level application from 60 to 120 kg N ha^{-1} and 120 to 180 kg N ha^{-1} significantly increased stomatal conductance of Nipponbare (Fig. 4).

Kano et al. (2011) and Tran et al. (2014) found that CSSL50 showed a positive and significant relationship between total root length and shoot dry weight only under mild drought stress conditions. However, in this study, only under well-watered conditions, the total root length of Nipponbare was found to be positively correlated with shoot dry weight at 120 kg N ha^{-1} (Fig. 5 b) and at 180 kg N ha^{-1} (Fig. 5 c) but not at 60 kg N ha^{-1} level application (Fig. 5 a). These facts strongly suggest that N application increased dry matter production due to the increased root system development under well-watered conditions. In other words, N application increased the total root length under well-watered conditions as contributed to the maintenance of its shoot dry matter production.

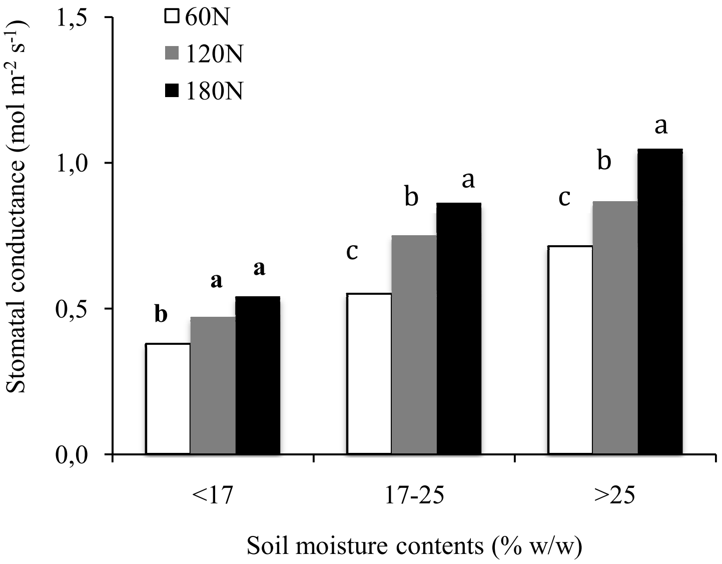
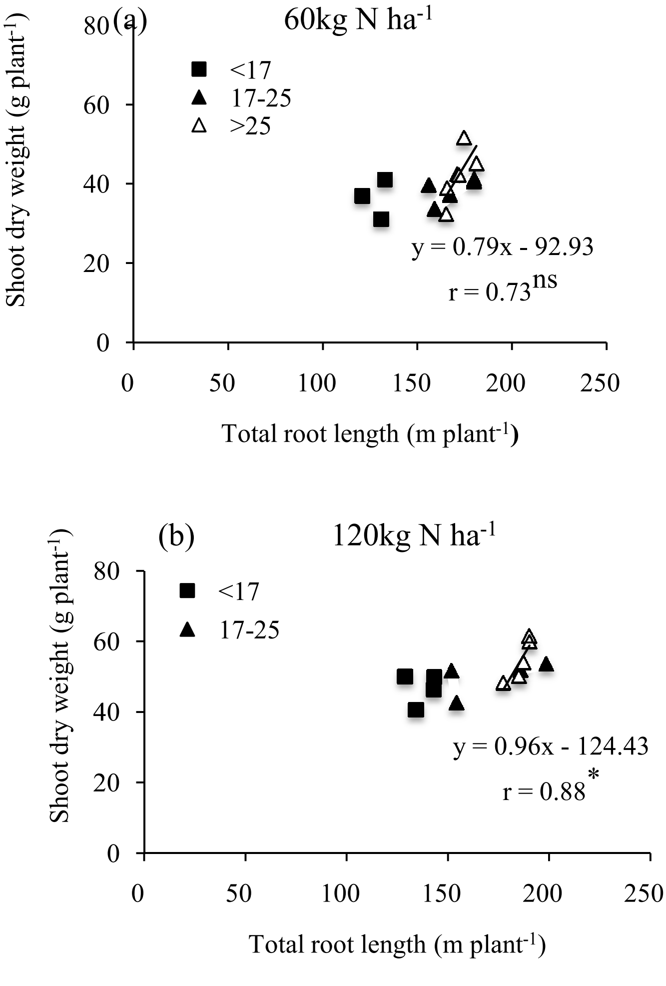


Fig.4. Effect of nitrogen level application 60 (□),120 (■) and 180 kg N ha^{-1} (■) on stomatal conductance of Nipponbare under different soil moisture content

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



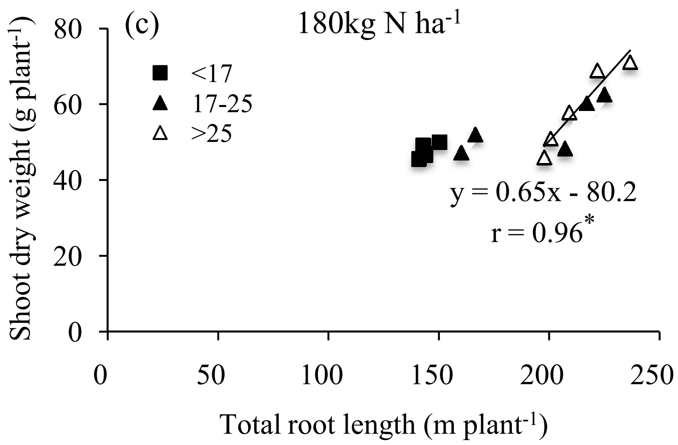


Fig. 5. Relationship between total root length and shoot dry weight of Nipponbare grown under <17% w/w of SMC (■), 17- 25% w/w of SMC (▲) and >25% w/w of SMC (∆) at 60 (a), 120 (b) and 180 kg N ha^{-1}(c)

Note: ■: y = 0.04x + 32.42, r =0,16^{ns} at 60N; y = 0.05x + 40.07, r = 0.24^{ns} at 120N; y = 0.39 x- 9.30, r = 0.76ns at 180N
▲: y = 0.19x + 7.40, r =0,59ns at 60N; y = 0.13x + 26.67 r = 0.63ns at 120N; y = 0.17 x + 20.16, r = 0.74ns at 180N.
*ns: not significant. *: indicates significance at P<0.05.*

4. CONCLUSIONS

In this study, we showed that ample amount of N application enhanced the total root length in response to well-watered condition. Consequently, the resulting increase in total root length due to promoted root system development was at least one of the causes for increased water and N uptake to maintain higher stomatal conductance and photosynthesis, and eventually higher dry matter production.

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