



RESEARCH PAPER

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Analysis of the relationship between root morphology and uptake of fertilizer nitrogen in rice (*Oryza sativa* L.)

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Abstract

Due to the roles of root in water and mineral uptake, it is one of the most important organs for plant growth. In order to analyze the morphological differences of the root in four Iranian cultivars of rice (Tabesh, Fajr, Tarom-e-Mahali and Neamat cultivars) and their relationship with nitrogen uptake, two experiments were conducted in greenhouse and laboratory conditions. In laboratory experiment cubes of sponge, to fix the grains on, which placed in a 30 cm depth Styrofoam container, containing Hogland nutrient solution, were used for hydroponic culture of the plants. One month after initiation of the sowing, the roots of seedlings were screened and the morphological characteristic including thickness, length, surface area, volume and the number of the forks of the roots were measured using image analysis methods implemented in WinRHIZOPro system. In the greenhouse experiment, the same cultivars as used in laboratory, were treated with heavy Nitrogen isotope (N^{15}) and compared. After germination, the seeds were planted in polybags containing 25 kg soil, in 4 replications. N^{15} labelled urea was applied at 150 kg N/ha in three equal parts and N use efficiency in rice grain and straw was measured at harvest. The results showed significant differences in root length, surface area, volume and the number of forks among the studied cultivars. The amount of Nitrogen in plant organs (derived from fertilizer applied), was found to range from 24.05 in Tarom-e-Mahali to 56.67 % in Neamat cultivars. A significant correlation was observed among the surface area, total root length, No of forks, biological yield and the amount of fertilizer Nitrogen uptake. The Neamat and Tabesh cultivars performed efficiently in fertilizer Nitrogen uptake.

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Introduction

Rice, *Oryza sativa* L., comprises the main food stuff for 50% of the world population in highly populated parts of the Asia, Latin America and Africa (Fageria, 2003). Rice is produced in both dry and water based farming and nearly 76% of the production comes from water based farming systems (Duan *et al.*, 2007).

During different stages of life cycle, plants need Nitrogen as a macronutrient. Nitrogen comprises about 2% of the dry weight of the plants and is incorporated in the structure of proteins, nucleic acids, Co-enzymes and many important cellular compounds such as chlorophyll and plant hormones (Williams *et al.*, 2001). The international Rice Research Institute has reported on the importance of the Nitrogen in determining the performance of the rice (IRRI, 2000).

The new systems of rice production are in need of consistent, environmentally safe and efficient management operations, and the role of nitrogen as a key factor to reach the optimal performance in rice yield in the new systems, is not deniable (Akbari *et al.*, 2008). If there is no congruence between the demands of the plant to nitrogen, during different stages of life cycle, and the usage of nitrogenous fertilizers, not only the high performance won't be achieved, but also because of the high rates of evaporation and washing effects occurring in paddies, nitrogen can be washed away and lead to reduced bioavailability and increased environmental pollution (Fageria and Baligar, 2001). Nitrogen is primarily absorbed as Ammonium and nitrate, and is, then, converted to other nitrogenous compounds (Malakuty *et al.*, 2010). Due to many important roles being played by nitrogen in plant life, its deficiency can depress the growth more than any other element. When the crop is supplied with enough amounts of nitrogen, growth rate, height, the number of the claws, the number of grains in each cluster and the percentage of the filled grains in each cluster will be improved, so it can be inferred that nitrogen affects all characters important in plant performance.

Owing to the effects of nitrogen on the leaf surface area and the photosynthetic enzymes, the increased levels of mineral nitrogen can lead to the increased photosynthesis and performance in rice (Peng *et al.*, 2001).

The amount of nitrogen uptake is dependent on the root surface area, which, in turn, depends on the total root length. Similar to the root surface area, the diameter of the root is also a determining factor in nitrogen uptake that is variable in different cultivars. The correlation between morphological attributes and nitrogen uptake rates has been proven (Zahara and Hanafi, 2009). A thick and deep root system has a positive bearing on rice performance (Yadav *et al.*, 1997). Therefore, the development of large root systems in rice has been, generally, suggested as one of the necessary factors to increase the rice grain performance. It has been determined that the high yielding cultivars of rice have higher primary root count, length and diameters with well developed secondary roots (Harada, 1997). The morphological characteristic of the root, the geometric shape of the root system in soil, inter-specific variation and competition are of importance in uptake of the underground resources (Lynch, 1995; Zahara and hanafi, 2009).

Chemical fertilizers are one of the expensive inputs, being utilized by the farmers to reach suitable levels of crop production. Nitrogen fertilizer is one of the agents, highly utilized in agriculture, and its overuse not only is not economically efficient, but also can lead to the ground water pollution via washing of the nitrogenous compounds (Peng *et al.*, 2001). The recycling rate of the inorganic fertilizers, by plants is low in majority of soil types. The uptake rate for inorganic fertilizers are about 50% or less for N, less than 10% for P and nearly 40% for K. such low uptake rates are due to the considerable lose of the nutrients via washing effect, flooding, sublimation and fixation in soil. These lose, potentially, can cause the deterioration of soil and water qualities, which, in turn, can lead to the overall environmental deterioration.

These are the reasons, necessitating the need to increase the Nitrogen uptake rate (Baligar and Fageria, 2001). With respect to above mentioned (expensive chemical fertilizers, water pollution) In this research, the root system in different cultivars of rice and the relationship between the morphological characteristic of root and nitrogen uptake was analyzed to determine characteristic of the root, important in nitrogen uptake and their effects on the seed production performance.

Material and methods

This study was conducted, simultaneously, in laboratory and greenhouse experiments, on 4 Iranian cultivars of rice (Tabesh, Fajr, Tarom-e-Mahali and Neamat) obtained from the Iranian National Rice Research Institute, using two completely randomized designs in 4 replications. In the first experiment, seeds were sterilized in 0.3% Hydrogen Peroxide (H_2O_2) for 30 minutes and then left in tap water. After germination, hydroponic culture using Sponge Nursery Technique was used to grow up the plants. To keep a constant temperature in the growth media, 30 cm deep Styrofoam boxes containing Hoagland's Solution were selected and the germinated seeds were fixed on 3×3×3 cm sponge cubes, which embedded on Styrofoam plates with a number of holes to insert the cubes in them. To prevent the leakage of the nutrient solution and penetration of light, the inner wall of the Styrofoam boxes were lined with a black nylon sheet. After filling the box with the Hogland nutrient solution, the Styrofoam plates with the sponge cubes and germinated seeds inserted on them, were put on the boxes. One month after planting, the newly germinated seeds were ready to be scanned.

All newly grown plants which had developed roots were scanned using image analysis method implemented in WinRHIZOPro, and the morphologic characteristic of the root including thickness, Total length, surface area, volume and the number of forks were determined. The laboratory condition was similar to natural condition (Kondo *et al.*, 2003). In the second experiment seeds with the same genotypes as the genotypes used in the first experiment were treated and compared using labeled nitrogen (N^{15}). This experiment was conducted using a completely randomized design in 4 replications in green house condition. Rice seeds were sterilized in 0.3% Hydrogen peroxide for 30 minutes and then left in tap water, to gain water. After germination, they were planted at the depths of 2-3 cm in plastic vases each containing 25 Kg soil. Irrigation was done once a day.

In order to measure the nitrogen uptake rate, heavy nitrogen isotope technique was used. An amount of 150 kg ha⁻¹, labeled urea source, enriched with 5% N-15 atom excess, was applied in three equal shares, one third in 15 days after planting, one third at tillering and one third at booting (Abbasi, 2010). Phosphorus (P) and Potassium (K) fertilizers were applied with an amount of 100 kg ha⁻¹ of each, at planting time. The wet weights of stem and grain were measured. The dry weight was measured in a ventilated oven at 70°C. When the grains and plant upgrond organs reached a constant weight, they were exited from the oven. The dried grain and straw were milled and after screening by 1mm mesh size screen, they were held in plastic sacks. Then the amount of the N^{15} was measured using an emission spectrophotometer. The amount of total nitrogen was measured using Kjeldahl method (Bremner and Mulvaney, 1982).

Calculations for the quantity of the nitrogen uptake from fertilizer was as follows (IAEA, 2006):

Determine of % ^{15}N abundance Where is transformed into atom % ^{15}N excess (a') by subtracting the natural abundance (0.3663 atom %N= a0) from the % N abundance (a) of the sample.

Plant % ^{15}N abundance, which is analyzed by emission spectrometr.

$$a' = a - a_0 \quad (1)$$

the fraction of the Nitrogen in the plant derived from the (labeled) fertilizer.

$$\%N_{dff} = \frac{\text{atom \% } ^{15}N \text{ excess}_{plant}}{\text{atom \% } ^{15}N \text{ excess}_{fertilizer}} \quad (2)$$

Dry matter (D.M.) yield for the whole plant and sub-divided into plant parts.

$$DM\ yield_{(kg/ha)} = FW_{(kg)} \times \frac{10000(m^2/ha)}{area\ harvested\ (m^2)} \times \frac{SDW_{(kg)}}{SFW_{(kg)}} \quad (3)$$

Where FW is fresh weight per area harvested and SDW and SFW are subsample dry and fresh weight, respectively.

$$N\ yield_{(kg/ha)} = DM\ yield_{(kg/ha)} \times \frac{\%N}{100} \quad (4)$$

Where %N is Total N concentration of the whole plant or plant parts.

$$Fertilizer\ N\ yield_{(kg/ha)} = N\ yield_{(kg/ha)} \times \frac{\%N_{dff}}{100} \quad (5)$$

$$\%Fertilizer\ N\ Utilization = \frac{Fertilizer\ N\ Yield_{(kg/ha)}}{Rate\ of\ N\ application} \times 100 \quad (6)$$

$$\%N\ Harvest\ Index = \frac{Grain\ N\ Yield_{(kg/ha)}}{plant\ N\ Yield_{(kg/ha)}} \times 100 \quad (7)$$

Analysis of the variance of the observations was conducted using SAS software and comparison of the means was done using LSD method.

Results and discussion

The studied cultivars of rice were significantly different in respect to the morphological characteristic of the root ($P < 0.01$). Nevertheless, there were no significant differences between the cultivars for the root diameter produced. The two Neamat and Tabesh showed significantly longer roots than those Fajr and Tarom. The most of differences tolerated number of forks and root surface area in rice cultivars. So that in Neamat with means of 2345.30 and 150.06 cm, respectively, had the highest amount and Significantly lower Number of forks and root surface area were observed for Tarom with means of 699.05 and 45.71 cm, respectively. Fagaria (2003) has, also, reported similar observations for the counts of the number of forks and root surface area in 20 rice genotypes. A significant difference between Nanguang and Elio cultivars was reported for root surface area (Fan *et al.*, 2010).

A significant difference has, also, been reported for the counts of the number of forks in wheat and Corn (Wang *et al.*, 2004). The highest mean root volume were observed in Neamat and there was no significant difference between others cultivars (tabel 1).

Significant differences were observed for plant height, number of tillers per pot, yield and the components of yield ($P < 0.01$; tables 3). All the studied cultivars were significantly different in their grain yield.

The highest (567.48 gr m⁻²) and the least (292.54 gr m⁻²) means yield were observed in Neamat and Tarom-e-Mahali, respectively. It has been indicated that the stronger root system in rice, can increase the grain yield (Yuan, 2001).

On the other hand, the highest and the least N Harvest Index percentage were observed in Neamat and Tarom-e-Mahali cultivars. There was no significant difference between the Neamat, Tabesh and Tarom-e-Mahali in their weight of grain thousand. The Tarom-e-Mahali due to its short panicle length and the limitation of the sink had the least yield, although this cultivar has the highest fertility percentage and the least unfilled grain number due to its lower seed production (Niknejad *et al.*, 2007). This finding is concordant with the results of the comparison between the Shafagh and hybrid GRH1 cultivars (Kazemi *et al.*, 2007). Therefore, although, the highest harvest index caused by the less biological yield was observed for Tarom. but its grain yield was lower, compared to the other cultivars. The lowest plant height observed in Tarom, confirms this observation.

All parametes of grain and straw related to the quantity of nitrogen uptake from fertilizer were significant ($P < 0.01$; table 2). The two Neamat and Tabesh showed significantly highest fertilizer nitrogen uptake percentage

(mean values of 56.67 and 40.24 %, respectively) than those Fajr and Tarom (24.36 and 24.05 %, respectively). (table 2). The results showed that the highest amount of the absorbed nitrogen was used for economic yield in Neamat and Fajr, but most of the absorbed nitrogen was used for biological yield in Tabesh. The results indicate that in the assessment and selection of the rice cultivars with outstanding morphological characteristics, not only the nitrogen uptake rate, but also the dedication of the absorbed nutrients such as photosynthetic elements or, on the other hand, the harvest index, which is of considerable importance, should be considered (Fagaria, 2010).

There was a positive correlation between the nitrogen consumption rate and the morphological characteristic of the rice root. The highest correlation was observed between nitrogen uptake rate and the roots number of forks ($R^2=0.92$), the other characteristic showing the next high values of correlation with nitrogen uptake were the total root length and root volume ($R^2=0.89$ and 0.86 respectively). A poor correlation ($R^2=0.59$) was obtained between the volume of root and the N uptake (Figure 1).

The morphology of the root can affect water and nutrient uptake in plants, significantly (Passioura and Wetselaar, 1972; Eghball *et al.*, 1993). In a farm experiment conducted on 10 cultivars of corn, a close relationship was observed between the nitrogen uptake and root length (Weisler and Horst, 1994). Although, the data on the relationship of the root morphology and nitrogen uptake in rice is scarce, but Lawler (2002) concluded that the increase of the volume and density of the root is effective in increasing the nitrogen uptake. The accumulation of nitrogen in plants has a close relationship with root characteristic, for which the main reason is the penetrance of the plants with larger roots in rice cultivars with higher nitrogen consumption rates (Fan *et al.*, 2010). In addition, the larger main roots are more effective in nitrogen uptake in corn (Liu *et al.*, 2008).

In nitrogen limited systems, the larger roots radiated from the major roots are more effective in nitrogen deposition (Wang *et al.*, 2004). In nitrogen efficient condition, the longer secondary roots are the main factors in nitrogen deposition (Singh and Talati, 2005).

Fan *et al.*, (2010) reported that the differences in dry weight and the morphology of the root, which are determining factors in nitrogen uptake, between Nanguang and Elio cultivars were highly significant and at tillering stage, the morphological attributes of the root were similar for different cultivars of rice.

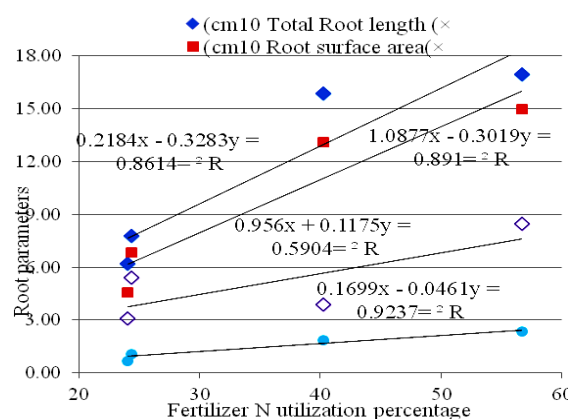


Fig. 1. Relationship between root parameters and fertilizer utilization percentage of rice.

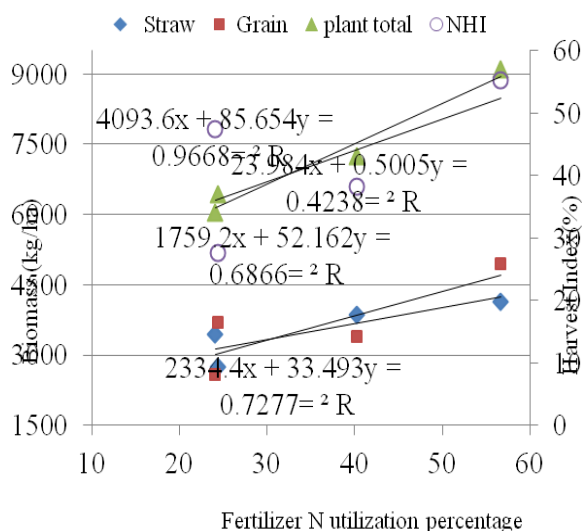


Fig. 2. Relationship between Biomass yield and fertilizer N utilization percentage in rice organs.

But at the stages of booting and heading, Nanguang variety had longer root and more root surface area compared to the Elio variety.

Especially after heading stage, the difference in the root characteristic was highly significant among different cultivars of rice (Fan *et al.*, 2010). The genotypic basis for the morphological differences of the root in various crops with different nitrogen consumption rates have been recorded for Corn (Wang *et al.*, 2004), Potato (Sattelmacher *et al.*, 1990), Wheat (Passioura and Welselaar, 1972) and a grass (Sullivan *et al.*, 2000).

The relationship between nitrogen consumption rate and performance indices were also analyzed. Poor correlation was observed between the harvest index, grain and straw yield and nitrogen consumption rate.

But there was a highly significant correlation between the biological yield and the percentage of the nitrogen consumption ($R^2=0.97$; Fig. 2).

In conclusion, based on the results observed in both experiments, it can be inferred that the morphological characteristic of the root in rice can affect more efficient nitrogen uptake. With the identification of the better rice cultivars based on the morphological attributes of the root system which are important in more and faster nitrogen uptake, in addition to the maintenance of the high yield, the nitrogenous fertilizers can be consumed more economically.

Table 1. Root parameters of rice cultivars, 30 days after germination.

Cultivars	Total Root length (cm)	Root surface area(cm)	Root diameter (mm)	Root Volume (cm ³)	No. of forks
Fajr	78.06 b	68.50 c	2.46 a	5.42 b	1096.55 c
Neamat	169.54 a	150.06 a	2.11 a	8.48 a	2345.30 a
Tabesh	158.73 a	130.91 b	2.04 a	3.91 bc	1884.05 b
Tarom	61.97 b	45.71 d	2.53 a	3.09 c	699.05 c

* Means followed by the same letter in the same column are not significant at the 1% probability level by LSD test.

Table 2. Means comparison of nitrogen uptake quantity characteristic from fertilizer.

Cultivars	%N	% ¹⁵ N excess	%Ndff	DMY (kg/ha)	NY (kg/ha)	FNY (kg/ha)	%FNU	%Ndffs
Fajr	2.18 b	2.22 b	33.83 c	6440.48 c	108.01 c	36.54 c	24.36 c	66.17 a
Neamat	3.05 a	2.97 a	45.41 a	9087.2 a	204.46 a	85.3 a	56.67 a	54.59 c
Tabesh	2.73 a	2.54 ab	37.31 bc	7254.17 b	161.77 b	60.36 b	40.24 b	62.69 b
Tarom	2.05 b	2.73 a	39.95 ab	6039.88 d	90.3 d	36.08 c	24.05 c	60.05 bc

* %N=% N in dry matter, %¹⁵N excess= Plant %¹⁵N abundance, which is analysed by emission, %Ndff = the fraction of the N in the plant derived from the (labelled) fertilizer, DMY= Dry matter yield per unit area, NY= N Yield, FNY= fertilizer N yield, %FNU= fertilizer N utilization percentage, %Ndffs= the fraction of the N in the plant derived from the (labelled) soil. Means followed by the same letter in the same column are not significant at the 1% probability level by LSD test.

Table 3. Means comparison of yield, components of yield, plant height and nitrogen harvest index

Cultivars	Plant height(cm)	No of tillers/pot	Panicle length(cm)	Weight/1000 Grains(gr)	Grain yield (gr/m ²)	N Harvest Index (%)
Fajr	81 c	6.37 b	28.11 a	22.23 b	490.77 b	27.63 c
Neamat	101 b	13.52 a	22.57 ab	29.19 a	567.48 a	55.33 a
Tabesh	117 a	9.66 ab	25.12 a	27.86 a	335.42 c	38.28 b
Tarom	87c	7.92 b	18.87 b	27.12 a	292.54 d	47.43 a

* Means followed by the same letter in the same column are not significant at the 1% probability level by LSD test.

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