

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.707.150>

The Selective Vulnerability of Rice Root System Architecture to Organic and Inorganic Nitrogen

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ABSTRACT

N fertilizers and high yielding varieties were major drivers of the enormous increase in rice productivity during the past 50 years. Despite increasing food production higher nitrogen use also contributed in environmental pollution and Increasing consciousness of conservation of environment and mitigation of climate change brought a major shift in cultivation practices of major crops towards organic agriculture. An important issue regarding the acceptance of organic agriculture is the question of productivity. In addition to readily available ammonium and nitrate ions, the soil of organic agriculture can contain a wide range of organic nitrogen compounds such as peptides, proteins, free amino acids, amino sugars and nitrogen heterocyclic compounds. The root system architecture (RSA) features are of utmost importance for increasing nitrogen use efficiency of future climate-resilient varieties. From a fundamental point of view, the influence of nitrogen on root development is still poorly understood. Modulating root architecture is a strategy that aims at developing crops that capture nutrients more efficiently and are thus suitable for sustainable agriculture with fewer fertilizer inputs. Our experiment aimed to study the responses of IR-28 root system architecture to the availability of different forms of nitrogen, including organic at seedling stage, so that we can understand up to what extent our current rice varieties, which are exclusively bred for intensive agriculture, are suitable for organic agriculture. From the experimental results it was indicated that root parameters viz. primary root length was significantly reduced due to the availability of nitrogen in nitrate and ammonia while increased with organic N. Main root angle was significantly increased with increasing N concentration and it became more steeper under the deficiency of N. Straightness of main root does not affected by the availability of N while the sources of nitrogen had significant effect and maximum straightness observed in organic N treated plant while minimum in ammonia treated. Lateral root number was increased with increasing nitrogen as nitrate and ammonia up to 100% as compared to organic N. Mean lateral root length was significantly affected by the sufficiency of nitrogen, while does not fluctuate with various forms of nitrogen. Sum of lateral root length was higher in nitrogen sufficiency. Lateral root density was significantly responding to availability and non-availability of nitrogen and significant reduction was observed in 0% N condition. Total root system size significantly influenced by sources and levels of nitrogen. Moreover, amongst root traits, total root system size was found least phenotypic plastic while lateral root density was ranked as highest phenotypic plastic trait due to nitrogen. The results indicate that combined nitrogen nutrition through nitrate and ammonia is most suitable for root system and seedling growth of rice as compared to the sole sources. The results of these study support the view that we need to breed varieties suited for organic agriculture and varieties such as IR-28, which is select and breed for high nitrogen input intensive agriculture, may not be efficient for organic nitrogen uptake and/or assimilation.

Keywords

Root system architecture, Root plasticity, Rice, Organic and inorganic nitrogen

Article Info

Accepted:

08 June 2018

Available Online:

10 July 2018

Introduction

Nitrogen (N) is a paramount element for crop productivity since it is a core component of many plant structures and for their metabolic processes. It is quantitatively the most important nutrient for plant development. Limited N availability has severe consequences for plant metabolism and growth resulting in lower biomass and yield of storage compounds (Epstein and Bloom, 2005). The 'green revolution' of the 1960s and 1970s helped agriculture to meet the food demands of a rapidly growing global population, through the development of dwarfed cereal cultivars, with high nitrogen responsiveness and better irrigation facilities. Past cereal production, including rice is mainly due to high application of N fertilizers together with the development of high yielding varieties. Approximately 85 to 90 Million Metric tons (MMt) of nitrogenous fertilizers are added to the soil worldwide annually up from 1.3 MMt in 1930 and 10.2 MMt in 1960 (Frink *et al.*, 1999) and this is predicted to increase up to 240 MMt by the year 2050 (Tilman *et al.*, 2002). Nitrogen fertilization has been used for decades to increase crop yield with relatively low efficiency since a considerable fraction (up to two-third) of N input accumulates as runoffs (Frink *et al.*, 1999), which dramatically affect the N cycle and associated processes (Vitousek *et al.*, 1997 and Galloway *et al.*, 2008). The Nitrogen Use Efficiency (NUE) which may be defined as the yield obtained per unit of available N in the soil has declined sharply with increasing application of nitrogenous fertilizer. In most intensive agricultural production systems, over 50% and up to 75% of the N applied to the field is not used by the plant and is lost by leaching into the soil (Raun and Johnson, 1999) and nitrogen use efficiency is only 33% (Abrol *et al.*, 2007). The NUE of cereals, including rice, may be low because of modern breeding

methods where selection trials are routinely carried out with sufficient to excess N provision often based on previous best practices and lines selected which respond to the applied N levels in the soil (Kamprath *et al.*, 1982). N fertilization introduced N into the environment largely that resulted in significantly negative environment consequences (Brown, 2011). Nitrogen lost from agricultural system will enter to groundwater, lakes, estuaries and coastal water where the reactive nitrogen can participate and induces death of aquatic life as well as harms human and animal population as its negatively influence on drinking water availability. The production and extensive application of N fertilizer also contributed in major environmental problems due to soil leaching and greenhouse gas emission that play a large role in ozone depletion and global warming (Donner and Kucharik, 2008). In addition to these negative environmental effects, synthetic nitrogen fertilizer is typically the single highest input cost for many crops, since commercial fertilizer production (via Haber Bosch method) is energy intensive process.

Agriculture sector activities (mainly nitrogen fertilizer use) are the main contributor of global anthropogenic N₂O emission (ca. 58%), soil deterioration and nutrients imbalance (Wuebbles, 2009). Nitrous oxide (N₂O) is the third most abundant greenhouse gas (GHG) with only carbon dioxide (CO₂) and Methane (CH₄) being most prevalent (Montzka *et al.*, 2011) and is a 300 times more potent GHG than CO₂ (Johnson *et al.*, 2007). India share 49% N₂O emission in 2005 (Out of 267 Gg, where Gg=1000000 kg) compared to 40% in 1985 (144 Gg) (Garg *et al.*, 2012). In order to reduce eutrophication and the costly component of crop production, there is an immediate need to reduce N fertilizer inputs. The concept of sustainable agriculture leads to conservation of natural resources with the

mitigation of climate change and increasing the productivity tend to move towards organic agriculture. It is based on minimizing the use of external inputs through use of on-farm resources efficiently compared to intensive agriculture and thus the use of synthetic fertilizers is avoided. Awareness of organic food for health conscious people steadily increases. There is an annual average growth rate of 20-25% (Ramesh *et al.*, 2005) increase in organic products demand. Worldwide over 130 countries produce certified organic products in commercial quantities (Kortbech-Olesen, 2000). An important issue to the acceptance of organic agriculture is found in the question of its low productivity. At present carrying capacity of organic agriculture only for 3-4 billion, well below the present world population (\approx 7 billion) and that is projected up to 9 billion for 2050 (Connor, 2008). To meet food requirements of this future population FAO estimates that food production will have to increase by 70 %.

In organic agriculture, crop productivity is mainly limited due to nitrogen availability which is not easily controllable (Owen and Jones, 2001). The N availability dependent on mineralization of crop residues and farm yard manures applied on the farm. In early crop growth stages when demand is low, N is lost while in later stages the demand from the plant is often much greater than the supply from mineralization and matching N need and mineralization one of the major limiting factors in organic agriculture system.

One of the basic principles of soil fertility management in organic agriculture is that crop nutrition depends on biologically derived nutrient. Organic residues of low nutrient content (FYM, vermicompost, green manure etc.) added to the soil surface or incorporated into soil undergo decomposition by soil microbes. In addition to the readily available ammonium and nitrate ions, soil of organic

agriculture can contain a wide range of organic nitrogen compounds such as peptides, proteins, free amino acids, amino sugars and nitrogen heterocyclic compounds (Mader *et al.*, 2002; Jones *et al.*, 2002). The organic nitrogen fraction typically comprises 0.1 to 0.5 % of total soil N (Barber, 1984).

NO₃⁻ and NH₄⁺ ions are usually considered to be the main N forms in soil solution taken up by crop plants and to a lesser extent as proteins, peptides or amino acids. Some agricultural crop species have been shown to absorb organic N (Okamoto and Okada, 2004), for example, in rice, the N uptake rate increases with organic N supply rather than nitrate application (Yamagata and Ae, 1996). Studies on plant grown in solution culture or upon excised roots have also demonstrated that uptake of organic N can occur at a rate comparable to or in excess of N uptakes from inorganic N sources (Chapin *et al.*, 1993; Raab *et al.*, 1999). Under organic agriculture crop productivity mainly limited due to nitrogen availability (Mader *et al.*, 2002) and additionally literature suggested that we need to breed differently for organic agriculture to increase their efficiency for nitrogen utilization (Sharma and Bardhan, 2017, Sharma *et al.*, 2017).

Moreover acquisition of the various N forms is regulated not only by their chemical nature and spatial availability in the soil, but also by root system architecture, transport system in the plasma membrane of root cells and mechanism that regulate the activity of N transport systems and root growth, depending on plant requirements. From a fundamental point of view, the influence of nitrogen on root development is still poorly understood. Modifying root architecture is a strategy that aims at developing crops that capture nutrients more efficiently and are thus suitable for sustainable agriculture with fewer fertilizer inputs. The present investigation aims to study

the responses of rice root system architecture to availability of different forms of nitrogen at seedling stage. However such experiment cannot be performed in the soils because of spatial and temporal variability of nitrogen in the soil, thus we choose to conduct our experiment in solid growing media under laboratory condition.

Materials and Methods

The study was conducted at the plant tissue culture laboratory, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India (20.9467° N, 72.9520° E) in September-October 2017. The rice seeds of IR-28 were provided by Regional Rice Research Station, Navsari Agricultural University, Vyara. In the study, polystyrene made optically clear and gamma irradiated pre-sterilized square petri dishes (125 x 125 x 20 mm³) having 139 cm² of growing area were used.

Growing condition

The seeds were surface sterilized by immersing in 0.1 percent mercuric chloride for 10 minute. Then seeds were washed thoroughly with deionized water and before placing in nutrient media, the seeds were soaked for 24 hours in deionized water. Three rice seeds were placed in one petri dish (Fig. 1).

The cultured dishes were placed in vertical orientation in the racks of culture room of the laboratory. The culture room was maintained at 25°C ±1°C, 60-70% RH with providing 12 hours of photoperiod at about 2000 lux by cool white fluorescent tube light.

Total 126 petri dishes were used for the experiment. The experiment was laid down in complete randomized design with three repetitions.

Growing media

Yoshida's solution (Yoshida *et al.*, 1976) was used as a growing media and each petri dish was poured with 100 ml volume. The culture media was substituted with different sources of nitrogen on the basis of molar mass and the pH was maintained at 5.7 after mixing all the stock solution. Before pouring the media into the square dish the media was autoclaved at 121°C for 2 hrs. 8 g/L Phytigel was added for the solidification of culture media.

Nitrogen treatment application

The experiment consist of fourteen treatments *viz.*, three levels of nitrogen sources (nitrate, ammonia and organic nitrogen) and their combination with four levels of concentrations (50, 75, 100 and 125% N) besides two controls (control – I, 100 % N as in Yoshida solution and control- II, 0% N, Yoshida solution).

According to the experimental treatments, all the individual stock solution prepared and calculations of different nitrogen sources and its level were calculated by using total nitrogen concentration in the standard Yoshida solution. In standard Yoshida solution, nitrogen was supplied by NH₄NO₃ (91.4 g/lit). As per our experimental treatments, the nitrogen was substituted by using different sources and desired concentration level were maintained on the basis of molecular mass (Table 1 to Table 3). NH₄NO₃, KNO₃, (NH₄)₂SO₄ and amino acid mixture (Glycine, L- Glutamic acid and L-Aspartic acid) were used for substitution of nitrogen from the Yoshida solution.

Root system analysis

Total 126 images of root system were captured by digital camera (Sony Cybershot) on 21 day after emergence (3 images per

treatment per repetition). These images were processed as per the guidelines of the Ez-Rhizo software (Armengaud *et al.*, 2009) and

the data obtained was converted to mean value for each parameter (Fig. 2).

Table.1 Substitution of nitrate as sole N source (g/lit)

Chemicals	50%	75%	100%	125%
KNO ₃	82.85 gm	82.85gm	82.85 gm	82.85 gm
CaNO ₃	38.10 gm	105.45gm	172.89 gm	236.44 gm
CaCl ₂	70.69 gm	39.04gm	7.33 gm	--
HCl	9.88 ml	27.36 ml	44.86 ml	48.91 ml

Table.2 Substitution of NH₄⁺ as sole N source (g/lit)

Chemicals	50%	75%	100%	125%
NH ₄ H ₂ PO ₄	29.71 gm	29.71 gm	29.71 gm	29.71 gm
(NH ₄) ₂ SO ₄	58.36 gm	96.02 gm	133.74 gm	133.74 gm
NH ₄ Cl	--	--	--	30.55 gm

Table.3 Substitution of organic nitrogen as sole N source (g/lit)

Amino acid	50%	75%	100 %	125 %
Glycine	28.56 gm	38.53 gm	57.13 gm	71.44 gm
L- glutamic acid	55.98 gm	83.93 gm	111.97 gm	140.01 gm
L- Aspartic acid	50.65 gm	75.92 gm	101.29 gm	126.67 gm

Other observations

Nitrogen content in shoot and root samples were observed by weight digestion method along with shoot and root dry weight per seedling at 21 days after emergence.

Statistical analysis

All mean values were subjected for statistical analysis for control vs rest design of factorial concept (Panse and Sukahtme, 1978) of the experiment and analysis was performed by department of agricultural statistics, N. M. College of Agriculture, NAU, Navsari. The significance of difference was tested by ‘F’ test at five per cent level. The critical differences was calculated whenever the

difference among treatment found significant.

Results and Discussion

Needless to say, thus the ability of plants to quickly and efficiently modulating its root architecture may determine its comparative success and productivity in nitrogen limiting environments. Under such condition plants activate foraging responses that induced morphological changes and modulated root system architecture, besides physiological and metabolic changes. Both nutritional status of the plant and the external nutrient availability can induce changes in overall root morphology (Giehl *et al.*, 2014). Root system architecture (RSA) defined as the spatial configuration of root system is the fundamental aspect in plant productivity

(Lynch, 1995). The ability of a plant to modify the RSA based on nutritional status of the surrounding, so called plasticity is currently the most accepted target trait for nitrogen use efficiency.

The results of primary root length (Fig. 3A) indicated significant increase in root length due to nitrogen unavailability. The primary root length of rice seedling was found significantly influenced by nitrogenous forms and their different levels. Results indicated that in control-I (100 % N, Yoshida solution) significantly minimum root length was observed while control-II (0% N, Yoshida solution) recorded significantly maximum root length. Deficiency in N results in a shift in dry matter in favor of root growth. (Ericsson 1995) and thus higher root growth was observed in control- II. Similarly in nitrogen deficiency, increase in primary root growth was observed by other workers (Linkohr *et al.*, 2002, Lopez-Bucio *et al.*, 2003). Various forms of nitrogen also influenced primary root length and minimum root length was observed with nitrate form which was statistically at par with ammonical form and control- I. Root proliferation and overall plant growth are usually greater with mixture of NH_4^+ and NO_3^- than with either form alone (Wang and Below 1992, Saravitz *et al.*, 1994, Schortemeyer and Feil, 1996) as indicated in 100 % N Yoshida solution (control-I). Rice is known as efficient for NH_4^+ (Wang *et al.*, 1993) as well as exceptionally efficient in absorbing NO_3^- also (Duan *et al.*, 2006). Maximum primary root length was recorded for organic nitrogen nutrition, which also exhibited maximum carbon allocation and up to 33 % of shoot biomass reduction was recorded as compared to control- I (100 % N Yoshida solution) (Fig. 5). This suggesting nitrogen starvation in IR-28 seedlings under organic nitrogenous nutrition. Root length under various levels also supported the above discussed fact that nitrogen deficiency

increases the root length and maximum and minimum root length were recorded with 50 % and 125 % levels of nitrogen, respectively. However, at 125 % of nitrogen, decreased in root length was observed with nitrate and ammonical form but not in organic form. Reduction in ammonical nitrogen may be attributed to its toxicity. Although rice is known as NH_4^+ tolerant species (Wang *et al.*, 1993) can be negatively affected by elevated NH_4^+ levels (Balkos *et al.*, 2010). Excessive NH_4^+ is also known to inhibit the growth of most crop species (Roosta and Schjoerring 2008). Infact stunted root growth is considered as principal symptom of ion toxicity (Gerendas *et al.*, 1997; Britto and Kronzucker, 2002, Balkos *et al.*, 2010). However, in the present study, at 125 % level of nitrogen, nitrate nitrogen found more toxic than ammonical nitrogen.

In several crop species, genetic variation in axial root growth angle is associated with rooting depth as in common bean and maize shallow growth angles enhances top soil foraging and acquisition of top soil resources such as phosphorus (Zhu *et al.*, 2005; Lynch 2011). In common bean, wheat, sorghum and rice, steep growth angles enhances subsoil foraging and water acquisition under terminal draught (Ho *et al.*, 2005; Manschadi *et al.*, 2008; Uga *et al.*, 2011; Mace *et al.*, 2012). In the present study, main root angle ($^\circ$) (Fig. 4G) tend to steeper in nitrogen deficit treatments and maximum angle was recorded for control-I while significantly narrow angle was recorded in control-II. Nitrate and ammonical nitrogen had similar effect on root angle while organic nitrogen treatment significantly decreases the main root angle. Previous reports suggesting no influence of nitrogen on root angle (Forde and Lorenzo, 2001), However, Trachasel *et al.*, (2013) reported decreased brace and crown root angle under low N condition in maize. In the present study, straightness of main root (Fig. 4H) was

not significantly influence by nitrogen availability, though form of nitrogen significantly influencing straightness of main root and in organic form, it was maximum as compared to ammonium and nitrate. This might be due to the fact that in organic nitrogenous treatment, seedling experiencing nitrogen limitation as supported by all other traits and thus root angle decrease more in organic and increases straightness as compared to ammonia and nitrate.

Lateral root number (Fig. 3B) of rice significantly varied in different forms of nitrogen nutrition and significantly maximum lateral root numbers were observed under nitrate nutrition while lowest number recorded with organic form of nitrogen. It has been shown that, nitrate stimulates lateral root growth by regulating auxin activity and it increases auxin accumulation in primary root tips (Vidal *et al.*, 2010). There are strong connections reported between auxin and nitrate signaling, which could cooperatively regulate lateral root development (Zhang *et al.*, 1999; Gutierrez *et al.*, 2007, Tian *et al.*, 2008; Krouk *et al.*, 2010; Mounier *et al.*, 2014).

As in the present study, along with lateral root number, sum of total lateral root length (Fig. 3D) was also recorded maximum in nitrate nitrogen. However, lateral root number, mean lateral root length and sum of lateral root length were significantly higher recorded in control-I (100% N Yoshida solution), where both nitrate and ammonical nitrogen were available. It seems to have complementary effects of availability of nitrate and ammonia on lateral root development because ammonium stimulates branching whereas nitrate stimulates lateral root elongation (Remans *et al.*, 2006; Lima *et al.*, 2010). Moreover, nitrogen deficiency shown to inhibit lateral root emergence as Krouk *et al.*, (2010) suggested that severe nitrogen limitation caused less auxin accumulation in

lateral root primordia and thus lateral root emergence is hampered. In present study, significantly lower lateral root development was observed in 50% nitrogen supply. Though, it seems that in present study, rice seedlings were not experiencing severe nitrogen deficiency as reported elsewhere that in severe deficiency, total lateral root length was decreased and completely absent of lateral root formation (Krouk *et al.*, 2010, Gruber *et al.*, 2013).

Moreover, lateral root length and sum of lateral root length was decreased at higher dose of nitrogen nutrition (Fig. 3 C and 3 D). It may be due to besides auxin signaling, high nitrogen may affect auxin concentration also. For instance, when external nitrate concentration was greater, the elongation of lateral root in maize was inhibited, which was due to reduction of auxin translocation from shoot to root in phloem (Tian *et al.*, 2008). Therefore, a reduced auxin level in root resulted in the inhibition of lateral root growth. Moreover higher concentration of nitrate also inhibited lateral root growth through ABA signaling (Signora *et al.*, 2001, Vidal *et al.*, 2010). Lateral root density of IR 28 seedling (Fig. 4E) was found more in nitrate as compared to ammonical and organic nitrogen nutrition.

However, at higher concentration of supply, in all forms of nitrogen, lateral root density was decreased. It may be attributed to root plasticity where plant roots adopt an economic saving model with lateral root inhibition to reserve energy and carbon skeleton for other usage.

Similar to our results, where at 100% of nitrogen nutrition, greater lateral root density, lateral root number and sum of lateral root length observed, in barley also nutrient rich 'patch' elicit in lateral root initiation and elongation (Drew, 1975).

Table.4 Shoot dry weight (mg/plant) of rice seedlings as influenced by various nitrogenous treatments

Level of nitrogen	Forms of nitrogen			Mean	ANOVA		
	NO ₃ ⁻	NH ₄ ⁺	Organic			S.Em±	CD at 5%
50%	20.2	20.5	18.0	19.6	Treatment	0.55	1.60
75%	24.1	22.5	18.0	21.5	Nitrogen forms (N)	0.28	0.80
100%	27.5	23.6	18.8	23.3	Level of nitrogen (L)	0.32	0.93
125%	18.9	19.3	17.6	18.6	N x L	0.55	1.60
Mean	22.6	21.5	18.1	20.73	0 % N vs Rest	0.41	1.18
Control-I (100% N Full Yoshida solution) mean	28.1				100% vs 0% Nitrogen	0.55	1.60
Control-II (0% N Yoshida solution -N) mean	17.5				CV %	4.55	

Table.5 Root dry weight (mg/plant) of rice seedlings as influenced by various nitrogenous treatments

Level of nitrogen	Forms of nitrogen			Mean	ANOVA		
	NO ₃ ⁻	NH ₄ ⁺	Organic			S.Em±	CD at 5%
50%	14.2	14.0	13.3	13.8	Treatment	0.45	1.30
75%	14.7	15.2	13.7	14.6	Nitrogen forms (N)	0.22	0.65
100%	17.5	18.2	14.0	16.6	Level of nitrogen (L)	0.26	0.75
125%	13.2	13.5	13.7	13.5	N x L	0.45	1.30
Mean	14.9	15.3	13.7	14.6	0 % N vs Rest	0.33	0.96
Control-I (100% N full Yoshida solution) mean	18.7				100% vs 0% Nitrogen	0.45	1.30
Control-II (0% N Yoshida solution - N) mean	13.5				CV %	5.24	

Table.6 Nitrogen content in shoot (mg.g⁻¹) of rice seedlings as influenced by various nitrogenous treatments

Level of nitrogen	Forms of nitrogen			Mean	ANOVA		
	NO ₃ ⁻	NH ₄ ⁺	Organic			S.Em±	CD at 5%
50%	1.92	1.49	1.29	1.57	Treatment	0.03	0.07
75%	2.15	1.63	1.25	1.68	Nitrogen forms (N)	0.01	0.04
100%	2.94	2.21	1.15	2.10	Level of nitrogen (L)	0.01	0.04
125%	1.33	1.46	1.09	1.29	N x L	0.03	0.07
Mean	2.09	1.70	1.20	1.66	0 % N vs Rest	0.02	0.05
Control-I (100% N Full Yoshida solution) mean	3.06				100 % vs 0% Nitrogen	0.03	0.07
Control-II (0% N Yoshida solution -N) mean	1.07				CV %	2.57	

Table.7 Nitrogen content in root (mg. g⁻¹) of rice seedlings as influenced by various nitrogenous treatments

Level of nitrogen	Forms of nitrogen			Mean	ANOVA		
	NO ₃ ⁻	NH ₄ ⁺	Organic			S.Em±	CD at 5%
50%	0.76	0.99	0.70	0.81	Treatment	0.02	0.06
75%	0.91	1.04	0.73	0.89	Nitrogen forms (N)	0.01	0.03
100%	1.36	1.14	1.01	1.17	Level of nitrogen (L)	0.01	0.04
125%	0.81	0.75	0.96	0.84	N x L	0.02	0.06
Mean	0.96	0.98	0.85	0.93	0 % N vs Rest	0.02	0.05
Control-I (100% N full Yoshida solution) mean	1.48				100% vs 0% Nitrogen	0.02	0.06
Control-II (0% N Yoshida solution - N) mean	0.52				CV %	3.91	

Figure.1 Root System Architecture of IR-28 at 21 days after emergence, under varied nitrogen sources and availabilities

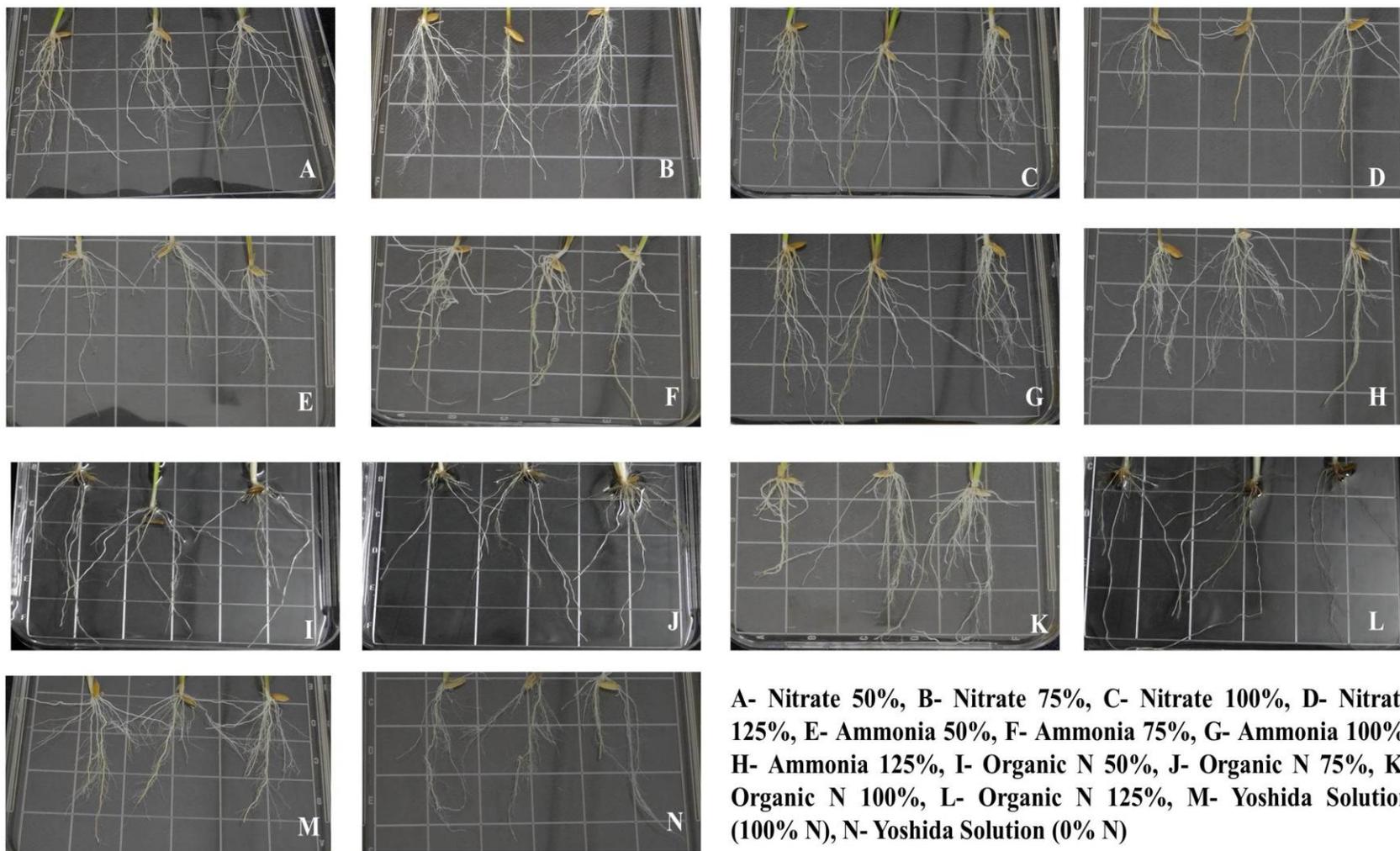


Table.8 Plasticity of root traits of rice seedlings as influenced by various nitrogenous treatments

Root traits	Plasticity value
Primary root length	0.030 ^{NS}
Main root angle	-0.053*
Straightness of main root	-0.016 ^{NS}
Lateral root number	-0.314**
Mean lateral length	-0.300 ^{NS}
Sum of lateral length	-0.298**
Lateral root density	-0.328**
Total root system size	-0.278**

Figure.2 Example of root system skeletonized image output of Ez-Rhizo for all nitrogenous treatments

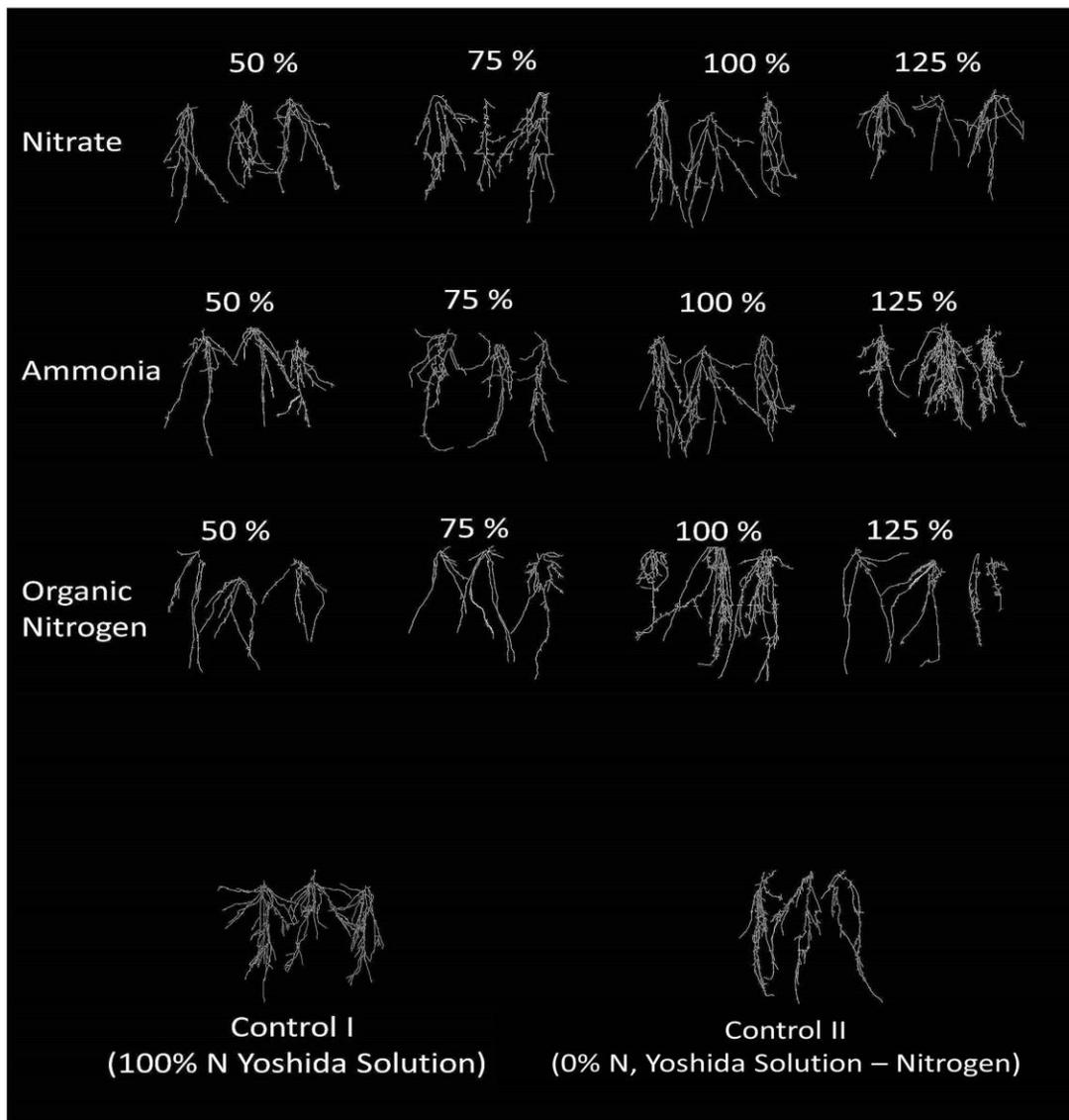


Figure.3 Primary root length (A), lateral root number (B), mean lateral root length (C) and sum of lateral root length (D) of rice (IR-28) seedling as influenced by various nitrogenous forms and availabilities. Any two means followed by a common alphabet are not significantly differed from each other by LSD at p=0.05

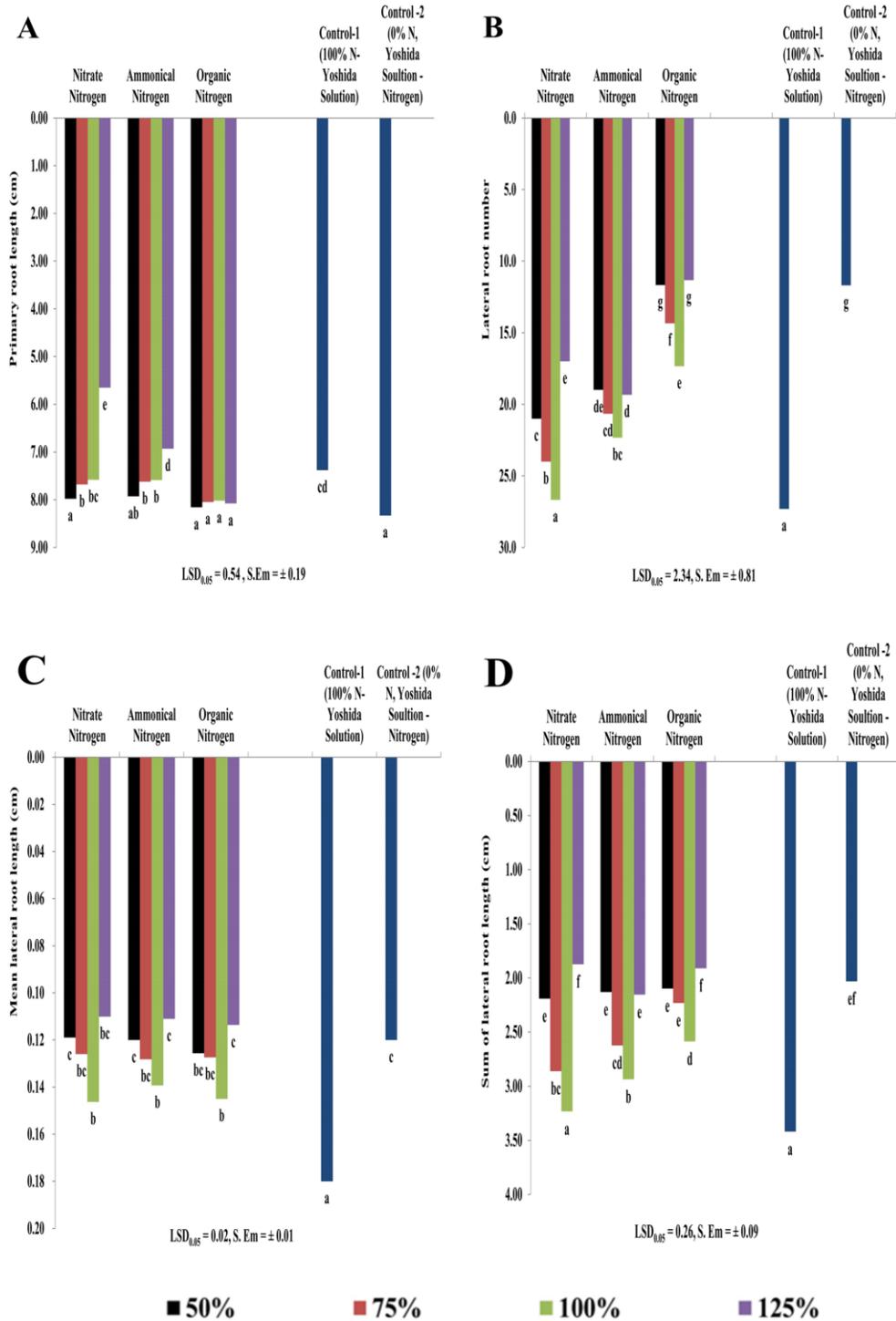


Figure.4 Lateral root density (E), total root system size (F), main root angle (G) and straightness of main root (H) of rice (IR-28) seedling at 21 days after emergence, as influenced by various nitrogenous forms and availabilities. Any two means followed by a common alphabet are not significantly differed from each other by LSD at $p=0.05$

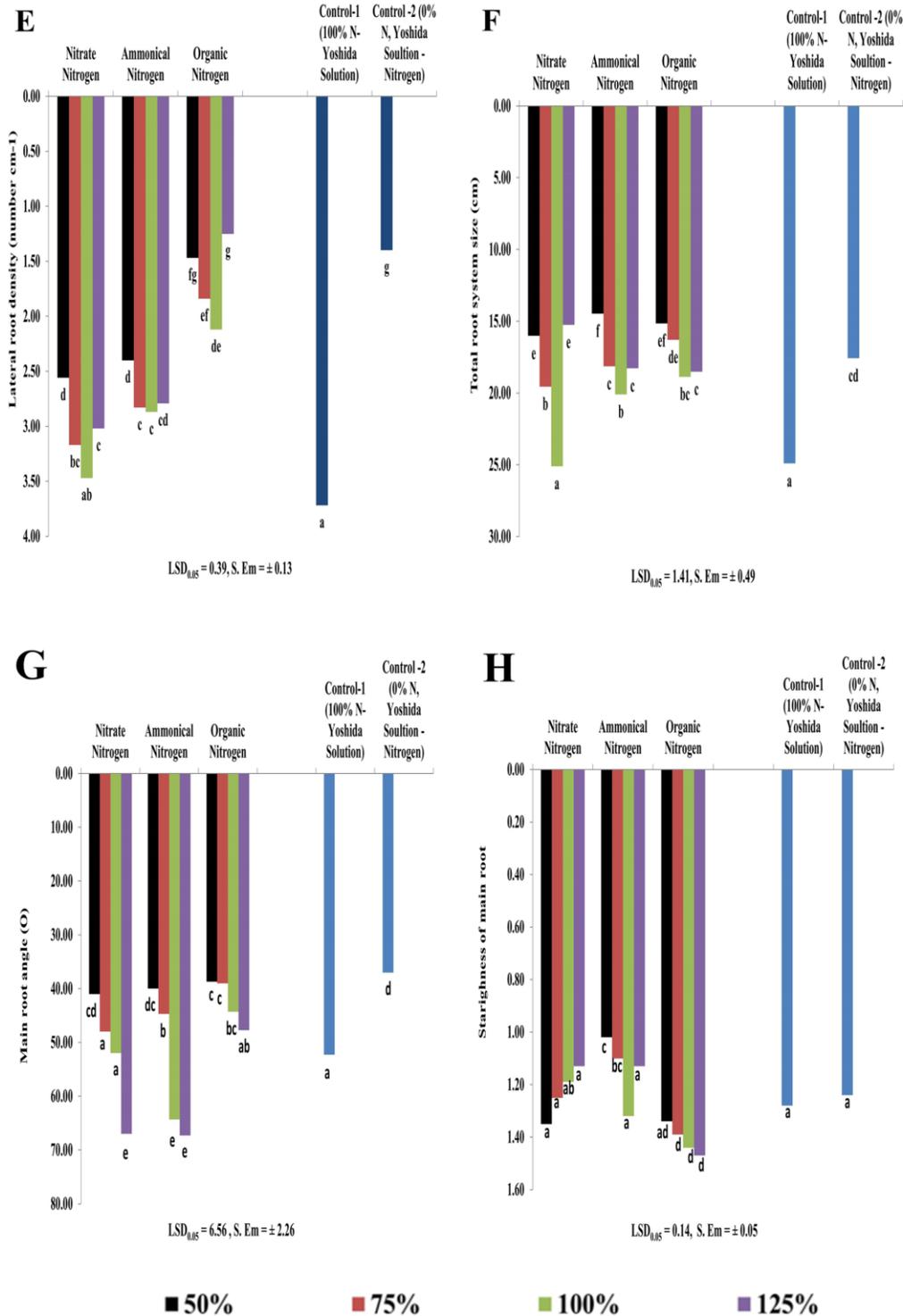
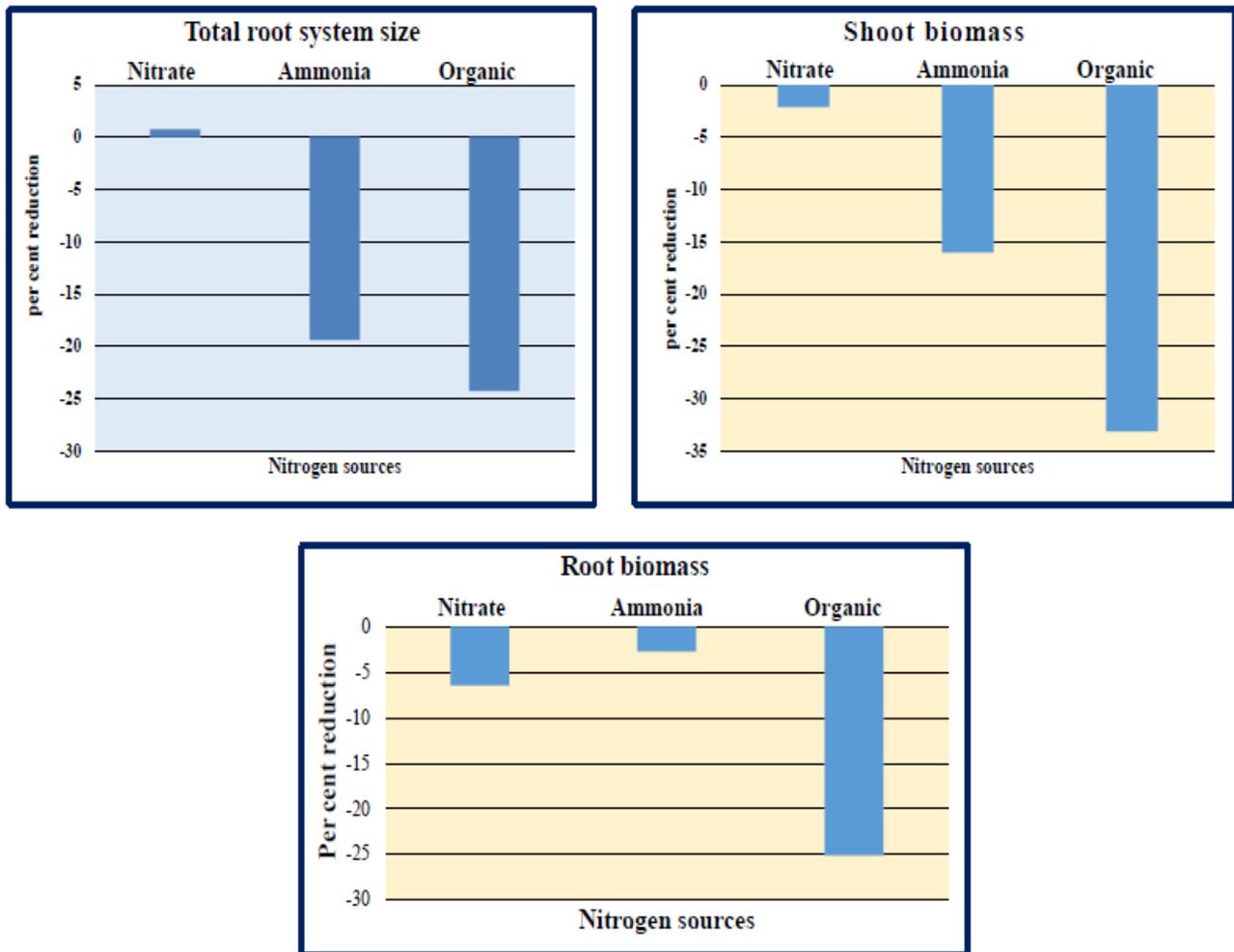


Figure.5 Percent reduction into total root system size, shoot biomass and root biomass of IR- 28 under 100% of nitrate, ammonical and organic nitrogen nutrition over control-I(100%N Yoshida solution)



High supply of N as with 125% level of nitrogen treatment, suppression of lateral root growth was also reported in many species. In *A. thaliana*, NO_3^- concentration over 10mM inhibits lateral root development by preventing meristem activation in newly emerged primordia (Nacry *et al.*, 2013). In the present study, at higher N level (125%), 32% reduction was reported in sum of lateral root length while 28% reduction recorded in lateral root number over 100% nitrogen nutrition (Fig. 3B and 3D). It may be due to arrestation of lateral root growth after emergence from the primary root (Zhang *et*

al., 1999). In the present study, IR-28 seedling shoots and root dry weight (Table 4 and 5) significantly influenced by nitrogenous form and their levels. Significantly maximum shoot and root dry weight were recorded for control-I (100% N Yoshida solution) where both NH_4^+ and NO_3^- forms were available. In sole treatment, nitrate form significantly increased shoot dry weight over ammonical and organic nitrogen nutrition while in root biomass effect of nitrate and ammonia were similar, though recorded higher root dry weight as compared to organic nitrogen.

Similar result trend was also reported for shoot and root nitrogen content (Table 6 and 7) with increasing levels of nitrogen, shoot and root dry weight along with N content increased up to 100% level. Nitrate form found to be superior over ammonical form for shoot biomass and it may be attributed to the fact that NH_4^+ assimilation demands more assimilates and excretion as ammonical ion, owing to its toxicity, require more assimilates, results in lower shoot biomass (Kronzucker *et al.*, 2001).

In maize also, nitrate form found superior over ammonical nitrogen nutrition for biomass (Bennett *et al.*, 1964). In our study, mixtures of amino acid showed nitrogen deficiency phenotype for most of the studied root traits and also reflected in shoot and root dry weight. However, shoot and root N content in organic form was significantly higher than control-II (0% N, Yoshida solution), though remain lower than ammonical and nitrate nitrogen. It indicated slower absorption of amino acids. However, Gioseffi *et al.*, (2012) reported in wheat that uptake rate of NO_3^- and NH_4^+ did not differ from each other and were generally twice as high as uptake rate of organic N. Moreover, amino acids also reported to inhibit nitrate reductase activity in corn roots and thus N assimilation process may also hindrance (Oaks *et al.*, 1977).

Root phenotypic plasticity

Root architectural plasticity is an important feature to confer adaptability across variable environments. In this study, root architectural plasticity of IR-28 seedling was evaluated under various nitrogenous forms and their level over 100 % nitrogen environment of Yoshida solution (Table-8) (Sandhu *et al.*, 2016). It aims to identify particular root trait which are highly plastic in nature and conferred adaptability of IR-28 seedling in

various nitrogen environment. Root trait phenotypic plasticity was calculated as the relative change in individual trait under different regimes of nitrogenous form as compared to control-I (100% N, Yoshida solution). Amongst root traits, all traits showed significant plasticity (at $P=0.05$ level) over control-I condition, except primary root length and straightness of main root. However, negative plasticity indicate that these traits were decreased under different level of nitrogen nutrition over control-I, which further signified synergistic effect of ammonical and nitrate nutrition over sole sources viz., ammonia, nitrate and organic N (amino acid mixture). Maximum root phenotypic plasticity was recorded for lateral root density while significant lowest root phenotypic plasticity was recorded for total root system size.

In this study, it is concluded that different forms of nitrogen viz. ammonia, nitrate and organic (mixture of amino acids), have remarkable and contrasting effects on root system architecture in rice. To the best of our knowledge, it is first of its kind report where organic nitrogen nutrition compared with inorganic nitrogenous sources on root system architecture. The results indicated that combined nitrogen nutrition through nitrate and ammonia is most suitable for root system and seedling growth of IR-28 as compared to sole source. Organic nitrogen nutrition was found least suitable, even at higher concentration where ammonia and nitrate showed inhibitory/toxic effects, root system of IR-28 in organic nitrogen treatment showed phenotype of nitrogen starvation. The results of these study support the view that we need to breed varieties suited for organic agriculture and varieties such as IR-28, which is select and breed for high nitrogen input intensive agriculture, may not be suitable for organic agriculture. However, to confirm responses of root system architectural traits to

organic nitrogen, we suggest to replicate the study with wild and/or land races of rice, which may have high uptake and assimilation efficiency for organic nitrogen.

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How to cite this article:

Jayeshkumar A. Bhabhor, Kirti Bardhan, Dhiraj P. Patel, Ajay V. Narwade and Harshad N. Chatrola. 2018. The Selective Vulnerability of Rice Root System Architecture to Organic and Inorganic Nitrogen. *Int.J.Curr.Microbiol.App.Sci*. 7(07): 1247-1265.
doi: <https://doi.org/10.20546/ijcmas.2018.707.150>