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Organic Nitrogen Effects on Root Architecture in Rice Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. Author KK wrote the manuscript, author HVP did the experiments and others help in framing and designing the experiments, providing the land races, other facilities, etc. All authors read and approved the final manuscript.

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ABSTRACT

The three land races *viz.* land races -1 (Lal Kada), land races -2 (Futiyu) and land races -3 (Kala Rata) were sown in organic media to investigate the uptake of nitrogen in the form of amino acid as substitute for inorganic nitrogenous fertilizers in their seedlings.The four concentrations (50%, 75%, 100% and 125%) of amino acid mixture (glycine, glutamic acid and aspartic acid) were applied to growing media and the roots architectural responses of 21 days of old seedlings were measured with the help of 2-D imaging software Ez-Rhizo after scanning of roots. Among four concentrations (50%, 75%, 100% and 125%) of amino acid mixture, the 100% amino acid mixture showed higher number of lateral roots, sum of lateral root length per seedling, lateral root angles, total root system size, mean lateral root length, lateral root density, nitrogen content in leaves and roots, root biomass and shoot biomass as compared to other percentage (50%, 75% and 125%) of amino acid mixtures. Moreover, land races -1 was found more responsive to amino acid mixture among the three land races.

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1. INTRODUCTION

Nitrogen (N) is a core component of many macromolecules in plant, constituents of nucleic acid, proteins, hormones, vitamins, etc. and quantitatively placed as the most important nutrient for growth and development of the plant [1].Limited and excess N availability has severe consequences for plant metabolism and growth resulting in lower yield of the crops [2], (Epstein and Bloom, 2005).In present era, there is demand of organic food for wellness of health and ecosystem because excess synthetic nitrogenous fertilizer led to environmental pollution and health hazard to mankind and animals [3]. The anaerobic rice production requires plenty of water and fertilizers due to leaching and seepage effects [4]. Application of inorganic fertilizer containing mineral nutrients such as nitrogen, phosphorus and potassium has been used profusely to increase yield of crops. Despite increasing food production, application of higher nitrogenous fertilizers in intensive agriculture also contributed to global warming [5]. Increasing consciousness of conservation of environment and mitigation of adverse effects of climate change brought a major shift in cultivation practices of major crops towards organic agriculture. Among different parts of plant, roots play important role in operating the various metabolic activity as well as anchoring and mechanical support [4]. All the metabolic activity depends on the supply of nutrient and water which are carried by the root system. They serve as the major interface between the plant and various biotic and abiotic factors in the soil environment by both sensing and responding to environmental cues [6]. The slow metabolic processes in plant under stress accelerated by changes made in root architecture and plant overcome the challenges posed by their sessile status [7]. This has been seen in the many ways where plants can dramatically alter their root architecture to optimize growth in a large variety of environmental and soil nutrient conditions [8].The modification in root architecture results in efficient uptake of nutrients and water thereby enhance stress tolerance, improve yield potential and decrease the need of heavy fertilizer application [9].Hence, Root architecture plays vital role in growth and development of plant and eventually affects yield potential of crops.

In present study the seeds of three landraces of rice were taken to study the root architecture in response to organic nitrogen nutrition in the form of amino acid mixtures. The root architecture system (RAS) studied with EZ-RHIZO software for the fast and accurate measurements. It was designed to detect and quantify multiple two dimensional root architecture system traits from seedlings, growing on a solid support agar medium [10]. Thus, experiments conducted for investigation of the effect of different concentration of organic nitrogen in the form of amino acid mixtures (glycine, L- glutamic acid, L-aspartic acid) on root system architecture of seedlings of land races of rice to support production of better variety through molecular breeding program.

2. MATERIALS AND METHODS

The seeds of three land races of rice *viz.* land races -1 (LR-1 Lal Kada), land races -2 (LR-2 Futiyu) and land races -3 (LR-3 Kala Rata) were put in square petri dish of size (125 x 125 x 20 mm) and having growth area of 139 cm^2 for germination. The experiment was carried out at Plant tissue culture laboratory, N. M. College of Agriculture, Navsari Agricultural University, Navsari (India). The temperature and relative humidity during the experiments recorded 20 ± 1^0C and 75±1%, respectively. Various root traits *viz*. primary root length, lateral root number, sum of lateral root length, lateral root angle, mean lateral root length, Nitrogen content in root and leaves, lateral root density, straightness of main root, total root system size, root and shoot biomass were measured to distinguish the effect of amino acids *viz.* Glycine, L- Aspartic acid, L-Glutamic acid on root architecture of rice seedlings.Yoshida's solution [11] 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 in the form of amino acids mixtures (Glycine, L-Glutamic acid and L-Aspartic acid) on the basis of molar mass and the pH was maintained at 5.7 after mixing.The four concentration (50%, 75%, 100%, 125%) of amino acid mixture with control (1)- 100% inorganic N in Yoshida solution and Control (2)- 0 % N in Yoshida solution excluding nitrogen source prepared and applied as source of nitrogen to the seeds of three landraces after disinfecting and soaking in water overnight. The roots images were scanned using HP Scanjet G2410 on 21 days after germination and processed into the EZ -Rhizo software.

2.1 Statistical Analysis

The data obtained from software was converted to mean value for each parameter with three repetitions under complete randomized design with factorial concept and statistically analysed as described by Pans and Sukahtme [12] for control *vs* rest design of factorial concept of the experiment.

3. RESULTS AND DISCUSSION

The primary root length and straightness of main root were found to increase due to lack of organic/inorganic nitrogen. They were significantly affected by different levels of amino acid mixture. These two traits found lower in control-I (100 % N) while they were recorded higher in control-II (0 % N) as compared to other treatments. Primary root length was found greater in 50% and 75% as compared to other percentage (100% and 125%) while straightness of primary root found comparatively higher in 125% of amino acid mixture (Fig. 1A and 1B). Deficiency in nutrient results in a shift in dry matter in favor of root length and straightness and it was found similarity with Wang et al. [13] where nutrient unavailability induce the length of roots for searching of minerals to the depth of soil hence higher root growth was observed in control-II (Fig. 1A). It has been reported in *Arabidopsis* that primary root growth highly

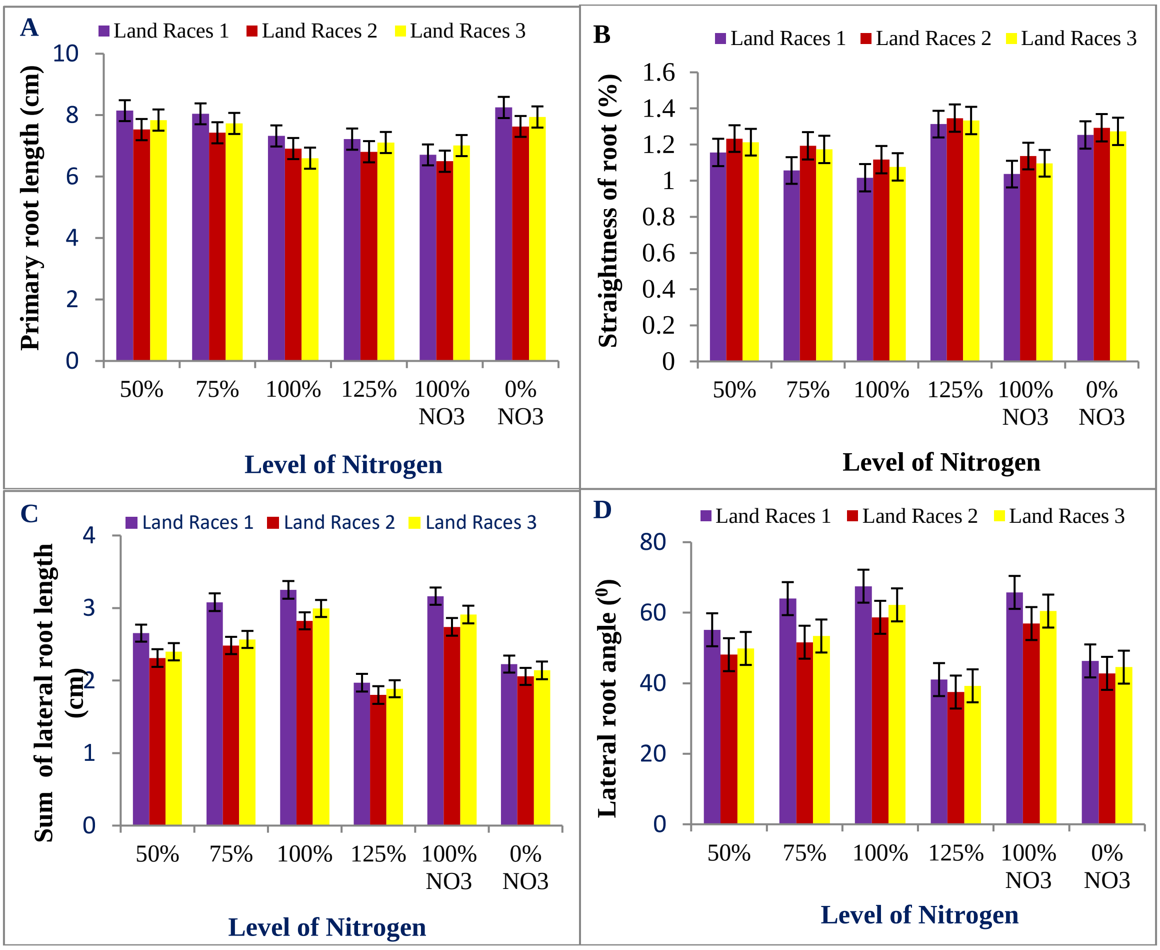

Fig. 1. (A) Primary roots length as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10) (B) Straightness of root as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10) (C) Sum of lateral root length as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10) (D) Lateral roots angle as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10)

influenced by nitrogen deficiency and increased in its length as compare to control [14,15]. Among land races primary root length in 100% treated seeds was found maximum in LR-1 and minimum root length was observed in LR-3 (Fig. 1A). Root length under various levels also supported the above discussed fact that lower dose of nitrogen increased the root length and higher dose (125%) decreased the root length (Fig.1A).These findings found conformity with Xin et al. [16] who reported that higher dose of Nitrogen retard root growth and lower dose enhanced the root growth in rice. It has been reported that at early stage of maize growth N deficiency stimulated root length and it was peaked before the tasseling [17]. Few crops showed parabolic relationship with supply of Nitrogen and root length and it has been reported in maize and spring wheat [18].

In several crop species, genetic variation in lateral 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 [19,20,21,22]. In common bean, wheat, sorghum and rice, steep growth angles enhances subsoil foraging and water acquisition under terminal drought [23,24,25,26]. In the present study, lateral root angle tend to steeper in nitrogen deficit treatments and maximum angle was recorded for control-I while significantly narrow angle was recorded in control-II (Fig. 1D). Trachasel et al. [27] reported that low N decreased brace and crown root angle under low

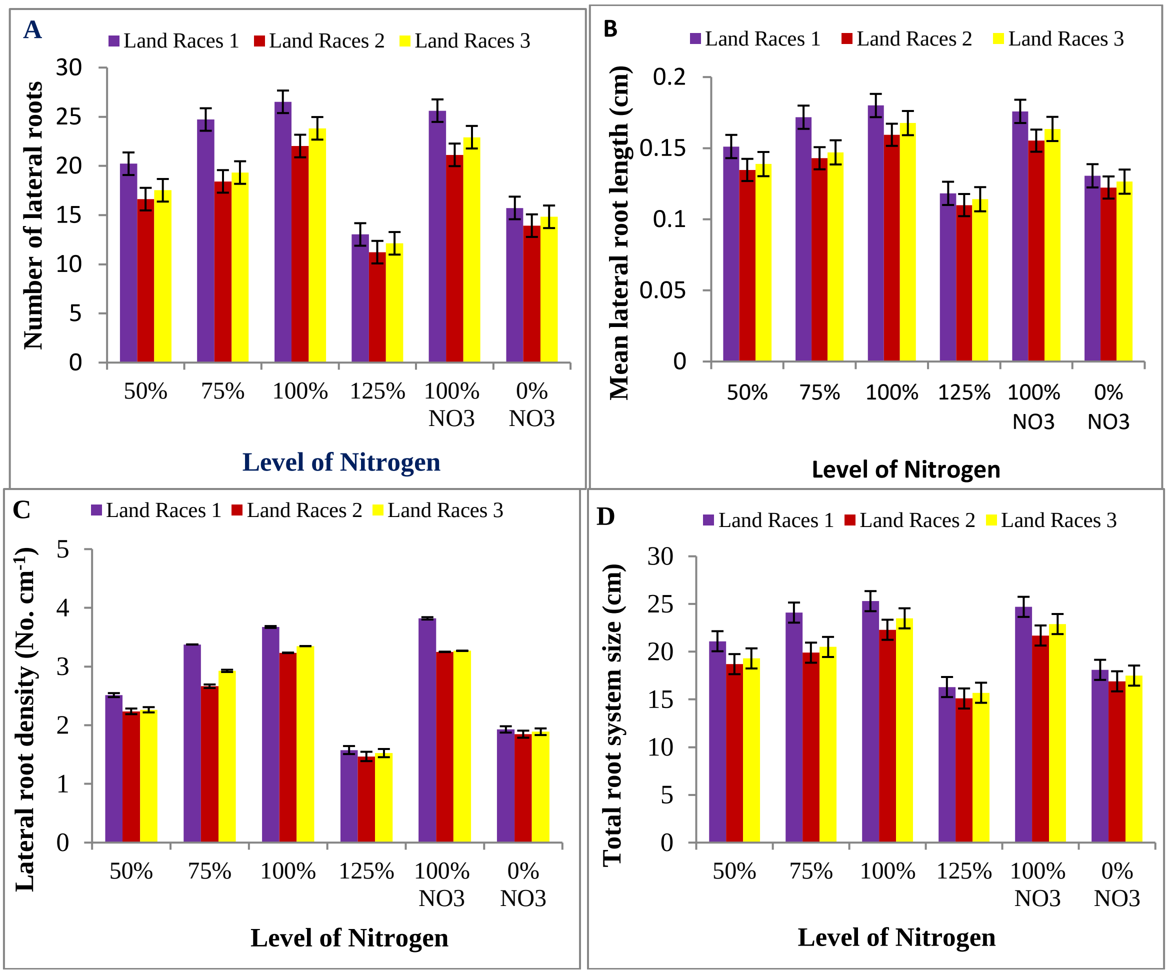

Fig. 2. (A) Number of lateral roots as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10). (B) Mean lateral root length as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10). (C) Lateral roots density as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10) (D) Total root system size as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10)

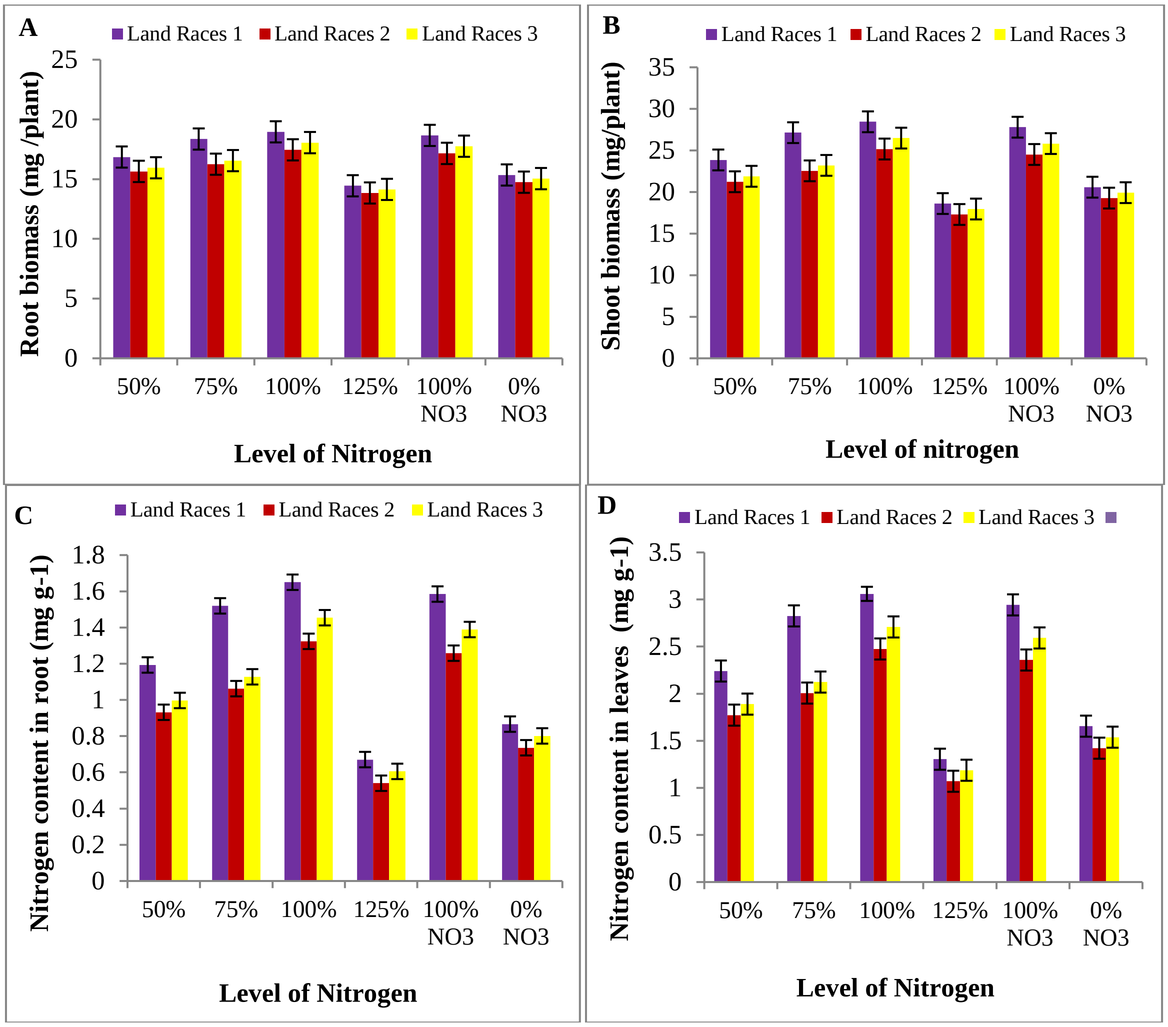

Fig. 3. (A) Root biomass as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10). (B) Shoot biomass as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10). (C) Nitrogen content in root as affected by different concentration of organic nitrogen and control ± Bars indicate SD (n = 10). (D) Nitrogen content in leaves as affected by different concentration of organic nitrogen and control. ± Bars indicate SD (n = 10)

N condition in maize. In the present study, low availability of nitrogen root angle decreased more (Fig. 1D) and increased straightness of roots (Fig. 1B). Among land races the 100% treated seeds, LR-1 was higher lateral root angle and lower straightness of main root as compared to other land races (LR-1 and LR-2) and other organic nitrogen concentrations (50% and 75%) treatments (Fig. 1D).

Lateral root of rice significantly varied with the level of organic nitrogen and its architecture viz. number of lateral root (Fig. 2A), mean lateral root length (Fig. 2B), sum of lateral root length (Fig. 1C) and lateral root density (Fig. 2C) were recorded higher in seedlings treated with 100% organic nitrogen followed by control-I. This resulted in increase of total root system size in 100% organic nitrogen treated seeds followed by control-I (Fig. 2D). The lowest number recorded with 125% organic nitrogen followed by control-II. Nitrogen deficiency shown to inhibit lateral root emergence as suggested by Krouk et al. [28] that severe nitrogen limitation caused less auxin accumulation in lateral root primordia and thus lateral root emergence is hampered. Gruber et al. [29] suggested that in severe deficiency of nitrogen formation of lateral roots decreased. Moreover, lateral root length and sum of lateral root length (Fig. 1C) was decreased at higher dose of organic nitrogen nutrition. It might be due to auxin signaling because higher nitrogen dose might affect auxin concentration and inhibit the formation of lateral root. Auxin plays a dominant role in the formation of lateral roots which is regulated by concentration of nitrogen. Hence, nitrogen and auxin signaling linked together and interaction between them with concentration dependent manner, exert positive and negative effects on the formation of lateral roots. The strigolactone an another phytohormone stimulate nitrate induced inhibition of PIN2 transcription gene in rice [30]. Thus, auxin transport obstructed in presence of excess nitrogen and formation of lateral roots suppressed. It was reported in maize that higher external amino acid concentration inhibit the elongation of lateral root it might be due to reduction of auxin translocation from shoot to root in the phloem [31]. Lateral roots density of LR-1 seedlings was found more as compared to LR-2 and LR-3 (Fig. 2C). However, at higher concentration of organic nitrogen supply, in all land races, lateral root density was decreased. Our results found similarity with Zhu et al. [7] where higher lateral root density, lateral root number and sum of lateral root length were reported.

High supply of N as with 125% level of nitrogen treatment, suppression of lateral root growth was also reported in many species. It may be due to arrestation of lateral root growth after emergence from the primary root [32]. Lateral root growth regulated by nitrate with specific transporter on plasma membrane mutating this gene retard the formation of lateral root [33]. It has been also reported in Cotton by Zhu et al. [7] that deficiency of nitrogen adversely affects the lateral root architecture result in suppression of growth and development of seedlings. The different research laboratory reported that lateral root density decreased under deficiency of nitrogen and showed parabolic relationship with concentration of nitrogen in cotton and sugar beet [34,35,36].

Higher root and shoot biomass were recorded in seeds treated with 100% amino acid mixture as compared to other treatments (Fig. 3 A, B). Similar result was also reported in root and leaves nitrogen content where100% amino acid mixture showed higher N content along with increased root and shoot biomass followed by control-1 and other treatments. LR-1 found to be superior over other two land races for biomass as well as nitrogen content in root and leaves (Fig. 3C, D).Similar result indicated in cotton where higher dose of Nitrogen increased the root and shoot biomass [34]. Total root and shoot biomass decreased under deficiency of nitrogen as reported by Schneider et al. [37] in maize.

4. CONCLUSION

Root architecture of plant greatly affects growth and development of the plant. Organic fertilizer production and utilization in agriculture tends to move the production of food grain towards health benefit and sound ecosystem. Providing amino acid mixtures for production of seedlings for transplanting rice would be a beneficial approach towards organic agriculture.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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