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Research Article

Nitrogen stress induced changes in root system architecture (RSA) in diverse wheat (T. *aestivum* L.) genotypes at seedling stage

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Abstract

Improvement of Nitrogen use efficiency (NUE) in field crops is very important for reducing the cost of production, sustainable agriculture, reduce energy consumption for the production of chemical fertilizer and mitigates the environment pollution. It is more so in case of major cereals like wheat, where the NUE is 40%. N Uptake, assimilation, utilization and remobilization are the components of NUE. N Utilization by shoot primarily depends on available resources which in turn determine by N uptake by root system. Amount of N forage by different genotype under different N regime is primarily determined by Root system architecture (RSA) and transporters. Here in this study, we are reporting a procedure of comparing N uptake in relation to root development and biomass among nine diverse genotypes of wheat seedling under N-stress and N-optimum condition. Biomass analysis showed significant increase in root dry weight due to N-stress in genotype HD-2967. In general shoots biomass reduced under N-stress condition. VL-401 exhibited significantly higher N-uptake under N-stress condition, where as it was least in case of GW-322 followed by Kalyansona. Fifteen RSA parameters were analyzed for all the nine genotypes, under both N-stress as well as N-optimum condition. All the RSA parameters except number of Forks and 2nd Order LR number showed higher values in N-stress as compare to N-optimum condition. However, six parameters namely TRS, PA, SA, Root volume, Main total length, and first order length increased significantly. Present study also identified two most contrasting genotypes VL-401 and WH-147 based on RSA.

Keywords: Wheat, nitrogen use efficiency, root system architecture, uptake and lateral roots.

1. Introduction

Plants take Nitrogen (N) from soil. Plant growth is most critically limited by N due to its low availability in soil. Some essential building blocks of life like nucleotide, cofactors, signaling molecules and protein contain nitrogen as their primary constituent. Therefore, quantitatively N is the most important nutrient and limiting factor for growth and development of plants (Kraiser *et al*., 2009). Crop yield gets seriously affected by inadequate Nitrogen, but excess of it not significantly contributes to yield but cause N pollution by means of leaching, surface runoff, denitrification, and emission of greenhouse gases (Amiour *et al*., 2012). Intake of nitrate-contaminated water causes health hazard (Almasri *et al*., 2007). Increased cost of food production and the eutrophication of many natural aquatic and terrestrial ecosystems (Anjana *et al*., 2007) is also the results of low recovery rate and high loss of fertilizer N. Rational application of N to avoid excessive fertilization together with the use of cultivars which efficiently use N sources have been proposed as a prime factor for improvement of NUE (Noulas *et al*., 2002). These desirable cultivars with greater NUE are thought to produce higher yields even at low N supply and have been called as efficient germplasms (Haefele *et al.*, 2008). Efficient farming techniques (Application of N fertilizer in several splits, use of coated chemical form of N fertilizer) when combined with NUE varieties (Varieties which absorb N from soil and metabolize them better) will helpful in fulfillment of long term objective for sustainable agriculture. (Hirel *et al.*, 2011). The NUE reported in case of cereals including wheat is only about 40% which means 60% of the applied fertilizer is lost to the environment, causing pollution in several means. (Raghuram *et al.*, 2007). Therefore, growing wheat cultivar with improved NUE will ultimately results in reduction of excessive fertilizer input and maintaining acceptable yield. Emphasis on growing these types of cultivar will be global requirement (Foulkes *et al.*, 2009). NUE is a function of multiple interacting genetic and environmental factors and an inherently complex character. Definition of NUE is total grain yield or biomass produced per unit of applied N fertilizer (Xu *et al.*, 2012). Different components of NUE are N uptake by root, assimilation, utilization or remobilization by shoot. NUE can be expressed as a ratio of output (total grain yield biomass, grain N, plant N) and input N in the form of fertilizers (Pathak *et al.*, 2008). There have been several reports suggesting genetic variability in NUE pertaining to genetic differences in N uptake and utilization efficiency in different crops including wheat (Namai *et al.*, 2009). Uptake of N from soil mainly depends upon the root system of the plant. Higher plants uptake N in the form of nitrate from root cell. This is the key and first step of N assimilation pathway from where most of the N for synthesis of different biomolecules is fulfilled. (Beevers *et al.*, 1980). For nitrate uptake, RSA and nitrate transporter plays extremely important role. The geometry of plant root system affects crop performance by affecting nutrient and water uptake efficiency, drought tolerance, tolerance to mineral toxicity and lodging tolerance. Therefore it is an important characteristic (Manske *et al.*, 2002). RSA varies between species, and also within species, subject to genotype and environment (Lynch *et. al.*, 1995). Development and architecture of roots hold potential for the exploitation and manipulation of yield and optimize agricultural land use (Den Herder *et al.*, 2010). RSA on a major scale include mainly the primary roots, lateral roots and accessory roots, which are the key determinant of nutrientand water use efficiency in plants. In a minor scale, RSA also includes root hairs that increase the surface area, alleviate uptake of water and nutrients (Bates *et al.*, 2000). The controlled environmental condition could be used to know the inherent mechanism and regulation for imparting efficiency in both terms of N uptake and utilization. In the current study, eight highly N-responsive genotypes were selected based on the field observation along with one popular genotype HD-2967. These nine genotypes were studied at their seedling stage under NO_3^- -optimum and NO_3^--stress conditions after growing them in nutrient-free media (a mixture of vermiculite and perlite) with external application of nutrient. Morphological characteristics like RSA, fresh and dry biomass of root and shoot, residual nitrogen content under both NO_3^--optimum as well as NO_3^--stress conditions were analyzed to decipher the N-responsiveness of the diverse wheat genotypes at seedling stage.

2. Materials and methods

2.1. Selection of genotypes

Based on field evaluation at ICAR-IIWBR, Karnal, eight wheat genotypes having diverse features for NUE along with one of the popular wheat genotypes HD-2967 have been selected for the study (Table 1).

Table 1. Wheat genotypes and their features used in the experiment

Genotypes	Features related to Nitrogen Use Efficiency (NUE)
WH-542, GW-322	High Nitrogen responsive genotypes
HS-277, WH-147	High Nitrogen use efficient
Sujata, VL-401	Poor Nitrogen use efficient
Kharchia	Least Nitrogen uptake and utilization ability
Kalyansona and HD-2967	Popular varieties during the 1980s and 2010s respectively

2.2. Growing condition for seedling

Briefly, the healthy seeds of all the selected genotypes were first rinsed with 70% ethanol for 3 min. and then surface sterilized using 0.5% Sodium hypochlorite for 3 min. After several washes with ddH_2O, the seeds were kept for germination in an incubator at 25±1°C in the dark. Three days old uniformly germinated seeds having the primary root length of approximately 1 cm were carefully transplanted in 4-inch pots containing 2:1 mixture of vermiculite: perlite after moisturizing with ddH_2O. The culture room growth condition was as mentioned by Sinha *et al.*, (2015). Murashige and Skoog medium (MS) (minus N) was used as nutrient media in which 8.00mM and 0.4mM nitrogen was added from $Ca(NO_3)_2.4H_2O$ and $NH_4^+NO_3^-$ respectively for N controlled condition while, for N-stress condition 0.08mM and 0.004mM nitrogen was added from $Ca(NO_3)_2.4H_2O$ and NH_4+NO_3^- respectively. The freshly prepared nutrient solution was applied as per the requirement during its growth phases for the entire 15 days period at three days interval.

2.3. Study of root system architecture

Roots of 15 days old seedling were scanned using EPSON PERFECTION V700 PHOTO scanner and images were analyzed by WinRhizo™ software 2013 (Regent Instrument Canada Inc. 2013) and fifteen different root parameter (Table 2) were studied as described by (Sinha *et. al.*, 2015). Three independent seedlings were sampled from each pot for scanning and each seedling was scanned three times.

Table 2. RSA parameter studied under present study with unit and definition

SN	Parameters	Unit	Definition
1	Total Root Size (TRS)	cm	Sum of the path length of primary root (PR) and lateral roots (LRs)
2	Projected Area (PA)	cm^2	2D measurement of thearea occupied by roots (Number of pixels multiplied by pixel area (pixel width* pixel height))
3	Surface Area (SA)	cm^2	Real surface of root that is in contact with the soil
4	Average Diameter	mm	Projected area divided by TRS
5	Root Volume	cm^3	π × (Half of the avg. diameter/2) 2 × TRS
6	No. of Tips	-	Total number of tips (where a link ends without any other link connection
7	No. of Forks	-	Total number of forks (where a link begins or ends on another link
8	Root Branch Angle	^{o}	Avg. branching angle of all links
9	MTL or MRS or MRP	cm	Path length of primary root (PRP)
10	First Order LR No.	-	Number of first order LRs (emerging from primary and seminal roots)
11	First Order Length	cm	Path length of First orderLRs
12	Second Order LR No.	-	Number of second order LRs (emerging from first-order LRs)
13	Second Order Length	cm	Path length of second order LRs
14	LRS	%	LR size: sum of path length of LRs as fraction of TRS
15	LR Density	cm^{-1}	LR density: first-order LR no. divided by PRP

2.4. Study of biomass

To know the effect of nitrogen on plant biomass, plants were divided into shoots and roots. Fresh and dry weight was measured by constant weight method after placing shoot and root samples in hot air oven at 75±2°C for approximately 16 hours.

2.5. Estimation of residual nitrogen

For estimation of residual nitrogen in pots, 5g of homogeneously mixed potting mixture was collectedf rom each pot after the experiment was over. The potting mixture (5g) was dissolved in 25ml 2M KCl solution for overnight (Janssen *et al.*, 2003). Once the potting mixtures were settled down, the supernatant was filtered through Whatman®1 filter paper and was used for measuring the nitrogen on auto analyzer (continuous flow auto analyzer, FOSS-FIA Star™5000).

2.6. Statistical analysis of data:

The standard statistical analysis was carried out to calculate the standard error of means (SEM) for each mean value and presented as error bar in each histogram. Subsequently, Least Significant difference at 5% was calculated for the treatment x genotype combination.

3. Results and Discussion

Based on field evaluation at ICAR-IIWBR, Karnal, eight genotypes along with one popular genotype HD-2967 (Table1) were used in the present study. In order to understand the relation of nitrogen uptake with plant biomass and RSA, we have grown these selected nine wheat genotypes under the laboratory condition to note the response of genotypes under nitrogen stress.

3.1. Effect of nitrogen stress on plant biomass

Root and shoot biomass was measured in all the nine genotypes under both N-optimal and N-stress condition. Root Fresh Weight (RFW) were mostly reduced, except in case of HD-2967, GW-322 and Kharchia under N-stress condition (Fig. 1a). Under N-stress most of the genotypes showed an increase in Root Dry Weight (RDW). However, it was only significantly increased in case HD-2967 (Fig.1b). Both Shoot Fresh Wight (SFW) and Shoot Dry Wight (SDW) were reduced under N-stress condition in all the genotypes under study, of which HS-277, GW-

322, WH-147 and Kharchia were significant for SFW and HS-277, WH-147, and Kharchia were significant for SDW(Fig. 1c and Fig. 1d). Also, the SDW under N-stress condition was at a higher level in case of HD-2967 and Kharchia (Fig. 1d).

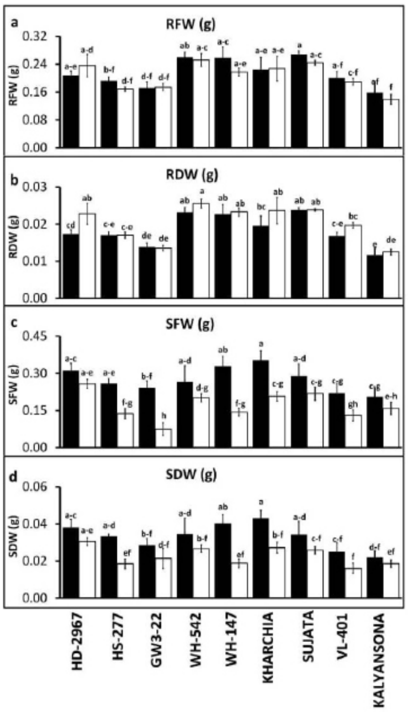

Fig. 1. Comparison of biomass as (a) RFW, (b) RDW, (c) SFW and (d) SDW under both N optimum (black bar) and N stress condition (white bar).

Considering wheat crop as a whole and combining the data of all the nine genotypes, root and shoot biomass was studied. It was observed that there is an increase in RDW, and the change was significant under N-stress. In general SFW and SDW decrease significantly under N-stress condition (Fig. 2).

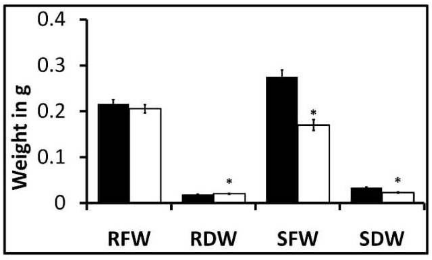

Fig. 2. Changes in root and shoot biomass of wheat seedlings under both N-optimum (black bar) and N-stress (white bar).

3.2. Residual nitrogen in pots: Estimation of residual N provides an idea about the N uptake by the different genotypes in the experimental condition (Fig. 3). Under the optimum condition maximum residual N was observed in HD-2967 followed by WH-542 and Kalyansona and least in case of VL-401. Genotype GW-322 showed maximum residual N followed by Kalyansona and least in case of VL-401 under N-stress condition.

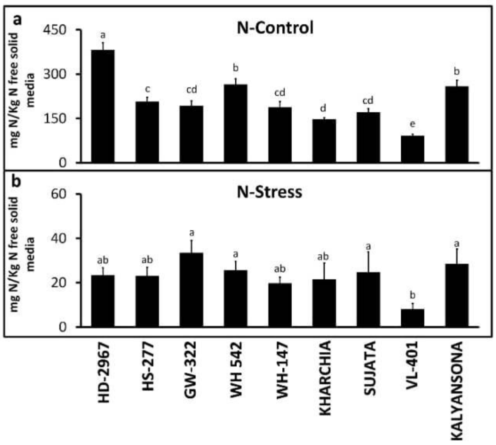

Fig.3. Comparison of residual nitrogen for diverse wheat genotypes, (a) N-control, and (b) N-stress condition.

3.3. RSA: In response to Nitrogen

RSA is a trait that represents considerable plasticity because of its sensitivity to environmental stimuli. RSA varies according to nutrients availability. For measuring the RSA in diverse wheat genotypes, fifteen parameters were recorded and studied (Table 2) under N-optimum and N-stressed condition. The scanned pictures of RSA for all the genotypes under study with 3 replications are presented in (Fig. 4) for visual comparison.

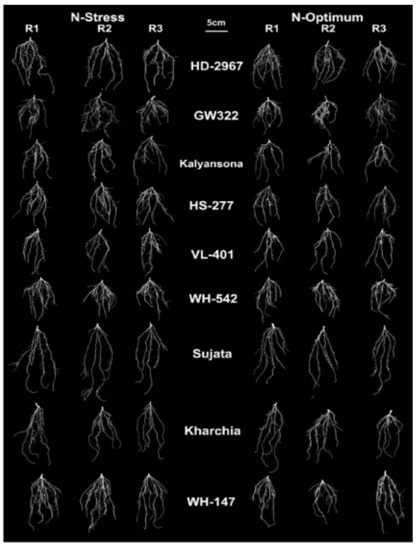

Fig. 4. Visual comparison of RSA for all the genotypes under study in both N-optimum and N–stress condition.

Total Root Size (TRS) has been increased, but not significantly, in all the genotypes except HD-2967 and Kharchia under stress condition. More TRS was observed in WH-147, HD-2967 and similar TRS was observed in GW-322 and WH-542 in both conditions. Intermediate TRS observed in Sujata and HS-277, while VL-401 and Kalyansona show low TRS. HD-2967 shown maximum TRS in N optimum condition (Fig. 5a). Projected area (PA) increased under N-deprived condition in all the genotypes under study except HD-2967, and Kharchia. When the Genotypes × Treatment interaction was analyzed, there was wide genotypic variation observed. Most of the genotypes did not show significant change. As per the analysis, only in case of WH-147, Kharchia and GW-322 significant change was observed. The highest value was observed in WH-147 under N-stress and the lowest value was in case of VL-401 (Fig. 5b). Surface area (SA)was maximum in WH-147 under N-stress condition with a significant difference from any genotypes under any of the treatments. Least surface area was found VL-401 under both N-conditions (Fig.5c). Average diameter showed decreasing trends except in a few genotypes, but there was no significant difference observed in any of genotypes under N-stress. Maximum average diameter was found in Sujata and least was recorded in GW-322 under both N- conditions. The genotypic difference was found obvious (Fig. 5d). Root volume also increased in all genotypes under N-stress condition compared to N-optimum except in Kharchia. Root volume was found higher in case of WH-147 (significant), Sujata, HD-2967 and Kharchia under both the conditions and lower level of root volume were reported in Kalyansona, HS-277, VL-401, and GW-322 (Fig. 5e). Number of tips showed non-significant genotypic variation. More number of tips were observed in WH-147, WH-542 and GW-322 and least number of tips observed in VL-401 and they were significantly different from each other irrespective of the treatments (Fig. 5f). Number of forksalso did not show significant genotypic variation. The fork number between N-optimum and N-stress condition was non-significant in all the genotypes except HD-2967 (Fig. 5g). Root branch angle did not show much variation among genotypes; neither they differed significantly under N-stress (Fig.5h). Number of forks and tips are closely related to each other as the number of forks automatically increases the number of tips. Main Total Length (MTL)/Main Root Size (MRS)/Main Root Path length (MRP) was showing genotypic variation and also increased in all genotypes under N-stress condition but significant difference was observed in VL-401 only (Fig. 5i). 1st Order LR emerges from the main root. First order LR number did not vary significantly under N-stress except VL-401 and WH-542. Genotypic variation was observed and most genotypes showed more LR number in N-stress than that of the N-optimum condition. Maximum first order LR number was observed in WH-542 in both conditions with a significant difference. Significantly lower number of LR was observed in case of VL-401 (Fig. 5j). 1st Order LR length is the length of lateral roots those emerging from main root. Only HS-277 showed a significant increase in 1st order LR length, though all genotypes showed an increase of this parameter under N-stress except HD-2967. Maximum first order length observed in HD-2967 and lowest in VL-401, which were significantly different from each other (Fig. 5k). Second order lateral root (LR) number and length showed significant genotypic variation under both N-optimum and N-stress conditions but in any of the individual genotype, the effect of N-stress was not significant (Fig. 5l and Fig. 5m). Lateral root size (LRS) was calculated as the sum of the path length of lateral roots as a fraction of TRS. Lateral root size has been increased insignificantly as a very small proportion in all genotypes under N stress condition except HD-2967, Kharchia and VL-401. However genotypic variation was significant under both the conditions (Fig.5n). LR density calculated as first order lateral root number divided by main root path length. All genotypes showed a significant change in LR density except HD-2967 and WH-147. More LR density observed in VL-401 under N optimum condition than under N-stress while more LR density observed in Sujata under N-stress condition. (Fig.6).

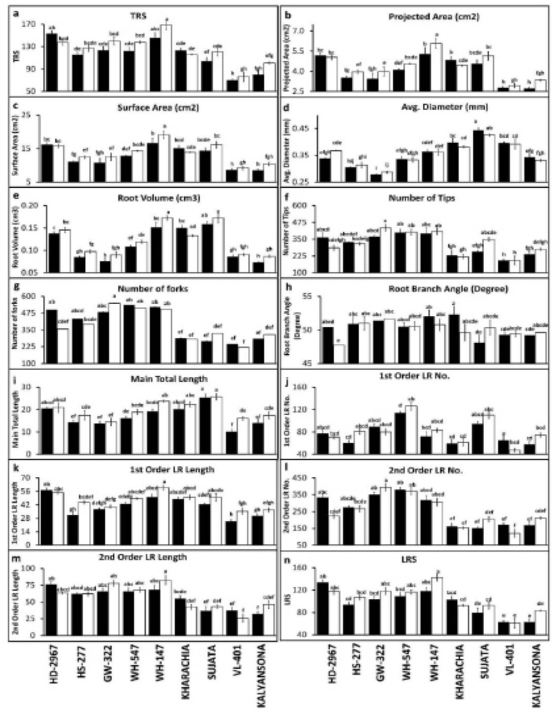

Fig. 5: RSA parameters in diverse wheat genotypes (a) TRS, (b) PA, (c) SA, (d) Avg. diameter, (e) Root volume, (f) No. of tips (g) No. of forks, (h) Root branch angle, (i) MTL, (j) 1st order LR no., (k) 1st order length, (l) 2nd order LR no.,(m) 2nd order LR length, (n) LRS, here N- optimum (black bar) and N-stress condition (white bar).

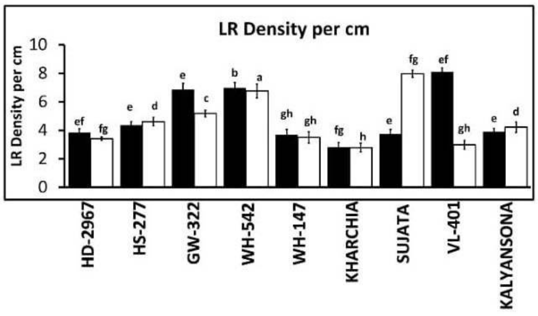

Fig. 6. Lateral root density in diverse wheat genotypes under both N- optimum (black bar) and N-stress (white bar).

3.4. RSA of wheat seedlings under N-stress: As in the case of root and shoot biomass, considering wheat crop as a whole and combining the data of all the nine genotypes, the RSA parameters were analyzed under N-stress Vs. N-optimum condition. It was observed that all the parameters increased under N-stress in comparison to N-optimum condition except Forks and second order LR number. However, only six parameters namely TRS, Projected area, surface area, root volume, main total length, and 1st order length increased significantly (Fig. 7).

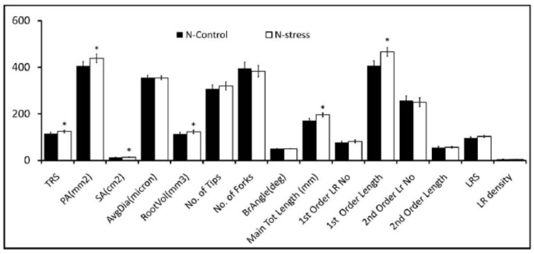

Fig. 7. Different parameters of Root System Architecture (RSA) of wheat seedlings under N-optimum (black bar) and N-stress (white bar).

The present study was completely dedicated in the root system, starting from its biomass to RSA both under N-optimum as well as N-stress condition. A diverse and contrasting set of genotypes based on filed evaluation was considered for the study. The study also showed lights on the root biology of wheat as a whole under N-stress, combining the information of all the diverse genotypes. However, most of the cases, the response of N-stress of 15 days old seedlings was not possible to corroborate with their filed evaluation grouping at maturity. This was mainly because, the seeds (endosperm) were not removed after germination and the seed reserve were sufficient enough for the plants to survived till 7-10 days (depending upon the varieties) without experiencing the N-stress due to the nutrient reserve. Hence, it was just a week's stress at the initial period of the wheat plants and it was attempted to record and observe the changes as well as genotypic differences in root penology through RSA at this initial stage of their growth. As the root system is mainly responsible for transport of nutrient from growing media to the plant, N-uptake for wheat would invariably depend on the RSA.

Root and shoot biomass: The general aspect which emerged about the wheat seedling was the reduction in shoot biomass (both fresh and dry weight), this is mainly because of more resource allocation towards root and reduction in shoot growth due to lack of supply of nitrogen. However, the genotype specific differences, which were observed here, were earlier explained by Kamiji *et al.* (2014). They studied to determine whether the accumulation of shoot biomass is the driver of greater N uptake in different wheat genotypes with higher vigorous growth, or whether greater N uptake leads to the greater growth. Under low N supply, differences in shoot biomass appeared to be the driver for the differences in the N-uptake rather than the differences in N-uptake generating differences in biomass. Kamiji *et al.*, (2014) also reported a poor correlation between shoot N uptake and shoot biomass was found under high N supply. This has implications for selection of genotypes for N-uptake efficiency. In the present study, HD-2967 was looking prominently different from other genotypes with respect to root biomass, especially root dry weight increased significantly under N-stress condition.

By measuring the left over nitrate (residual nitrogen) after the experimental period was over, it could be inferred that VL-401 genotype had the maximum uptake capacity under both N-optimum as well as N-stress condition. Lowest N-foraging capacity was in WH-542 and Kalyansona under both the conditions, though the least uptake under N-optimum condition was in case of HD-2967. Interestingly, all the three genotypes were high yielding nitrogen responsive (high input responsive) genotypes. This suggests the low uptake characteristics of these genotype at seedling stage itself and the rebyindicating the higher N-input requirement for their higher yield. Biomass in relation with the uptake of nutrient has been explained very critically in a review by Poorter *et al.*, (2012), where the biomass allocation patterns to leaves, stems and roots in vegetative part were reported. Garnier (1991) stated mentioned that growth can be analysed from a nutrient perspective and relative growth rate (RGR) is then given by the product of the net uptake rate of nitrogen (N) per unit root mass (NIR, nitrogen intake rate), the concentration of N in the plant (PNC) and the fraction of biomass invested in roots (RMF, root mass fraction): $RGR = NIR \times 1/PNC \times RMF$. Though the results of the present study could highlight the relative N-uptake and biomass in the seedling stage, it would be clearer when if the N-content in different parts are measured as an extension of this study in future.

Understanding the development and architecture of roots holds potential for the manipulation of root characteristics to both increase food plant yield and optimize agricultural land use. Smith *et al.*, (2012) reviewed that the majority of research conducted in cereals focused on the species *O. sativa* and *Z. mays* while wheat is relatively unexplored. In that context, the present work is very important for wheat as a cereal crop with special emphasis under N-stress.

Root got spreads in a larger area under N-stress condition that is why projected area increased in all genotypes under stress condition except in HD-2967 and Kharchia. WH-147 showing a significant difference in the projected area which is high N use efficient genotypes. The increase is area occurs when the total length of main and/ or lateral root increases and branch angle of lateral root changes to more horizontal. In the present study also the reason might be the same. Due to this, root got spreads in a larger area under N- stress condition. Exception was in HD-2967 and Kharchia. Clearly, both the cases root length was the main factor which strongly correlated with the projected area (Fig.5b), and this in turn reflected in other parameters for these two genotypes such as surface area and root volume (Fig.5c and fig.5e). Highest value of root volume and diameter was strongly correlated in the case of Sujata. But the increase of both the parameters under stress seems to be insignificant. Among the length and diameter, length was having a correlation with the projected area, volume and surface area. Only a few reports were found on N-stress and RSA. In cucumber, there were an insignificant increase in root length and PA, SA, root volume and average diameter got reduced under N- stress. Of which decrease in root diameter was only significant (Bai-Ge *et al.*, 2012). It is obvious that the parameters depend on species, and even there is wide variation among the genotypes in the present study. Hence, studying individual genotypes are extremely important.

From the RSA study with the nine diverse genotypes, it was observed that the genotypic variations are quite wide with respect to all the recorded parameters. This was also reported for different *Brachypodium* accessions under N and P-deprivation. Significant differences in RSA between two *Brachypodium* accessions grown on nutrient-rich, low-N and low-P conditions was found. More specifically, one accession maintained axile root growth under low N, while the other accession maintained lateral root growth under low P. These traits resemble the RSA of crops adapted to low-N and -P conditions, respectively (Ingram *et al.*, 2012). Most contrasting genotypes in terms of RSA were WH-147 and VL-401, mainly with respect to root length and related parameters like projected area, root volume, surface area etc.. The percentage increase in value for these parameters under nitrogen stress was also highest in case of VL-401. Number of root tips and forks were also found contrasting between these two genotypes with WH-147 having the highest range and VL-401 having the lowest range. Hence, these two genotypes would be worth studying at a molecular level for N-uptake, especially at their seedling stage. Two other genotypes, which were different from others, are HD-2967 and Kharchia.Theyshowed a decrease in root length under N-stress. HD-2967 needs detailed investigation for the above reason. However, Kharchia is known for salinity and draught tolerance, and also their root growth is considerably high even in normal (N-optimum) condition (Fig. 4). Both the cases, the branch angle reduced (significantly in case of HD-2967) under N-stress, which means, the lateral and branch roots were more vertical under stress than that of the N-optimum condition, and this would logically penetrate more soil depth even when the length is same. The reasoning of reduction in the length in case of HD-2967 can be justified by the fact that it might be getting compensated with the decrease in branch angle, and thereby doing a better resource management by this genotype. Similar observation on root angle was made in case of common bean and suggested as synergistic interaction, under low-P conditions from having long root hair length combined with shallow root angle which might be probably better than each phene in isolation (Miguel, 2011). It has also been proposed that Root Cortical Aerenchyma (RCA) may also be synergistic with root phenes that enhance soil exploration in different soil domains, such as root angle (Saengwilai *et al.*, 2014). Specially under low nitrogen, maize root growth angles become steeper (Trachsel *et al.*, 2013). These parameters of RSA combined with root dry weight (which increased significantly) in case of HD-2967 puts this genotype under a different root system. Length is decreasing while dry weight is increasing is really an important aspect, which indicated that there is some solute accumulation in the root cell under N-deprivation, which is nothing to do with root growth.

Root volume analysis made two contrasting group of genotypes, which hold good both under N-optimum as well as stressed condition. High volumes were found in case of Sujata, WH-147 and HD-2967 and lower root volume in case of Kalyansona, GW-322, VL-401 and HS-277. Commonalities among the genotypes within the group and differences between genotypes from the two different groups in relation to uptake will also be an important aspect to study further. Like other parameters, LR density also showed genotypic difference between N-optimum and N-stress condition. Under the present study overall root volume increased, which reflected in root architecture but not the weight significantly. However, root dry weight increase indicates that the priority of the wheat seedlings was towards root at the cost of shoots. This also reflected in overall result in shoot biomass (both fresh and dry weight). Priority of root is mainly for the better uptake of nutrient, and it started from the beginning of the growth stage i.e. at 15 days old seedlings in wheat. This study reveals that mainly 1st Order length contributed significantly toward the other significantly different parameters. When the nutrient is deprived in the solid media, the root extends in search of nitrogen and the root length increases. It is reported that nitrogen can affect root development either as a result of changes in the external concentration, or through changes in the internal nutrient status of the plant. Low soil N stimulates root elongation in maize (Liang *et al.*, 2005); Lateral root elongation, Gao *et al.* (2015), through the signaling pathway. Present work on root biomass and RSA could be the basis for future identification of important genetic components of RSA traits under nutrient limitation using a mapping population derived from the two contrasting wheat genotypes, as reported in case of *Brachypodium* (Ingram *et al.*, 2012).

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Author's contribution

CKN has actually done the most part of the work, G and AB has carried out the estimation of residual nitrogen, SKS has suggested for detail designing the experiment, KV has grown the materials in field from where the genotypes were selected, PKM has overall idea of the research experiment and guidance as group leader.

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