Indian J. Genet. Plant Breed., (2022); 82(4): 391-402
doi: 10.31742/ISGPB.82.4.2

ISSN: 0975-6906

www.isgpb.org

RESEARCH ARTICLE

Comparative analysis of differential root system architecture in tetraploid and hexaploid wheat under optimal and limiting nitrogen conditions

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Abstract

The variation in nitrogen (N) uptake efficiency is influenced by many factors once the plant root comes in contact with the soil. A study to compare root traits and N uptake among wheat genotypes belonging to hexaploid (*Triticum aestivum*) and tetraploid (*T. durum* and *T. dicoccum*) species at three depth zones (0-30 cm, 30-60 cm and > 60 cm) and two N levels [(limiting N: N_{50} (50 kg ha^{-1}) and optimal N: N_{100} (100 kg ha^{-1})] was conducted using image-based phenotyping and analysis. The response of a species for a trait was dependent on the N level as well as the rooting depth. In all the three species, root surface area, crossings and root biomass showed higher mean value at N_{50} than at N_{100}. However, total root length, root length density and specific root length showed higher mean value at N_{50} only in *T. aestivum* and *T. durum*. The variance for root traits at N_{50} and N_{100} varied with the rooting depth and species studied. The effect of N level was significant for all the N uptake traits including above ground biomass, N (%), above ground N and Nitrogen uptake efficiency (NUpE). For NUpE, only the genotype effect was significant. Principal component (PC) analysis classified root length traits and N uptake traits along PC1 at both the N levels. However, distribution of genotypes across the two PCs was different at the two N levels. Among bread wheat lines RAJ 4248, UAS BW-13357 and GW322 performed better in terms of N uptake efficiency. ECI26374, MLT DW RF7 and ICARDA RI 15 among durum wheat genotypes and DDK 50421, DDK50332 and DDK-50404 among *T. dicoccum* genotypes were found to have superior performance for NUpE. The current study delineates the importance of evaluating roots at different depths instead of whole root systems and using competitive N levels in NUE research so that the N use efficient varieties can perform equally well in the field at moderate N levels.

Keywords: Nitrogen use efficiency, tetraploid, hexaploid, wheat, N uptake efficiency, root length density, root biomass

Introduction

Nitrogen (N) fertilizer is one of the most important inputs in cereal crop production. It constitutes about 57.1% of agricultural fertilizer consumption worldwide and 65.1% in India (FAO 2019). As per the Indian Fertilisation Association assessment 2014-15, 18.2% of the global consumption of nitrogenous fertilizers was applied to wheat alone. In India, 23.5% of the total domestic consumption of N fertilizer is applied to wheat (Heffer et al. 2017). This undue application of nitrogenous fertilizers has led to lower N use efficiency (NUE) for cereal crops in India (21%) in comparison to the global figures of 35% (Omara 2019). Wheat (*Triticum aestivum* L.) being one of the earliest domesticated crops is consumed globally and is grown in a wide range of environments (Meena et al. 2022). Inadequate availability of N in wheat leads to destruction of chlorophyll, stunted growth and shortened juvenile phase. However, the line between adequate availability and deficiency of N in the soil is not very clear and depends upon many environmental and management factors. The organisation of plant root system in space and its growth pattern over time is governed by genetic factors as well as external stimuli. Increased lateral branching of roots in soil patches enriched with nutrient has been observed in many cases (Forde and Lorenzo 2001).

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How to cite this article: Dubey S., Patil M., Desai S.A. and Biradar S. 2022. Comparative analysis of differential root system architecture in tetraploid and hexaploid wheat under optimal and limiting nitrogen condition. Indian J. Genet. Plant Breed., **82**(4): 391-402.

Source of support: Nil

Conflict of interest: None.

Received: May 2022 **Revised:** Sept. 2022 **Accepted:** Nov. 2022

Maximum growth of the root system occurs from early node elongation till anthesis after which root growth slows and transitions to senescence (Ghimire et al. 2020). Shoot and root growth respond differently to N availability (Arora et al. 2001).

NUE is a complex trait comprising two key components, N uptake and N utilization efficiency which are complex traits in themselves, each involving many physiological processes and biochemical pathways (Hawkesford 2017). The variance in Nitrogen uptake efficiency (NUpE) is more influenced by G x E interaction than NUtE (Cormier et al. 2013). NUE has increased over years due to indirect selection for yield (Cormier et al. 2016). Phenotyping the above ground portion of the plant is easy and instruments are also available today for large scale and precise measurement of a large number of plants at various growth stages and under different stress conditions. However, root phenotyping is challenging, and its technology has not grown at the same pace as for other plant traits. Large-scale root phenotyping is an altogether different domain. The health and vigor of roots for absorbing water and nutrients can be measured using quantitative characters such as root length, root biomass, root length density, root diameter. Root phenotyping is cumbersome and requires specialized methods and instruments. These include soil column culture (Tomar et al. 2016; Ren et al. 2017), pots, transparent pot culture, shovelomics (Boudiar et al. 2021), rhizotrons or growth chambers (Ghimire et al. 2020), rhizotubes (Jeudy et al. 2016), rhizoboxes (Liu et al. 2021) or hydroponic culture with modified Hoagland solution (An et al. 2006; Ranjan et al. 2019; Zhang et al. 2019). However, it has been often observed that techniques for screening seedling root traits correlate well with rooting depths until vegetative stages only and not at reproductive stages (Watt et al. 2013; Paez-Garcia et al. 2015). Despite high rate of early nitrate uptake, the uptake dynamics at later stages of crop growth has a substantial role towards total N uptake in wheat (Pang et al. 2015). Nevertheless, techniques that involve soil as the growth medium mimic to some extent the actual environment in which the plants grow and hence give a clear picture of root response under different stress conditions. Therefore, the present experiment was conducted under moderate N (N_{50}) and control N (N_{100}) conditions using soil as the growth medium to assess the impact on root growth and foraging capacity. Wheat genotypes vary with respect to the extent of metabolic and molecular changes that lead to differential growth of roots under low N conditions (Xu et al. 2019). Uppal et al. (1991) observed considerable genetic variability for nitrogen percentage and total plant nitrogen in grain as well as straw of wheat varieties when evaluated at 45 days of sowing, 90 days of sowing and at harvest (Tomar et al. 2016; Ranjan and Yadav 2020; Boudiar et al. 2021). It has been reported in various studies that polygenes, each with minor effects govern the basic structure of root traits and their modification with soil conditions (Lynch 2019; Ranjan et al. 2022). The variation in heritability values for root traits across studies also reveal the quantitative nature of root traits as the genotypic effect of root traits is masked by the soil conditions. (Passioura 2012). Under non-limiting N conditions, root diameter, total root length, root surface area and root volume can be selected in early generation but under N limiting conditions, the selection for these traits has to be postponed to later segregating generations in wheat (Ranjan et al. 2022). In the present study, a diverse set of tetraploid and hexaploid wheat genotypes were used for evaluation of root traits at different rooting depths and two levels of N supply (N_{50} and N_{100}).

Materials and methods

Plant material and experimental site

The study was carried out at AICRP Wheat, MARS, UAS Dharwad, Karnataka, India. The experimental site is located at 15.4889° N and 74.9813° E latitude and 661 metres above mean sea level. Polyvinyl chloride (PVC) pipes (120 cm height and 22 cm diameter) were used for the study and mounted in open field. The procedures described by Ranjan et al. (2019) were used to conduct the experiment. The amount of available Nitrogen was also analysed in the soil sample using alkaline potassium permanganate method and was found to be 87.2 kg ha^{-1}. The experiment was laid out in completely randomised design with two treatments of Nitrogen (moderate N: N_{50} (50 kg ha^{-1}) and control N: N_{100} (100 kg ha^{-1}). At sowing, all the soil columns were fertilised with a mixture of N:P:K (50:60:40 kg ha^{-1}). At tillering, only the control or N_{100} columns were fertilised with 0.26 g (@ 50 kg ha^{-1}) urea per column. The columns were watered using tap water using a 1-L beaker to ensure equal amount of water supply to all the genotypes at both the treatments.

Forty-two genotypes of wheat comprising 20 of bread wheat (*T. aestivum* L.), 11 of durum (*T. durum* Desf.) and 11 genotypes of *khapli* or *samba* wheat (*T. dicoccum*) were used for the experiment. The details of the genotypes including their origin and pedigree information have been outlined in Supplementary Table S1. Bread wheat and durum genotypes, evaluated earlier for their yield and NUE response, were included to study their root traits and to serve as checks. The genotypes, UAS BW-13355, UAS BW 13358, UAS BW 13354, UAS BW 13359, UAS BW 13356 and WH 1022 have been reported to have high NUE; HD 2967 and K9107 have been reported to show moderate NUE, while C306, Bijaga yellow and Amruth are reported to have low to moderate NUE. Amruth, Bijaga yellow, UAS 446, DDK 1029 and NP-200 are recommended checks for the rainfed region. The remaining genotypes represented currently cultivated as well as old varieties of the three wheat species obtained from CIMMYT and AICRP wheat trials for abiotic stress. The durum and dicoccum wheat genotypes included in the study have been reported superior with respect to water use efficiency and drought tolerance in breeding trials.

Root sampling and Imaging

The experiment was terminated just after the completion of anthesis. Plant height, above ground biomass, N (%), above ground Nitrogen (g), N uptake (g N plant^{-1}) and N uptake efficiency (g N g^{-1} N) were recorded for four replications per treatment. The roots were separated from the soil as per Ranjan et al. (2019). The above ground portion was then separated from the roots, sun dried for two days and then oven dried at 65^{0}C for 3 days. Shoot N (%) was estimated using micro-Kjeldahl method. Above ground nitrogen was calculated as: Shoot N (%) x above ground biomass plant^{-1}. Nitrogen uptake was calculated as: Above ground Nitrogen/ Total N supply and Nitrogen uptake efficiency was calculated as: NUP/Total N supply. Total N supply was estimated as fertiliser N + soil mineral Nitrogen.

The roots of each genotype were divided into 3 parts (top (0–30 cm), middle (30-60 cm) and bottom (>60 cm), spread on a wet paper towel and labelled. The root sampling was done as suggested by Oliveira et al. 2000. Once the root sampling for all genotypes was completed, the root samples were retrieved from the freezer and allowed to come to room temperature before starting the image capture. Each of the root samples were scanned using the EPSON flatbed root scanner. The commercial software WinRHIZO™ (Regent Instruments Inc., Canada, 2013 (Arsenault 1995) was used for analysing the image data. The following parameters obtained from the imaging data recorded for four replications per treatment combination were used for further analysis: Total root length (TRL) (in cm), surface area (SA) (cm^2), average diameter (AD) (mm), root volume (RV) (cm^3) and number of crossings. The root length data from top, middle and bottom zone was used for the calculation of root length density or root length per unit soil volume. After completion of image capture, the root samples along with their identity labels were oven dried at 70^{O}C for 36 hrs and the root biomass (RB) was recorded using an electronic weighing balance. Specific root length (SRL) was calculated using the formula: SRL (cm/cm^3) = Total Root length/ Root biomass

Statistical analysis

The three species were considered as three different groups for two-way analysis of variance. Hence, two factors were evaluated *i.e.,* N level (two levels-N_{50} and N_{100}) and wheat species (three levels *i.e., T. estivum, T. durum* and *T. dicoccum*). Two-way ANOVA was done using the type III sum of squares method. All the analyses were carried out invariably in R (R Studio Team 2022). Descriptive statistics were first calculated separately for each species at the three depth zones and then a combined analysis was done for the three root zones. The combined analysis included the above ground and N uptake traits. The assumption of normality was tested by observing normal probability plots and trait histograms as well as conducting Shapiro–Wilk test. The Pearson correlation coefficient was estimated using the combined root data. Principal component analysis was also done on the combined data for root traits and above ground traits.

Results

Analysis of variance for root traits and N uptake traits

The analysis of variance was carried out to study the influence of two factors, *i.e.,* Nitrogen level (N_{50} and N_{100}) and wheat species under study on root traits at different depths and N uptake in wheat (Table 1).

Total root length was significantly dependent on the type of wheat species, N level as well as their interaction. Compared to $N_{100,}$ the average root length was higher at N_{50} in *T. aestivum* and *T. durum* but not in *T. dicoccum*. The surface area of the root zone at different depths was not substantially affected by any of the two factors considered. However, in a combined analysis using the total surface area of the root zone, the type of wheat species significantly affected the root surface area and the highest values were recorded in *T. dicoccum*. Average diameter at the three root depths significantly varied with the wheat species. In the middle zone (30–60 cm), a significant interaction effect was observed. Based on the combined data, *T. aestivum* recorded comparatively lower values for this trait than the other two species. Root length density was found to be an important character in the top and middle part of the root zone as the individual factors and their interaction was significant for this trait. Root volume varied with N level in the middle portion and with species in the bottom zone of the root. The number of crossings was highly dependent on the N level supplied. Higher number of root crossings formed at $N_{50.}$ The interaction effect was also significant for this trait. The root biomass was highly affected by the two factors and their interaction in the middle zone of growth. A higher root biomass was recorded at lower levels of N supply. The N level was highly significant for specific root length in the top portion and its interaction in the top and middle portion of the root. The interaction effect was also significant in the top and bottom portions. However, the species effect was also significant for this trait in the bottom zone of the root. The N level as well as type of wheat species significantly influenced the expression of all the above ground traits including above ground biomass (g), above ground N (g), NUpE and NUP. However, for plant height, only the species effect was pronounced.

A combined analysis of the above ground and N uptake traits Table 2 showed that average plant height was not affected by the applied N and the variation in this trait was genotype dependent. The variation in above ground

Table 1. Two-way analysis of variance for root traits analysed in the (a) Top (1-30 cm), (b) Middle (30-60 cm) and (c) Bottom (>60 cm) zone of the soil column during rabi 2020-21

Response	df	Total Root length (cm)	Surface Area(cm²)	Avg. Diam. (mm)	Root length density (cm/m³)	Root Volume (cm³)	Crossings	Root biomass (g)	Specific root length (g/cm)
(a) Top (1-30 cm)									
Intercept	1	261.50***	284.67***	333.27***	261.50***	135.15***	9.66**	100.10***	70.95***
N_level	1	14.24***	0.99	0.48	14.24***	0.00	27.60***	0.01	12.22***
Wheat_species	2	2.66*	1.56	1.23	2.66*	0.70	1.31	0.75	0.06
N_level:Wheat_species	2	5.17**	0.07	4.81*	5.17**	0.71	3.27*	2.69*	5.89**
Residuals	78								
(b) Middle (30-60 cm)									
Intercept	1	169.30***	170.00***	257.36***	169.30***	73.46***	16.92***	91.93***	18.62***
N_level	1	0.28	0.92	0.19	0.28	0.06	9.06**	21.38***	4.69*
Wheat_species	2	0.72	2.36	8.72***	0.72	5.38**	0.02	8.05***	1.23
N_level:Wheat_species	2	4.49*	1.68	3.17*	4.49*	0.37	0.19	2.87*	0.84
Residuals	72								
(c) Bottom (>60 cm)									
Intercept	1	61.18***	1.48***	145.50***	60.76***	92.76***	3.28*	26.15***	35.57***
N_level	1	0	2.16	0.32	0	3.19*	7.53**	0.03	0.16
Wheat_species	2	0.19	0.53	2.75*	0.23	1.08	1.32	1.56	3.91*
N_level: Wheat_species	2	0.9	0.73	2.32	0.96	0.28	2.75*	0.44	2.49*
Residuals	34								

Avg. = Average, Diam. = Diameter

biomass and N (%) was significant only for N level, while the variation in above ground N and NUP was significant for both the tested factors. The NUpE was only significant for the genotype. These results indicate the importance of genotype in breeding for NUE.

Analysis of root parameters at different depths

The descriptive statistics for important traits namely, root length density, root biomass and specific root length for *T. aestivum*, *T. durum* and *T. dicoccum* at different rooting depths is presented in Table 3-5, respectively. The statistics for remaining root parameters for the three species are given in Supplementary Tables S2, S3 and S4. Table 6 shows combined descriptive statistics of the complete root zone along with above ground and N uptake traits. The box plots for root length density, root biomass and specific root length at the two N levels at different depths are depicted in Fig 1. The box plots for other root traits are shown in Supplementary Fig. 1.

Top (1-30 cm)

In *T. aestivum* and *T. durum*, the average root length was higher at N_{50} than at N_{100}. The root length density showed a trend similar to the total root length in the top portion of the root zone. However, for bread wheat and durum wheat, it was higher at N_{50} than at N_{100}. The variation for this trait was significantly higher at N_{100} in tetraploid wheat than at N_{50}. The average root biomass was significantly higher at N_{50} for tetraploid wheat than hexaploid wheat species. The variation for this trait was dependent on the level of N supply. The specific root length was higher at N_{50} for *T. durum* and *T.aestivum*, but not so in *T. dicoccum*.

The variance for this trait also showed a similar trend. From the present investigation, it is clear that root evaluation for nutrient use/uptake efficiency should consider evaluation at different depths to have a clear idea about the importance of each trait.

Middle (30-60 cm)

Tetraploid species showed higher values for root length density at N_{50} while hexaploid genotypes showed higher values for this trait at N_{100}. Also, the variation for this trait was also higher at N_{100} in tetraploid wheats whereas hexaploid species showed more variation at N_{50}. The root biomass was higher at N_{50} than at N_{100} in the middle zone of the root. On the contrary, the variance for this trait was higher at N_{100} than at N_{50}. Specific root length was higher at N_{100} for all the species, but the differences between the two N levels were not significant for the genotypes of dicoccum wheat. Variation for this trait was found to be higher at N_{100}.

Table 2. Two-way analysis of variance for *T. aestivum*, *T. durum* and *T. dicoccum* genotypes for root traits and N uptake traits using combined data of the three depth zones of the soil column during *rabi* 2020-21

Response	Df	TRL (cm)	SA (cm^2)	AD (mm)	RLD (cm/ m^3)	RV (cm3)	Crossings	RB (g)	SRL (g/ cm^3)	PH (cm)	AGB (g)	N (%)	AGN (g)	NUP (g plant-1)	NUpE (gg-1)
Intercept	1	135.20***	157.59***	579.84***	135.20***	116.49***	16.00***	135.48***	158.46***	3124.57**	1082.72***	595.14***	546.76***	473.54***	367.52***
N level	1	0.71	0.14	0.02	0.7107	0.22	23.19***	1.63	0.27	0.0007	36.64***	31.99***	67.57***	16.25***	1.27
Wheat species	2	2.14	4.53**	3.37**	2.135	5.37**	0.4	4.10**	0.98	25.80***	0.95	1.69	3.57**	3.093*	2.40*
N level:wheat species	2	0.29	0.4	0.07	0.2942	0.3	0.57	1.3653	0.01	0.29	1.28	0.10	0.74	0.97	1.81
Residuals	78														

Bottom (>60 cm)

In the bottom root zone of *T. aestivum* and *T. durum* genotypes, most traits did not show significant variation between the two levels of N supply except the number of root crossings which was higher at N_{50}. The variation for this trait was also higher at N_{50}. Among the genotypes of *T. dicoccum*, the root traits showed higher values at N_{50} than at N_{100}, though variance for most traits (except crossing and SRL) was higher at N_{100} than at N_{50}.

A combined analysis of the complete root system showed that the traits total root length, average diameter, root length density, crossings and root biomass showed a higher average response at N_{50}. Also, the variation for all the traits except SRL was higher at N_{50}. A higher above ground biomass was observed at N_{100}. As expected, the average value for N uptake traits (N in %), above ground N, and N uptake were higher at N_{100}. The NUE was higher at N_{50}. However, the variation for all the above ground traits was higher at N_{100}.

Correlation analysis

Change in N level changed the intensity of association between the root traits but the direction of association was same at two N treatments. Except average diameter, all the root traits correlated positively with each other and with the N uptake traits (Table 7). Average diameter did not correlate significantly with any N uptake trait at N_{50}. Plant height showed a negative correlation with specific root length. It did not correlate with any other traits. A negative correlation was observed between root biomass and specific root length due to the method of its calculation. All the above ground and N uptake traits correlated significantly with each other. However, above ground biomass showed no association with N (%). At N_{100}, the pattern of correlation among root traits and between root traits and above ground traits was maintained. Average diameter showed a negative correlation with root length related traits and N uptake traits. Specific root length showed a positive correlation with N (%). Above ground biomass showed a negative correlation with N (%) but a positive correlation with other N uptake traits.

Principal component analysis

Principal component analysis was carried out for all the genotypes and complete root systems separately for the two Nitrogen levels (Fig. 2). At N_{50}, the first two principal components explained 71.6% variation in the analysed traits. The first principal component (54.4%) comprised of total root length (11.30%), surface area (11.47%), root length density (11.30%), above ground Nitrogen (11.035) and root volume (9.75%). The second principal component (17.2%) was more related to specific root length (30.69%), number of crossings (16.59 %), average root diameter (13.92%), root biomass (12.15%) and plant height (10.87).

At N_{100}, the first two principal components explained 67.8% of the variance in the analysed traits. PC1 (53.2%) was mainly loaded by of total root length (11.93%), surface area (10.87%), root length density (11.93%), N (%) (10.36%), above ground N (10.09%) and NUP (10.09 %) and NUpE (10.09%). The second principal component (14.6%) was related to above ground biomass (37.7%), specific root length (9.03%), above ground N (9.82%), NUP (9.82%) and NUpE (9.82%). Hence, the loading of traits along the PCs was different at the two N levels. The distribution of individuals also varied with the N level. Only *T. dicoccum* and *T. aestivum* genotypes were distributed across the two PCs at N_{50} while individuals from all the three species were found across the two PCs at N_{100}.

Superior genotypes were identified for NUpE and the percentage change at N_{50} for various root traits and N uptake traits was estimated in comparison to control condition (N_{100}). The top performing genotypes have been listed in Table 8 along with the checks. Detailed data on all the traits is represented in Table

Table 3. Descriptive statistics (DS) for root traits in the top (1-30 cm) depth for *T. aestivum, T. durum* and *T. dicoccum* genotypes

DS	N level	*T. aestivum*			*T. durum*			*T. dicoccum*		
		RLD (cm/m^3)	RB (g)	SRL (g/cm)	RLD (cm/m^3)	RB (g)	SRL (g/cm)	RLD (cm/m^3)	RB (g)	SRL (g/cm)
Minimum	N50	133540.85	0.35	1091.42	116078.82	0.34	857.22	145334.89	0.90	788.85
	N100	87578.75	0.54	907.78	91198.78	0.50	806.80	125132.18	0.76	875.19
Maximum	N50	319861.94	1.97	6215.26	220303.62	1.73	3776.34	224324.75	2.78	2124.30
	N100	268964.38	1.80	2823.35	267075.72	1.36	2917.17	290049.16	1.80	2633.08
Mean	N50	231109.80	0.99	3046.47	178245.35	1.16	1963.16	177197.14	1.70	1150.98
	N100	173769.76	0.98	1919.77	154911.20	0.95	1795.51	201753.12	1.15	1851.37
σ	N50	51391.57	0.49	1671.36	33397.64	0.52	1053.44	29410.21	0.55	422.22
	N100	49154.12	0.35	596.35	57234.61	0.34	720.77	56116.35	0.33	559.16
S.E.	N50	11491.50	0.11	373.73	10069.77	0.16	317.62	8867.51	0.17	127.30
	N100	10991.20	0.08	133.35	17256.88	0.10	217.32	16919.72	0.10	168.59
C.V.	N50	0.22	0.50	0.55	0.19	0.44	0.54	0.17	0.32	0.37
	N100	0.28	0.36	0.31	0.37	0.36	0.40	0.28	0.28	0.30

Table 4. Descriptive statistics (DS) for root traits in the Middle (30-60 cm) root zone for *T. aestivum, T. durum* and *T. dicoccum* genotypes

DS	N level	*T. aestivum*			*T. durum*			*T. dicoccum*		
		RLD (cm/m^3)	RB (g)	SRL (g/cm)	RLD (cm/m^3)	RB (g)	SRL (g/cm)	RLD (cm/m^3)	RB (g)	SRL (g/cm)
Minimum	N50	36383.13	0.40	668.86	154014.97	0.39	2813.25	141684.80	0.42	2780.41
	N100	89281.43	0.02	2703.70	84265.27	0.06	2095.00	92517.64	0.13	1549.43
Maximum	N50	338251.59	0.68	7277.71	262483.34	0.66	6255.97	381533.10	0.65	7369.38
	N100	295928.33	0.69	122990.09	265757.38	0.60	25288.31	303809.23	0.80	16386.49
Mean	N50	181987.69	0.50	3790.93	212382.02	0.49	4524.72	278232.58	0.53	5363.66
	N100	202072.93	0.30	13816.29	170498.61	0.21	12419.91	182216.25	0.45	5616.30
σ	N50	77943.89	0.08	1777.01	33442.19	0.09	1223.07	77527.11	0.08	1382.13
	N100	61587.78	0.15	27454.98	66598.20	0.17	7792.93	57966.17	0.22	4037.06
S.E.	N50	17881.56	0.02	407.67	11147.40	0.03	407.69	23375.30	0.02	416.73
	N100	14129.21	0.03	6298.60	22199.40	0.06	2597.64	17477.46	0.06	1217.22
C.V.	N50	0.43	0.16	0.47	0.16	0.17	0.27	0.28	0.16	0.26
	N100	0.30	0.50	1.99	0.39	0.80	0.63	0.32	0.48	0.72

Table 5. Descriptive statistics for root traits in the Bottom (> 60 cm) root zone for *T. aestivum, T. durum* and *T. dicoccum* genotypes

DS	N level	*T. aestivum*			*T. durum*			*T. dicoccum*		
		RLD (cm/m^3)	RB (g)	SRL (g/cm)	RLD (cm/m^3)	RB (g)	SRL (g/cm)	RLD (cm/m^3)	RB (g)	SRL (g/cm)
Minimum	N50	98126.53	0.03	4765.01	115323.59	0.10	5847.64	182294.97	0.13	4781.98
	N100	92371.25	0.11	2114.94	142428.19	0.09	15936.93	95281.75	0.10	6122.70
Maximum	N50	326237.25	0.46	29916.53	229843.39	0.33	14070.87	343333.72	0.39	24970.79
	N100	305823.44	0.44	21963.46	218087.21	0.13	20658.85	322481.83	0.40	10042.61
Mean	N50	202901.94	0.22	13687.15	179990.37	0.21	9953.81	239457.25	0.25	11121.27
	N100	201134.76	0.21	12495.23	188262.46	0.11	18009.75	176353.09	0.23	7985.87
σ	N50	82818.26	0.16	8880.49	47668.46	0.11	3521.40	66717.95	0.09	6311.69
	N100	69312.53	0.12	6560.84	34186.97	0.02	1957.50	91020.83	0.12	1357.32
S.E.	N50	29280.68	0.06	3139.73	108.82	0.19	46.05	23588.36	0.03	2231.52
	N100	24505.68	0.04	2319.61	73.21	0.05	14.67	32180.72	0.04	479.88
C.V.	N50	0.41	0.72	0.65	0.26	0.52	0.35	0.28	0.35	0.57
	N100	0.34	0.56	0.53	0.18	0.18	0.11	0.52	0.50	0.17

Table 6. Descriptive statistics for root traits and Nitrogen uptake traits using combined data of the three depth zones of the soil column during *rabi* 2020-21

DS	N level	Total Root length (cm)	Surface Area(cm^2)	Avg Diam (mm)	Root length density (cm/m^3)	Root Volume (cm^3)	Crossings	Root biomass (g)	Specific root length (g/cm)	Plant Height (cm)	Above ground biomass (g)	N (%)	Above ground Nitrogen (g)	N uptake (g/plant)	NUpE (gg-1)
Minimum	N50	1170.075	185.840	0.324	38692.941	2.254	4937.500	0.339	1091.423	65.050	10.557	0.601	0.068	0.124	0.225
	N100	919.284	167.109	0.308	30399.593	2.497	2167.500	0.565	1295.982	68.250	13.981	0.746	0.132	0.163	0.202
Maximum	N50	9237.922	1061.565	0.631	305486.825	12.306	82065.000	3.768	5727.254	98.600	20.834	1.527	0.209	0.379	0.687
	N100	8896.474	1064.928	0.705	294195.561	12.662	53017.500	2.692	7568.988	101.250	29.198	1.780	0.444	0.549	0.679
Mean	N50	5129.756	575.267	0.429	169634.802	6.092	32463.024	1.798	3087.920	82.540	15.961	0.829	0.132	0.240	0.435
	N100	4472.039	558.878	0.427	147884.873	6.040	14179.619	1.404	3340.784	83.305	20.385	1.198	0.243	0.300	0.371
σ	N50	1878.988	213.563	0.072	62135.853	2.562	17244.587	0.679	1169.158	8.908	2.417	0.151	0.029	0.053	0.096
	N100	1770.108	214.676	0.083	58535.307	2.616	9674.370	0.485	1402.395	8.826	3.250	0.278	0.064	0.079	0.098
S.E.	N50	289.934	32.953	0.011	9587.770	0.395	2660.898	0.105	180.405	1.374	0.373	0.023	0.004	0.008	0.015
	N100	273.134	33.125	0.013	9032.194	0.404	1492.788	0.075	216.394	1.362	0.501	0.043	0.010	0.012	0.015
C.V.	N50	0.366	0.371	0.168	0.366	0.421	0.531	0.378	0.379	0.108	0.151	0.182	0.220	0.220	0.220
	N100	0.396	0.384	0.193	0.396	0.433	0.682	0.345	0.420	0.106	0.159	0.232	0.263	0.263	0.263

Table 7. Pearson's correlation coefficients at N_{50} (in pink) and N_{100} (in blue) for root and above ground and Nitrogen uptake traits using combined data of the three depth zones of the soil column during *rabi* 2020-21

Traits	TRL (cm)	SA (cm^2)	AD (mm)	RLD (cm/m^3)	RV (cm^3)	Crossings	RB (g)	SRL (g/cm^3)	PH (cm)	AGB (g)	N (%)	AGN (g)	NUP (g plant-1)	NUpE(gg-1)
TRL (cm)	1	0.92**	-0.22	1**	0.82**	0.69**	0.52**	0.38**	0.22	0.25	0.75**	0.74**	0.74**	0.74**
SA (cm^2)	0.92**	1	0.07	0.92**	0.95**	0.54**	0.64**	0.21	0.32**	0.21	0.81**	0.74**	0.74**	0.74**
AD (mm)	-0.34*	-0.04	1	-0.22	0.29	-0.38**	0.11	-0.35**	0.26	-0.08	-0.06	-0.07	-0.07	-0.07
RLD(cm/m^3)	1**	0.92**	-0.34*	1	0.82**	0.69**	0.52**	0.38**	0.22	0.25	0.75**	0.74**	0.74**	0.74**
RV (cm^3)	0.77**	0.93**	0.24	0.77**	1	0.44**	0.59**	0.14	0.36**	0.19	0.72**	0.67**	0.67**	0.67**
Crossing (g)	0.67**	0.68**	-0.1	0.67**	0.64**	1	0.05	0.54**	0.03	0.03	0.42**	0.34*	0.34*	0.34**
RB (g)	0.53**	0.62**	0.02	0.53**	0.6**	0.41**	1	-0.54**	0.41**	0.28	0.55**	0.6**	0.6**	0.60**
SRL (g/cm^3)	0.56**	0.41**	-0.45**	0.56**	0.26	0.31*	-0.33*	1	-0.3**	-0.08	0.16	0.08	0.08	0.08
PH (cm)	0.24	0.27	0.13	0.24	0.24	0.11	0.35*	-0.08	1	0.27	0.11	0.27	0.27	0.27
AGB (g)	-0.04	-0.05	-0.11	-0.04	-0.11	-0.05	0.21	-0.19	0.06	1	0	0.69**	0.69**	0.69**
N (%)	0.83**	0.74**	-0.31*	0.83**	0.61**	0.56**	0.44**	0.46**	0.21	-0.16	1	0.72**	0.72**	0.72**
AGN (g)	0.69**	0.61**	-0.31*	0.69**	0.48**	0.44**	0.51**	0.27	0.22	0.5**	0.76**	1	1	1
NUP (g/plant)	0.69**	0.61**	-0.31*	0.69**	0.48**	0.44**	0.51**	0.27	0.22	0.5**	0.76**	1**	1	1**
NUpE (gg-1)	0.69**	0.61**	-0.31*	0.69**	0.48**	0.44**	0.51**	0.27	0.22	0.5**	0.76**	1**	1**	1

Table 8. Top performing checks and genotypes identified based on Nitrogen uptake efficiency for root traits and nitrogen uptake traits at moderate (N_{50}) and control (N_{100}) Nitrogen supply in *T. aestivum*, *T. durum* and *T. dicoccum*

S. no	Genotype	N level	NUpE (gg^{-1})	RLD (cm/m^3)	SRL (g/cm^3)	S.No	Genotype	NUpE (gg^{-1})	RLD (cm/m^3)	SRL (g/cm^3)	S.No	Genotype	NUpE (gg^{-1})	RLD (cm/m^3)	SRL (g/cm^3)
	T. aestivum						*T. durum*					T. dicoccum			
15	WH 1022	N_{50}	0.52	165378.8	4846.88	31	Amruth	0.55	152492	3505.64	33	NP 200	0.62	261605	2835.6
		N_{100}	0.42	211433.5	6012.84			0.32	66126.4	1529.23			0.26	187500	3784.91
6	UASBW-13354	N_{50}	0.51	280211.3	4310.7	30	UAS 446	0.48	188729	2211.22	42	DDK-1029	0.49	212323	3000.86
		N_{100}	0.32	161651.7	3954.81			0.49	252579	4928.04			0.39	142243	2498.66
7	UASBW-13359	N_{50}	0.46	269180.6	4932.42	27	Bijaga Yellow	0.3	203534	2553.38	41	DDK-50421	0.69	289138	2320.19
		N_{100}	0.42	234414.9	3108.47			0.3	189075	6336.02			0.36	184267	2977.32
12	RAJ4248	N_{50}	0.49	191327.2	3567.21	22	EC126374	0.48	156888	4128.22	33	DDK-50332	0.62	263449	3811.77
		N_{100}	0.54	214985.8	5179.6			0.33	105532	2487.86			0.46	225173	2529.39
14	UASBW-13357	N_{50}	0.48	305486.8	3943.46	24	MLT DW RF 7	0.43	177302	2620.38	39	DDK-50404	0.51	236989	3867.77
		N_{100}	0.52	217997.2	4800.11			0.42	190746	3604.23			0.52	169824	2472.69
18	GW 322	N_{50}	0.48	133277.1	1779.45	23	ICARDA RI 15	0.39	185358	5727.25					
		N_{100}	0.3	145181.2	4212.65			0.35	151036	3458.52					

S5, S6 and S7. Among *T. aestivum* lines the best performing checks were WH1022, UASBW-13354 and UASBW-13359. RAJ 4248 (12), UAS BW-13357 (14) and GW322 (18) showed good performance for NUpE and performed as good as the checks.Among durum wheat genotypes, the checks Amruth (31) and UAS446 (30) showed showed good NUpE than the evaluated genotypes. ECI26374 (22), MLT DW RF7 (24) and ICARDA RI 15 (23) were the top performing genotypes. NP200 and DDK 1029 were the top performing checks among dicoccum wheat. The genotypes DDK 50421, DDK50332 and DDK-50404 were the top genotypes for NUpE. DDK 50421 surpassed the checks for NUpE.

Discussion

The importance of root system for attaining higher Nitrogen use efficiency in crop plants is unquestioned. The plant material used in the study was intended to compare the three species of wheat for root traits relevant to Nitrogen uptake. This study presents a practical research approach by using two levels of N and evaluation of roots at different depths of soil. Many studies focussing on root system evaluation consider N0 as one of the treatments (Svoboda and Haberle 2006; Giorgio and Fornaro 2012). Relying only on soil N reduces the probability of identifying genotypes which might perform well at N50 and provide comparable grain yield. In a long-term experiment with contrasting fertilisation systems, Svoboda et al. (2020) found that potential for improvement in the root system could only be observed in the N fertilised plots and not in the N0 treatment. Some root traits are greatly influenced by differential N application while others are not influenced at a significant level. In order to give a satisfying N yield at lower levels of N supply, the roots modify specific traits of their architecture at different soil depths to scavenge for available N (Giorgio and Fornaro 2012).

In the top 30 cm root zone, higher values were observed for root length density and specific root length in *T. aestivum* and *T. durum* at $N_{50,}$ though the average diameter was lower. This shows the tendency of roots in these species to expand the root system in the form of fine roots to acquire the nutrients. Dicoccum wheat has a naturally extensive root system. At lower levels of N supply, the increment in root length could not suffice for the N requirement of the plant. Hence, the roots tend to expand their diameter to strengthen N uptake. Fatholahi et al. (2020) found that *T. dicoccum* wheats accumulate more biomass but were less responsive to N supply, as evidenced by lower N uptake, utilization, remobilization, use efficiency, grain yield, and harvest index in comparison to the improved durum and bread wheats particularly in the presence of sufficient N supplies. During domestication, the hexaploid species tended to allocate less biomass to root system. Moving towards the middle portion of the root, the tetraploid

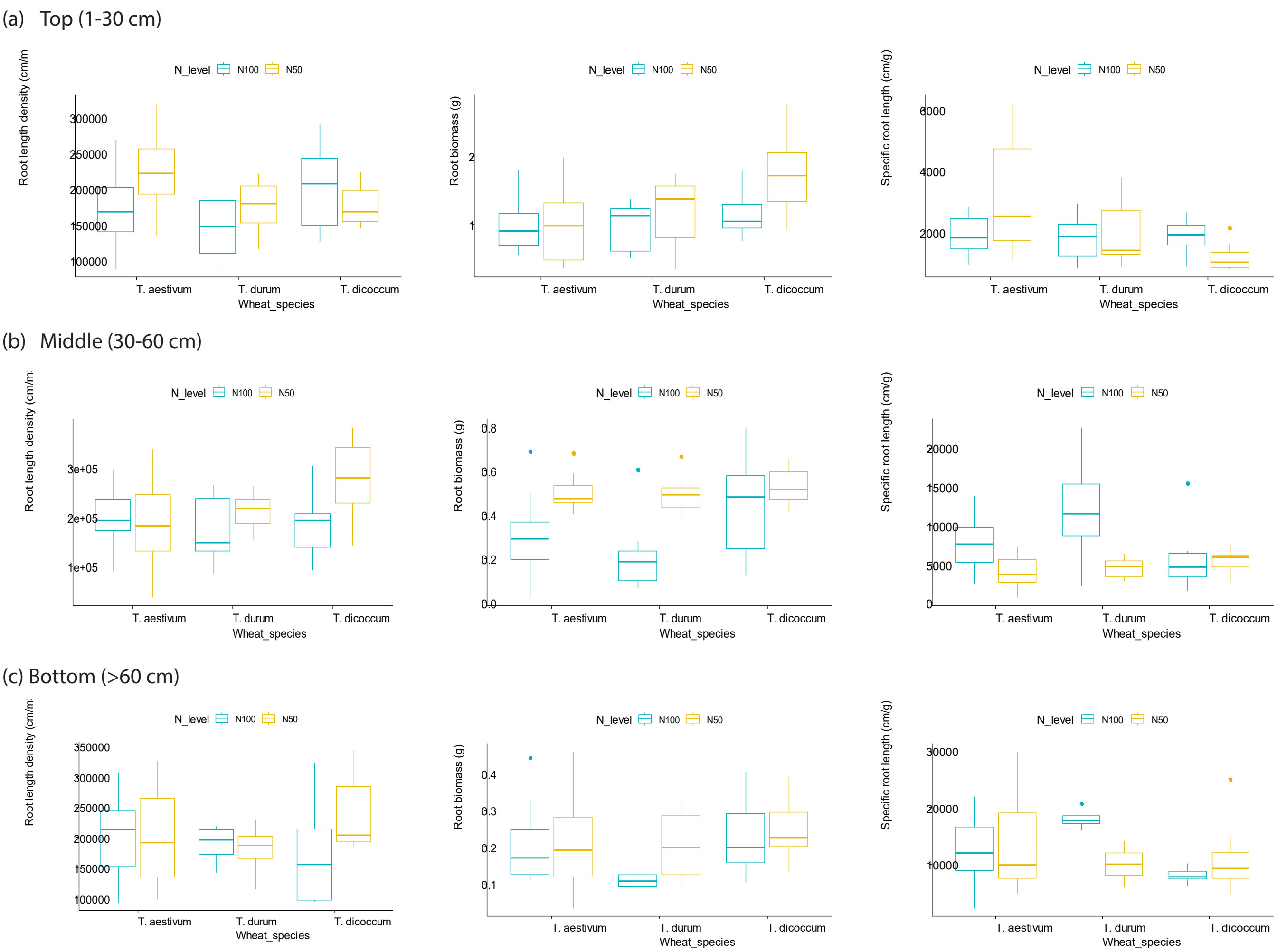

Fig. 1. Boxplots for root length density, root biomass and specific root length for *Triticum aestivum, Triticum durum* and *Triticum dicoccum* at N_{50} and N_{100} ¬for (a) Top (1-30 cm), (b) Middle (30-60 cm) and (c) Bottom (>60 cm) zone of the soil column. Median values are represented by horizontal lines splitting the boxes; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; dots represent outliers

wheats show higher total root length, surface area and root length density at N_{50} than hexaploid wheat. Increasing root length density has been suggested as a viable approach to improve N uptake in many crops (Garnett et al. 2009). The changing importance of different root traits at different depths across the three species is seen here. During the process of domestication, the hexaploid species allocated less biomass to root system (Gui et al. 2021). Substantial variability was observed for the number of crossings at both the N levels and all the depths. The interaction of N level x wheat species for this trait is significant in the top and bottom portions of the root zone. Higher root biomass at N_{50} in tetraploid wheats seems to be due to increment in root diameter. In the middle root zone, the roots of *T. aestivum* wheat react to limited N by increasing root diameter, durum wheats increase diameter as well as length and *T. dicoccum* wheats improve their root diameter to sustain plant growth. The greater root biomass in this zone is attributed to higher average root diameter in this zone. Many of the aestivum and durum wheat genotypes could not extend their roots beyond 60 cm in the bottom zone of the root. Here, the *T. dicoccum* wheat genotypes were more prominent. All the important root traits (except root diameter and root volume) showed higher mean values at N_{50} in this zone in *T. dicoccum* wheat. Additionally, the results from the combined analysis of variance show that few root traits like total root length which showed variation at a given depth were not significantly different among the species and N levels in the combined analysis. On the other hand, traits like surface area and root volume assumed greater importance here. The rest N uptake traits showed expected results at the two N levels and Nitrogen uptake efficiency was found to be greater at limited N supply. Higher above ground biomass but lower root biomass at N_{100} shows that at sufficient levels of N supply, the plant roots do not proliferate vigorously and still can maintain the shoot dry weight specified by the genotype. On the other hand, at N_{50}, the root system of Nitrogen uptake efficient genotypes tends

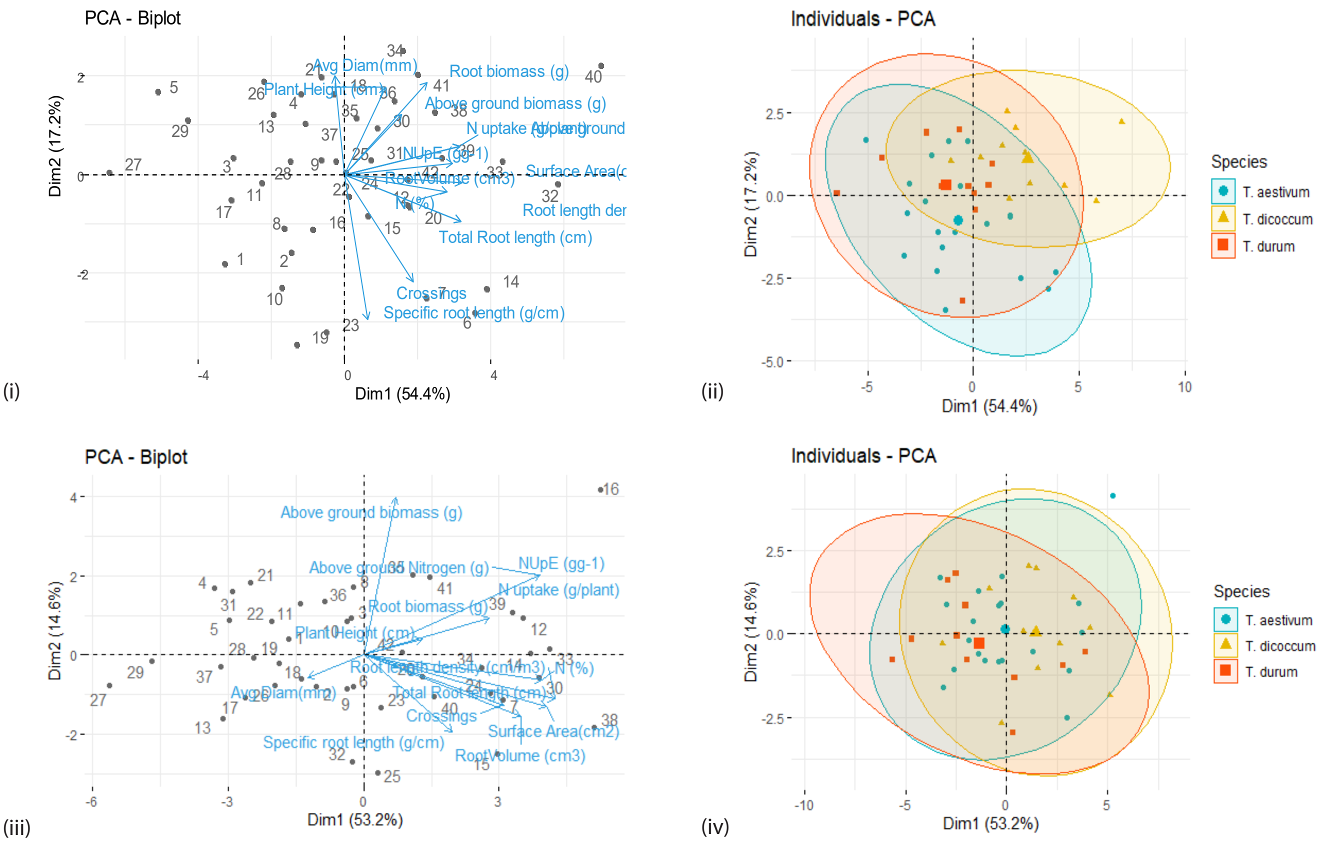

Fig. 2. Biplot of the first two principal components (i) (N_{50}) and iii (N_{100})), PCA of individuals (ii) (N_{50}) and iv (N_{100})) based on root traits and Nitrogen uptake traits for Triticum aestivum, Triticum durum and Triticum dicoccum genotypes. Genotypes coded with numbers as in Supplementary Table S1

to scavenge horizontally as well as vertically to acquire and make available sufficient N to build the minimum biomass specified by the genotype and maintain the C:N metabolism.

In general, the direction of correlation among the root traits and N uptake traits was not affected by the applied N. The correlation matrix suggested a negative relationship of total root length, number of crossings, SRL with average root diameter and between root biomass and specific root length. Similar relationships between root diameter and other root traits have been reported in other studies (Awad et al. 2018; Ghimire et al. 2020). Also, plant height is not much affected by the N levels and is a genotype dependent trait. Semi-dwarfing alleles did not significantly affect any root system trait in a study on winter wheat (Awad et al. 2018). A positive correlation was observed between total root length and N uptake shows the importance of root length for efficient N uptake. Similar relationship was observed by Ranjan et al. (2019).

Reduction in dimensions using PC analysis led to the capture of traits with high variance in the principal components. The distribution of variables in the PC biplot and the angles between the vectors was similar to the expectations from the correlation matrix. From the two PC analyses it is clear that some genotypes changed position in the PC plot when N supply was reduced from N_{100} to N_{50}. Genotypes that displayed good response for both the PCs and a consistent position in the PC plot for the two treatments are putative candidates for selection and breeding of N efficient genotypes. In the current experiment, among *T. aestivum* genotypes WH1022 (15), UASBW-13354(6) and RAJ 4248 (12) showed comparable NUpE to the the checks. Amruth (31), UAS 446 (30) and ECI26374 (22) among durum wheat genotypes and DDK50420, NP200 and DDK50332 among dicoccum genotypes were found to have superior performance for NUpE and were identified as best candidates for root system and N uptake traits. From the ranking of the genotypes, it can be inferred that higher N uptake efficiency might translate into a higher NUE if a genotype shows higher N utilisation efficiency. For instance, WH1022 shows moderate NUE in our phenotypic studies (unpublished data) but displays superior N uptake. On the other hand, UASBW 13359 has consistently performed well for total NUE and is recommended as a check, but is ranked at seventh place for N uptake. However, the current experiment underscores the importance of NUpE in breeding for NUE.

The present study sheds light on the importance of root traits variation in wheat at different depths across tetraploid and hexaploid wheat genotypes for improvement in NUE. Higher total root length and root length density and a compact root system are important characters relevant to efficient N uptake. Durum wheat genotypes were found to be relatively sensitive to N_{50} condition than *T. dicoccum* and *T. aestivum* genotypes. The tetraploid wheat genotypes showing better response for root morphological and N uptake traits as compared to the hexaploid wheat genotypes prove that their genome carries useful genetic variation for nitrogen use efficiency. The A and B genomes of the tetraploid (*Triticum* spp.) wheats are not much exploited as compared to A and B genomes of hexaploid *Triticum* species which have been demesticated (domestication syndrome) than tetraploid *Triticum* species and, therefore, can be utilized for wheat improvement with respect to NUE. However, very limited studies have been carried out on the genetic control of nitrogen use efficiency in wheat because of the complex nature of the trait. Han et al. (2015) reviewed the factors that interact with N uptake; and whether the genetic gain in NUE can be explained by NUpE or NUtE depends on the level of N supply and the time of study and it further depends on the available N in the soil. In a study on hexaploid wheat, the Qtls associated with NUE is determined whether they co-segregated with GS1 gene (glutamine synthetase) and NADH-GOGAT. Roots are directly associated with soil, water and nutrient uptake and root architecture for sustainability and adaptability for higher crop yield (Voss-Fels et al 2017). Halder et al. (2021) advocated an improved root system is essential for which they have identified candidate genes for root traits in hexaploid wheat; they further advocated marker assisted breeding.

The findings of this study will aid the breeders to focus on important root traits relevant to particular wheat species for improving NUpE and to select appropriate rooting depth and selection methodology for their experiment. A practical approach for future breeding programmes would be to use moderate N treatments for identification of superior genotypes rather than extremely high or extremely low N treatments. The moderately low level of N application allows the tolerant/NUP efficient genotypes to outcompete their counterparts and show superior performance in terms of better root growth performance and/or N uptake efficiency. In addition to this, the use of synthetic wheat for crossing programmes might help to broaden the genetic base of existing wheat cultivars in terms of nutrient use efficiency.

Authors' contribution

Conceptualization of research (SB, SAD); Designing of the experiments (SAD, SB, SD, MP); Contribution of experimental materials (SB, SAD); Execution of field/lab experiments and data collection (SD, SB, MP); Analysis of data and interpretation (SB, SAD, SD); Preparation of manuscript (SAD, SB, SD).

Supplementary material

Supplementary Tables S1 to S7 and Supplementary Fig. S1 are provided online www.isgpb.org.

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Supplementary Table S1. A list of 42 genotypes used in the study, their origin, pedigree and Nitrogen Use Effeciency response

S. no	Genotype name	Pedigree
Triticum aestivum		
1	UAS 304	SERI/CEP 80120//KAUZ/PBW 343
2	PBW 343	ND/VG 9144//KAL/BB/3/YCO"S'/4A/EE#S"S"
3	UAS BW-13355	SUP 152//ND643/2*WBLL1
4	UAS BW-13358	SITE/MO//PASTOR/3/TILHI/4/WAXWING/KIRITATI
5	UAS 323	SITE/MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC
6	UAS BW-13354	PASTOR//HXL7573/2*BAU/3/WBLL1
7	UAS BW-13359	ND/VG 9144//KAL/BB/3/YCO"S'/4A/EE#S"S"
8	UAS BW-13356	CHEN/AE.SQ//2*OPATA/3/FINSI
9	PBW 175	HD 2160/WG 1025
10	RAJ 1972	HD2195/HD2160
11	K 9107	K 8101/K 68
12	RAJ 4248	RAJ4083/WR765
13	DBW 14	RAJ 3765/PBW 343
14	UAS BW-13357	SUP 152//ND643/2*WBLL1
15	WH 1022	WH-283/UP-2338[4281][4314]
16	HD 2967	ALD/COC//URES/HD2160M/HD2278
17	C 306	RGN/CSK3 //2* C591/3/C217/N14 //C28
18	GW 322	GW 173/GW 196
19	UAS BW-13175	BAJ #1/3/KIRITATI//ATTILA*2/PASTOR*2/4/MUTUS*2/TECUE #1
20	UAS BW-13170	KUTZ*2//KFA/2*KACHU
Triticum durum		
21	UAS DW 30217	
22	EC126374	Exotic collection
23	EC45306	Exotic collection
24	MLT DW RF 7	BYBLOS/6/PLATA_6/GREEN_17/3/CHEN/AUK//BISU*2/5/PLATA_3//CREX/ALLA/3/SOMBRA_20/4/SILVER_14/ MOEWE/9/CBC 509 CHILE/6/ECO/CMH76A.722//BIT/3/ALTAR 84/4/AJAIA_2/5/KJOVE_1/7/AJAIA_12/F3LOCAL(SEL. ETHIO.135.85)//PLATA_13/8/SOOTY_9/RASCON_37//WODUCK/CHAM_3
25	Bijaga Yellow	M. LOCAL/GAZA
26	IDON VAR 1	
27	IDON VAR18	
28	UAS DW-31344	GUAYACANINIA/GUANAY/8/GEDIZ/FGO//GTA/3/SRN_1/4/TOTUS/5/ENTE/MEXI_2//HUI/4/YAV_1/3/ LD357E/2*TC60//JO69/6/SOMBRA_20/7/JUPARE C 2001/9/BAROYECA ORO C2013
29	IDSN 7013-18	JUPARE C 2001*2/IM/6/ADAMAR_15//ALBIA_1/ALTAR 84/3/SNITAN/4/SOMAT_4/INTER_8/5/SOOTY_9/ RASCON_37/7/GUAYACAN INIA/KUCUK/4/ARMENT//SRN_3/NIGRIS_4/3/CANELO_9.1
30	UAS 446	DWR 185/ DWR 2006//UAS 419
31	Amruth	ANLC/GAZA
Triticum dicoccum		
32	NP 200	Selection from Germplasm lines
33	DDK-50332	Selection from Germplasm lines from SomaningNingappaKenjal, Mugalkod
34	DDK-50366	Selection from Germplasm lines from KadappaParappaMalakannavar, Kopadatti (Yadawad) Tq.Gokak
35	DDK-50383	Selection from Germplasm lines from Hanumanth L. Pulyagol, Belagali
36	DDK-50388	Selection from Germplasm lines from Kareppa Kodli, Harogeri
37	DDK-50391	Selection from Germplasm lines from GurupadappaRudrappaHuddar, Mahalingpur
38	DDK-50403	Selection from Germplasm lines from Beerappa P Kurubali, Dhavaleshwar
39	DDK-50404	Selection from Germplasm lines from MurageppaBaramappa Latur, Malali
40	DDK-50420	Selection from Germplasm lines from VirupakshappaMugalkod, Arabhavi
41	DDK-50421	Selection from Germplasm lines from MahalingappaKankanwadi, Hirenandi, Tq-Gokak. Dist- Belgaum
42	DDK-1029	DDK 1012/HW 1093//276-15

Supplementary Table S2. Descriptive statistics (DS) for root traits analysed at three soil depths for Triticum aestivum genotypes during rabi 2020-21 at Dharwad

DS	N level	TRL (cm)	SA (cm^2)	AD (mm)	RLD (cm/m^3)	RV (cm3)	Crossings	RB (g)	SRL (g/cm^3)
(a) Top (1-30 cm)									
Minimum	N50	1346.09	181.10	0.298	133540.85	1.43	3815.50	0.35	1091.42
	N100	882.79	126.75	0.307	87578.75	1.28	1393.50	0.54	907.78
Maximum	N50	3224.21	395.57	0.721	319861.94	4.72	38561.00	1.97	6215.26
	N100	2711.16	391.67	0.681	268964.38	5.01	10799.00	1.80	2823.35
Mean	N50	2329.59	259.22	0.424	231109.80	2.64	14321.38	0.99	3046.47
	N100	1751.60	239.26	0.448	173769.76	2.64	4223.70	0.98	1919.77
σ	N50	518.03	62.92	0.112	51391.57	0.98	9719.37	0.49	1671.36
	N100	495.47	65.26	0.103	49154.12	0.97	2409.37	0.35	596.35
S.E.	N50	115.83	14.07	0.025	11491.50	0.22	2173.32	0.11	373.73
	N100	110.79	14.59	0.023	10991.20	0.22	538.75	0.08	133.35
C.V.	N50	0.22	0.24	0.265	0.22	0.37	0.68	0.50	0.55
	N100	0.28	0.27	0.231	0.28	0.37	0.57	0.36	0.31
(b) Middle (30-60 cm)									
Minimum	N50	366.74	77.68	0.280	36383.13	0.73	398.00	0.40	668.86
	N100	899.96	155.35	0.247	89281.43	1.22	2795.00	0.02	2703.70
Maximum	N50	3409.58	340.99	0.552	338251.59	4.17	30514.00	0.68	7277.71
	N100	2982.96	328.03	0.517	295928.33	3.39	15832.00	0.69	122990.09
Mean	N50	1834.44	190.02	0.373	181987.69	1.88	12968.03	0.50	3790.93
	N100	2036.90	219.69	0.358	202072.93	2.05	6336.53	0.30	13816.29
σ	N50	785.67	74.30	0.075	77943.89	0.93	8674.08	0.08	1777.01
	N100	620.80	53.57	0.064	61587.78	0.61	3259.72	0.15	27454.98
S.E.	N50	180.25	17.05	0.017	17881.56	0.21	1989.97	0.02	407.67
	N100	142.42	12.29	0.015	14129.21	0.14	747.83	0.03	6298.60
C.V.	N50	0.43	0.39	0.201	0.43	0.49	0.67	0.16	0.47
	N100	0.30	0.24	0.179	0.30	0.30	0.51	0.50	1.99
Bottom (>60 cm)									
Minimum	N50	989.12	72.64	0.301	98126.53	0.78	1227.00	0.03	4765.01
	N100	931.10	156.46	0.289	92371.25	1.40	1471.50	0.11	2114.94
Maximum	N50	3288.47	250.83	0.525	326237.25	2.94	41774.50	0.46	29916.53
	N100	3082.70	371.13	0.458	305823.44	4.15	10963.50	0.44	21963.46
Mean	N50	2045.25	190.11	0.392	202901.94	1.89	19093.69	0.22	13687.15
	N100	2059.96	242.96	0.368	201134.76	2.57	6072.06	0.21	12495.23
σ	N50	834.81	66.06	0.080	82818.26	0.65	16111.82	0.16	8880.49
	N100	705.36	69.64	0.061	69312.53	1.04	3079.09	0.12	6560.84
S.E.	N50	295.15	23.36	0.028	29280.68	0.23	5696.39	0.06	3139.73
	N100	249.38	24.62	0.021	24505.68	0.37	1088.62	0.04	2319.61
C.V.	N50	0.41	0.35	0.205	0.41	0.34	0.84	0.72	0.65
	N100	0.34	0.29	0.165	0.34	0.41	0.51	0.56	0.53

Supplementary Table S3. Descriptive statistics (DS) for root traits analysed at three soil depths for *Triticum durum* genotypes during rabi 2020-21 at Dharwad

DS	N level	Total Root length (cm)	Surface Area (cm^2)	Avg Diameter (mm)	Root length density (cm/m^3)	Root Volume (cm^3)	Crossings	Root biomass (g)	Specific root length (g/cm)
(a) Top (1-30 cm)									
Minimum	N50	1170.07	167.58	0.391	116078.82	1.59	4937.50	0.34	857.22
	N100	919.28	156.95	0.281	91198.78	1.88	1812.50	0.50	806.80
Maximum	N50	2220.66	334.27	0.712	220303.62	5.49	24400.00	1.73	3776.34
	N100	2692.12	319.73	0.705	267075.72	4.73	7179.00	1.36	2917.17
Mean	N50	1796.71	255.23	0.545	178245.35	3.23	15377.59	1.16	1963.16
	N100	1561.50	227.87	0.506	154911.20	2.85	4124.32	0.95	1795.51
σ	N50	336.65	53.65	0.123	33397.64	1.18	6455.30	0.52	1053.44
	N100	576.92	51.43	0.135	57234.61	1.02	2124.15	0.34	720.77
S.E.	N50	101.50	16.18	0.037	10069.77	0.35	1946.35	0.16	317.62
	N100	173.95	15.51	0.041	17256.88	0.31	640.46	0.10	217.32
C.V.	N50	0.19	0.21	0.226	0.19	0.36	0.42	0.44	0.54
	N100	0.37	0.23	0.267	0.37	0.36	0.52	0.36	0.40
(b) Middle (30-60 cm)									
Minimum	N50	1552.47	168.89	0.289	154014.97	1.15	1266.00	0.39	2813.25
	N100	849.39	121.07	0.280	84265.27	0.90	1465.50	0.06	2095.00
Maximum	N50	2645.83	341.04	0.499	262483.34	3.51	21035.00	0.66	6255.97
	N100	2678.83	264.54	0.513	265757.38	2.43	16634.00	0.60	25288.31
Mean	N50	2140.81	217.57	0.361	212382.02	2.03	11459.11	0.49	4524.72
	N100	1718.63	181.52	0.352	170498.61	1.66	5819.72	0.21	12419.91
σ	N50	337.10	53.47	0.066	33442.19	0.87	7286.74	0.09	1223.07
	N100	671.31	47.26	0.091	66598.20	0.58	4812.84	0.17	7792.93
S.E.	N50	112.37	17.82	0.022	11147.40	0.29	2428.91	0.03	407.69
	N100	223.77	15.75	0.030	22199.40	0.19	1604.28	0.06	2597.64
C.V.	N50	0.16	0.25	0.182	0.16	0.43	0.64	0.17	0.27
	N100	0.39	0.26	0.259	0.39	0.35	0.83	0.80	0.63
(c) Bottom (>60 cm)									
Minimum	N50	1162.46	119.71	0.304	115323.59	1.12	4629.00	0.10	5847.64
	N100	1480.54	138.24	0.278	142428.19	1.25	5869.50	0.09	15936.93
Maximum	N50	2316.82	204.00	0.366	229843.39	1.91	11024.00	0.33	14070.87
	N100	2267.02	243.71	0.427	218087.21	2.16	31942.00	0.13	20658.85
Mean	N50	1814.30	166.69	0.335	179990.37	1.49	7668.25	0.21	9953.81
	N100	1956.99	198.03	0.351	188262.46	1.90	13780.63	0.11	18009.75
σ	N50	480.50	35.53	0.026	47668.46	0.32	2622.83	0.11	3521.40
	N100	355.37	50.32	0.061	34186.97	0.44	12307.70	0.02	1957.50
S.E.	N50	240.25	2.65	0.045	108.82	0.26	29.85	0.19	46.05
	N100	177.69	3.22	0.103	73.21	0.30	160.65	0.05	14.67
C.V.	N50	0.26	0.21	0.078	0.26	0.22	0.34	0.52	0.35
	N100	0.18	0.25	0.174	0.18	0.23	0.89	0.18	0.11

Supplementary Table S4. Descriptive statistics (DS) for root traits analysed at three soil depths for *Triticum dicoccum* genotypes during *rabi* 2020-21 at Dharwad

	N level	Total Root length (cm)	Surface Area(cm^2)	Avg Diameter (mm)	Root length density (cm/m^3)	Root Volume (cm^3)	Crossings	Root biomass (g)	Specific root length (g/cm)
(a) Top (1-30 cm)									
Minimum	N50	1464.98	211.78	0.487	145334.89	2.39	4212.00	0.90	788.85
	N100	1261.33	177.03	0.298	125132.18	1.65	2729.00	0.76	875.19
Maximum	N50	2261.19	428.50	0.692	224324.75	5.08	16297.00	2.78	2124.30
	N100	2923.70	385.66	0.659	290049.16	5.33	24379.00	1.80	2633.08
Mean	N50	1786.15	286.19	0.599	177197.14	3.72	10498.59	1.70	1150.98
	N100	2033.67	273.00	0.442	201753.12	3.09	7635.73	1.15	1851.37
σ	N50	296.45	70.74	0.058	29410.21	0.82	3680.26	0.55	422.22
	N100	565.65	72.31	0.113	56116.35	1.17	6161.33	0.33	559.16
S.E.	N50	89.38	21.33	0.018	8867.51	0.25	1109.64	0.17	127.30
	N100	170.55	21.80	0.034	16919.72	0.35	1857.71	0.10	168.59
C.V.	N50	0.17	0.25	0.097	0.17	0.22	0.35	0.32	0.37
	N100	0.28	0.26	0.256	0.28	0.38	0.81	0.28	0.30
(b) Middle (30-60 cm)									
Minimum	N50	1428.18	158.06	0.303	141684.80	1.13	3610.00	0.42	2780.41
	N100	932.58	149.35	0.284	92517.64	1.46	1789.00	0.13	1549.43
Maximum	N50	3845.85	488.76	0.478	381533.10	5.55	32218.00	0.65	7369.38
	N100	3062.40	404.06	0.830	303809.23	5.70	15214.00	0.80	16386.49
Mean	N50	2804.58	292.74	0.390	278232.58	2.96	14494.50	0.53	5363.66
	N100	1836.74	253.18	0.501	182216.25	3.09	6356.59	0.45	5616.30
σ	N50	781.47	106.50	0.062	77527.11	1.56	9729.94	0.08	1382.13
	N100	584.30	87.06	0.194	57966.17	1.47	3906.54	0.22	4037.06
S.E.	N50	235.62	32.11	0.019	23375.30	0.47	2933.69	0.02	416.73
	N100	176.17	26.25	0.058	17477.46	0.44	1177.87	0.06	1217.22
C.V.	N50	0.28	0.36	0.160	0.28	0.53	0.67	0.16	0.26
	N100	0.32	0.34	0.387	0.32	0.48	0.61	0.48	0.72
(c) Bottom (>60 cm)									
Minimum	N50	1837.53	180.92	0.281	182294.97	1.39	2997.00	0.13	4781.98
	N100	990.45	133.05	0.326	95281.75	1.17	1141.50	0.10	6122.70
Maximum	N50	3460.80	414.49	0.391	343333.72	3.48	32616.50	0.39	24970.79
	N100	3352.20	361.86	0.744	322481.83	3.45	10502.50	0.40	10042.61
Mean	N50	2413.73	240.06	0.349	239457.25	2.16	12972.88	0.25	11121.27
	N100	1833.19	231.92	0.454	176353.09	2.44	4560.94	0.23	7985.87
σ	N50	672.52	78.33	0.036	66717.95	0.71	9319.99	0.09	6311.69
	N100	946.16	89.99	0.151	91020.83	0.79	3694.90	0.12	1357.32
S.E.	N50	237.77	27.69	0.013	23588.36	0.25	3295.11	0.03	2231.52
	N100	334.52	31.82	0.053	32180.72	0.28	1306.35	0.04	479.88
C.V.	N50	0.28	0.33	0.104	0.28	0.33	0.72	0.35	0.57
	N100	0.52	0.39	0.332	0.52	0.32	0.81	0.50	0.17

Supplementar Table S5. Response of top performing genotypes identified based on Nitrogen uptake efficiency for root traits and nitrogen uptake traitsin *Triticum aestivum* at moderate (N_{50}) and control (N_{100}) Nitrogen supply

S. no.	Genotype	N level	NUpE (gg^{-1})	TRL (cm)	SA (cm^2)	AD (mm)	RLD (cm/m^3)	RV (cm^3)	Crossings	RB (g)	SRL (g/cm^3)	PH (cm)	AGB (g)	N (%)	AGN (g)	NUP (g $plant^{-1}$)
Triticum aestivum																
15	WH 1022	N_{50}	0.52	5001.06	579.78	0.45	165378.83	6.47	20601.50	1.03	4846.88	71.15	17.57	0.89	0.16	0.29
		N_{100}	0.42	6393.75	825.91	0.39	211433.47	8.86	28189.00	1.06	6012.84	68.25	15.61	1.78	0.28	0.34
6	UAS BW-13354	N_{50}	0.51	8473.59	859.15	0.37	280211.28	8.77	82065.00	1.97	4310.70	72.80	15.99	0.96	0.15	0.28
		N_{100}	0.32	4888.35	758.64	0.44	161651.67	7.33	12966.50	1.24	3954.81	75.75	21.50	0.98	0.21	0.26
7	UAS BW-13359	N_{50}	0.46	8140.02	736.29	0.35	269180.60	7.04	60457.00	1.65	4932.42	87.30	15.03	0.92	0.14	0.25
		N_{100}	0.42	7088.71	736.94	0.33	234414.90	6.69	20813.50	2.28	3108.47	84.95	15.51	1.76	0.27	0.34
12	RAJ 4248	N_{50}	0.49	5785.73	706.63	0.45	191327.21	8.54	58577.00	1.62	3567.21	84.90	16.73	0.88	0.15	0.27
		N_{100}	0.54	6501.17	759.72	0.41	214985.81	7.74	16486.50	1.26	5179.60	84.60	23.63	1.49	0.35	0.44
14	UASBW-13357	N_{50}	0.48	9237.92	976.44	0.38	305486.82	9.99	74782.50	2.34	3943.46	77.10	15.39	0.94	0.14	0.26
		N_{100}	0.52	6592.24	817.02	0.43	217997.21	9.78	16500.00	1.37	4800.11	73.80	21.19	1.61	0.34	0.42
18	GW 322	N_{50}	0.48	4030.30	490.06	0.43	133277.13	5.51	15684.50	2.26	1779.45	75.55	18.74	0.77	0.14	0.26
		N_{100}	0.30	4390.28	518.68	0.40	145181.16	4.65	12792.00	1.04	4212.65	76.50	20.93	0.94	0.20	0.24
	% Change		16.24	13.43	-1.55	1.54	13.43	2.83	189.72	31.76	-14.26	1.07	-15.98	-37.35	-46.22	-20.87

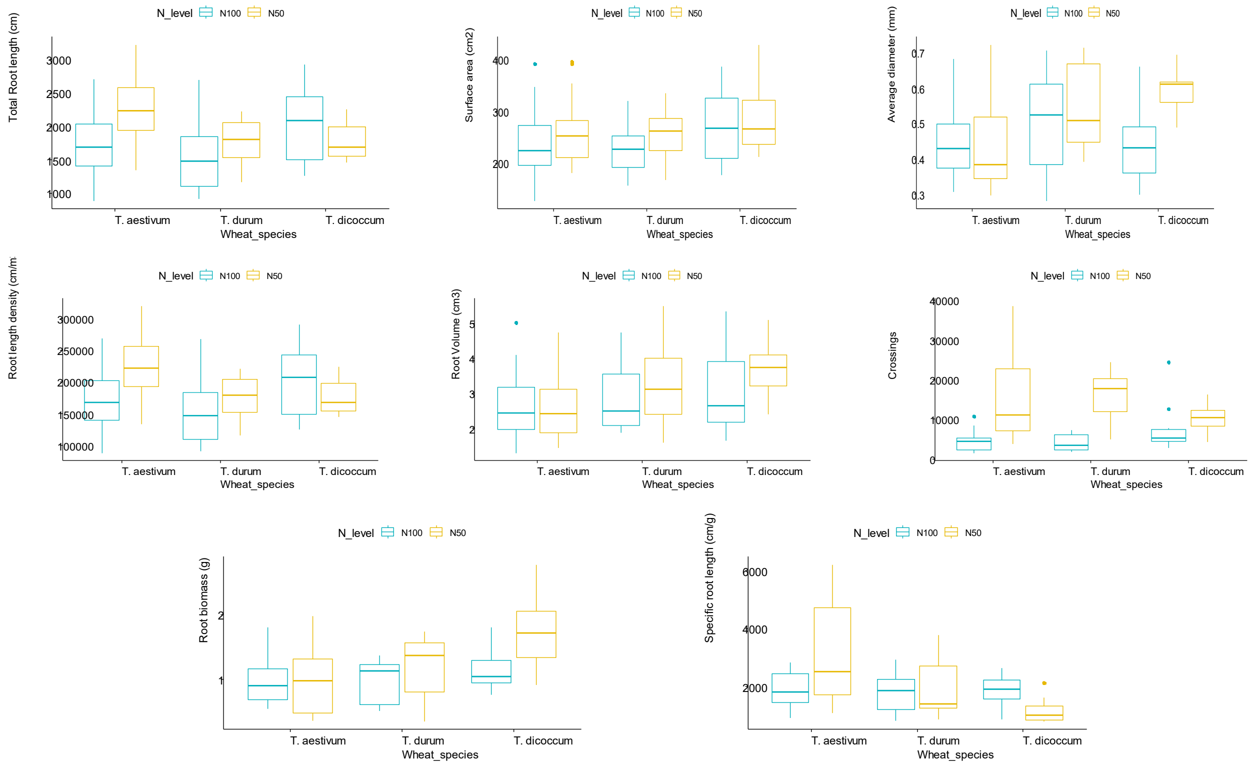
Supplementar Table S6. Response of top performing genotypes identified based on Nitrogen uptake efficiency for root traits and nitrogen uptake traitsin Triticumdurum at moderate (N50) and control (N100) Nitrogen supply

S. no.	Genotype	N level	NUpE (gg^{-1})	TRL (cm)	SA (cm^2)	AD (mm)	RLD (cm/m^3)	RV (cm^3)	Crossings	RB (g)	SRL (g/cm^3)	PH (cm)	AGB (g)	N (%)	AGN (g)	NUP (g $plant^{-1}$)
31	Amruth	N_{50}	0.55	4611.34	432.41	0.34	152491.56	3.18	37059.00	1.32	3505.64	93.25	20.83	0.80	0.17	0.30
		N_{100}	0.32	1999.66	363.05	0.57	66126.39	4.48	9392.50	1.31	1529.23	76.35	25.20	0.83	0.21	0.26
30	UAS 446	N_{50}	0.48	5707.18	620.56	0.39	188729.45	6.13	17619.00	2.58	2211.22	76.70	17.35	0.85	0.15	0.27
		N_{100}	0.49	7637.97	735.36	0.31	252578.51	5.94	21387.50	1.55	4928.04	85.10	18.10	1.77	0.32	0.40
27	Bijaga Yellow	N_{50}	0.30	6154.86	808.29	0.47	203533.80	9.15	28947.00	2.41	2553.38	97.60	10.56	0.87	0.09	0.17
		N_{100}	0.30	5717.62	611.15	0.38	189074.79	6.31	14541.00	0.90	6336.02	98.35	13.98	1.38	0.19	0.24
22	EC126374	N_{50}	0.48	4744.29	549.12	0.44	156887.87	6.50	27475.50	1.15	4128.22	75.30	18.38	0.79	0.15	0.26
		N_{100}	0.33	3191.30	384.41	0.40	105532.39	3.82	9158.00	1.28	2487.86	77.15	22.26	0.96	0.21	0.26
24	MLT DW RF 7	N_{50}	0.43	5361.61	602.84	0.41	177301.90	6.26	29424.50	2.05	2620.38	76.85	15.85	0.83	0.13	0.24
		N_{100}	0.42	5768.15	694.69	0.41	190745.64	7.94	53017.50	1.60	3604.23	81.85	20.01	1.39	0.28	0.34
23	ICARDA RI 15	N_{50}	0.39	5605.22	591.20	0.37	185357.92	5.75	51077.50	0.98	5727.25	72.70	13.98	0.84	0.12	0.21
		N_{100}	0.35	4567.32	637.86	0.49	151035.70	9.22	24917.00	1.32	3458.52	78.25	18.66	1.22	0.23	0.28
	% Change		19.31	11.43	5.19	-5.35	11.43	-1.94	44.70	31.61	-7.15	2.47	42.01	217.67	42.94	-14.63

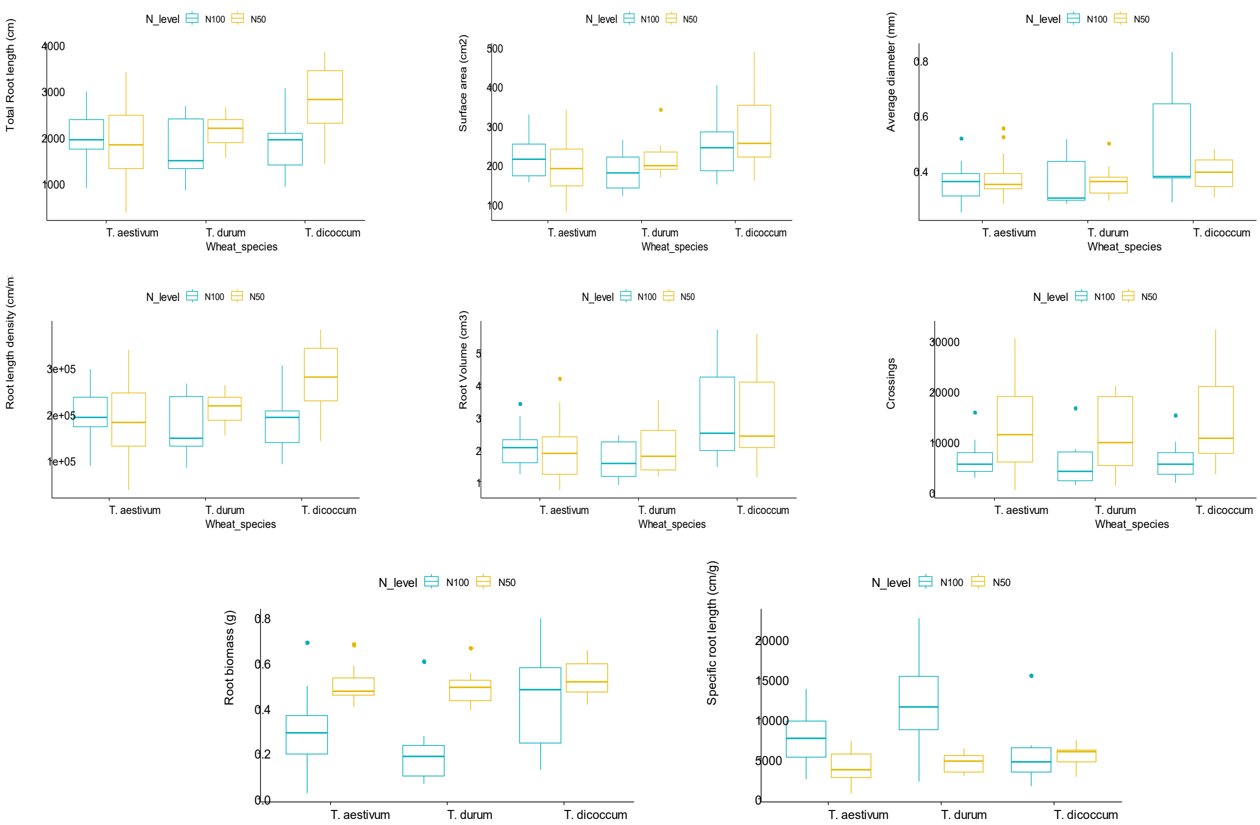
Supplementary Table S7. Response of top performing genotypes identified based on Nitrogen uptake efficiency for root traits and nitrogen uptake traitsin Triticumdicoccum at moderate (N_{50}) and control (N_{100}) Nitrogen supply

S. no.	Genotype	N level	NUpE (gg^{-1})	TRL (cm)	SA (cm^2)	AD (mm)	RLD (cm/m^3)	RV (cm^3)	Crossings	RB (g)	SRL (g/cm^3)	PH (cm)	AGB (g)	N (%)	AGN (g)	NUP (g $plant^{-1}$)
Triticum dicoccum																
33	NP 200	N_{50}	0.62	7910.94	1045.67	0.44	261605.11	10.74	42979.00	2.79	2835.60	80.05	12.39	1.53	0.19	0.34
		N_{100}	0.26	5669.99	700.98	0.53	187499.51	10.40	12028.50	1.50	3784.91	88.30	16.27	1.04	0.17	0.21
42	DDK-1029	N_{50}	0.49	6420.64	623.55	0.40	212322.83	6.17	48274.00	2.14	3000.86	87.60	17.21	0.87	0.15	0.27
		N_{100}	0.39	4301.44	592.55	0.50	142243.31	7.69	33318.00	1.72	2498.66	85.70	21.53	1.18	0.25	0.32
41	DDK-50421	N_{50}	0.69	8743.53	1061.57	0.51	289137.85	12.31	30964.50	3.77	2320.19	90.20	16.89	1.24	0.21	0.38
		N_{100}	0.36	5572.23	813.67	0.48	184266.79	7.85	20348.00	1.87	2977.32	101.25	17.91	1.33	0.24	0.29
33	DDK-50332	N_{50}	0.62	7966.69	753.60	0.41	263448.88	8.04	43565.00	2.09	3811.77	93.20	20.21	0.93	0.19	0.34
		N_{100}	0.46	6809.22	1062.25	0.59	225172.68	12.66	22746.50	2.69	2529.39	93.90	23.38	1.30	0.30	0.37
39	DDK-50404	N_{50}	0.51	7166.56	804.56	0.46	236989.47	9.78	29230.50	1.85	3867.77	89.95	19.03	0.82	0.16	0.28
		N_{100}	0.52	5135.47	778.02	0.46	169823.91	9.84	11388.50	2.08	2472.69	98.50	20.29	1.68	0.34	0.42
	% Change		47.04	39.00	8.65	-13.13	39.00	-2.87	95.35	28.20	11.03	-5.70	-13.74	-17.55	-31.74	0.18

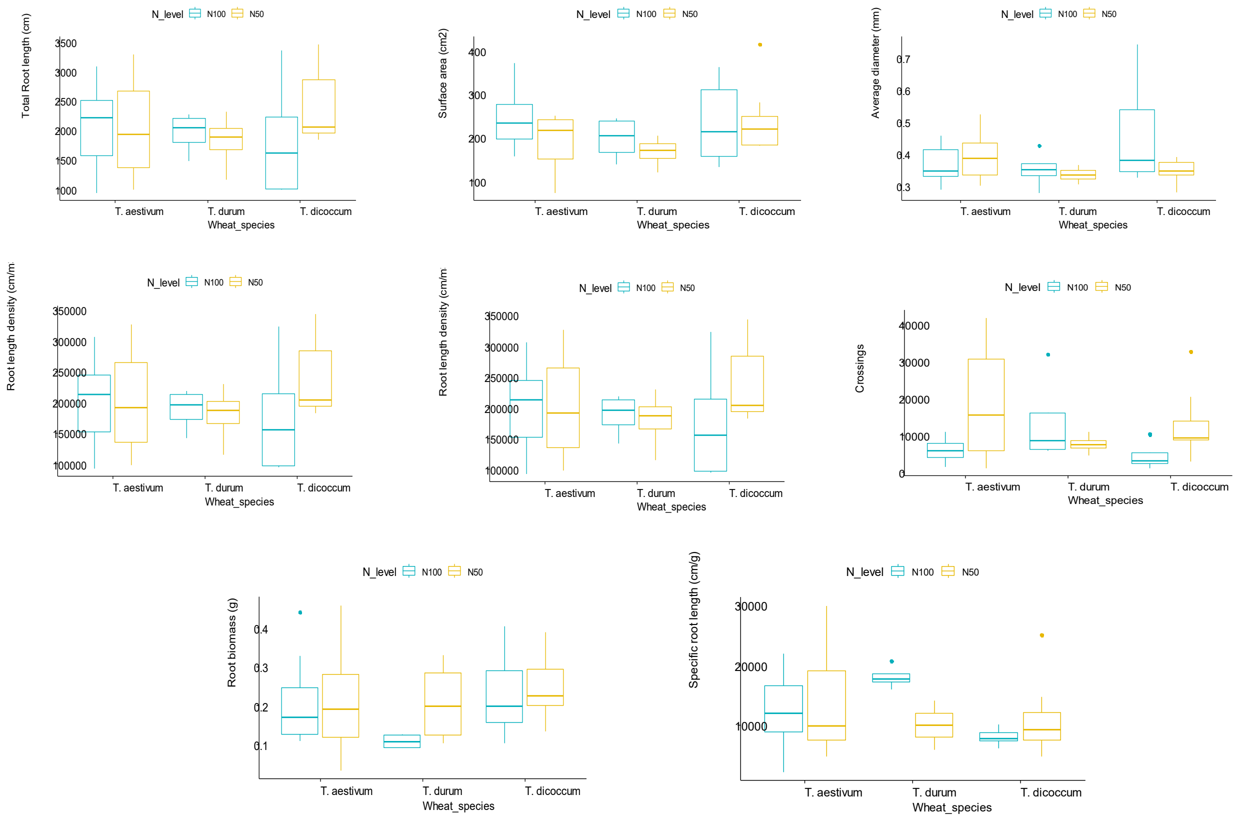
(a) Top (1-30 cm)



(b) Middle (30-60 cm)



(c) Bottom (>60 cm)



Supplementar Fig. S1. Boxplots for root traits for Triticum aestivum, *Triticum durum* and *Triticum dicoccum* at N50 and N100 ¬for (a) Top (1-30 cm), (b) Middle (30-60 cm) and (c) Bottom (>60 cm) zone of the soil column . Median values are represented by horizontal lines splitting the boxes; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; outliers are represented by dots. Variable names coded as in Supplementary Table S1