

Genotypic variations in root traits of wheat varieties at phytomer level

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Abstract

The aims of this study were to investigate genotypic variations in root traits at phytomer level of wheat varieties and for recommending a few root traits as selection parameters in future breeding programs. Two separate experiments were conducted to measure their root traits for hydroponically grown wheat plants. In Experiment 1, main axis length, root hair density and diameter differed from phytomer to phytomer at 60 days after sowing for two varieties, *Shotabdi* and *Sonalika*. Density of first order laterals at their axis of origin, dry weights of roots and shoots and root:shoot ratio varied significantly among 8 varieties. In Experiment 2, number of root bearing phytomer, total number of adventitious roots, main axis length at root bearing phytomer 1 and 2 (youngest roots were the reference point and numbered as phytomer 1), length of first order laterals at phytomer 3, root hair density and dry weights of roots and shoots were significantly different among varieties. PC1 (principal component 1) resulted in significant variation among varieties for number of live leaves, new roots appeared, number of root bearing phytomer, total number of adventitious roots, root dry weight and shoot dry weight. PC2 yielded significant difference among varieties for live leaves, main axes length at phytomer 1 & 2, number of new roots, root hair density and diameter. Selection of varieties based on main axes length at the youngest phytomer & root hair density per unit surface area along with dry weights of roots and shoots could be recommended for future breeding program as these four parameters consistently resulted in significant variation among varieties.

Keywords: Wheat, Phytomer, Root hairs, Lateral roots

Introduction

Root architecture plays an important role in agricultural productivity by modifying the efficiency of nutrient uptake of the plants from the soil (Lynch, 1995). It is reported previously that some root traits are directly related to grain yield of wheat, e.g., diameter of the roots, are directly related to harvest index and biomass at maturity (Richards and Passioura, 1989), seminal root number is strongly related to grain mass (Bengough *et al.*, 2006) and shallow & deep root weight and root biomass are positively correlated with grain yield (Ehdaie *et al.*, 2012). There is widespread evidence that root architecture and different root characteristics of many crop species varies among genotypes (Crush *et al.*, 2007; Hebbar *et al.*, 2014; O'Toole and Bland, 1987; Whalley *et al.*, 2013). In a few quite recent studies the importance of studying root architectural traits has been emphasized for adaptation of the crop varieties to various abiotic stress conditions. O'Toole and Bland (1987) described the significance of genotypic variation for adaptation to diverse environments and also in different edaphic niches in agriculture. Manschadi *et al.* (2006) examined root system characteristics of two wheat genotypes contrasting in tolerance to water limitation and assessed the functional implications on adaptation to water-limited environments and reported that drought-tolerant wheat had a compact root system, while the standard wheat variety had a more uniform rooting pattern and greater root length at depth. Manschadi *et al.* (2008) evaluated the genotypic variability exists among 30 wheat genotypes based on root system architecture quantified through fractal analysis for drought tolerance and seminal root characteristics. In their study, genotypic variations existed among wheat genotypes for growth angle and number of seminal roots at the seedling stage. Mediterranean wheat varieties had wider growth angle compared to the genotypes adapted to the deep clay soils. Besides, drought stress implications of adventitious root development and root anatomical development towards salinity resistance is also described (Maggio *et al.*, 2001; Saqib *et al.*, 2005). Saqib *et al.* (2005) reported that under saline-waterlogged conditions the development of adventitious nodal roots and cortical root aerenchyma improves Na^+ exclusion and salt resistance.

Previous studies identified a few root traits which are responsive towards different stress factors with variability among genotypes (e.g., Hurd, 1968; Hurd, 1974; Richards and Passioura, 1989). Hurd (1968) reported root growth differences among seven Canadian bread wheat cultivars. In the study of O'Brien (1979) ten wheat genotypes differed in their pattern of root development for the length of main axis at 7 and 8 weeks' of growth. The author reported that length of root main axis was influenced by tiller number and rate of tiller production. In some other previous studies length and number of both nodal and seminal roots, root elongation rate, rooting depth and vertical proportions of root weights were significantly different among genotypes as reviewed by O'Toole and Bland (1987).

Even though root traits directly affects economic yield of wheat and varies among wheat genotypes but majority of the previous studies ignored measuring traits related to lateral roots for varietal screening probably because: i) root measurements are time consuming and laborious, ii) efficient and easy measuring technique is unavailable and also may be, iii) most responsive and 'representative' root traits are yet to identify. On the otherhand, studying root growth and development at the phytomer level and at the phyllochron time-scale is a comparatively newer concept (Robin, 2011; Robin *et al.*, 2010) which can be adopted to identify the variable root traits hydroponically to recommend for the future breeding programs. As far as the literature explored none of the previous studies included traits related to root hairs for assessing genotypic variation. This study therefore, attempts to investigate root growth and development of 13 wheat varieties at the phytomer level to select out the variable root traits, including the traits related to root hairs.

Materials and Methods

The experiment was conducted at the Department of Genetics and Plant Breeding, Bangladesh Agricultural University. Seeds of thirteen elite wheat varieties were collected from the Wheat Research Centre of the Bangladesh Institute of Agricultural Research, Gazipur (Table 1). Two separate experiments were conducted during *rabi* season (November to February) of 2012 – 2013 in Bangladesh. Experiment 1 was conducted with 8 selected varieties out of 13. Seeds of the selected varieties were placed for germination on 26 November 2012. Experiment 2 was started one month later from 26 December 2012. Seeds were germinated in clean tap water floated in foam net inside the plastic trays in both experiments (Fig. 1a). Around 200 seeds were germinated per tray for each variety in order to select 15 healthy seedlings with synchronized leaf appearance at transplanting in hydroponic nutrient solution. Seed germination process took 3 to 4 days. Seedlings were transplanted in hydroponic solution following a completely randomized design with 15 and 10 replicates for each of the thirteen varieties in Experiment 1 and Experiment 2, respectively. There were 10 individual trays; each tray contained 13 individual plants, for setting up the whole experiment in both Experiment 1 (8 varieties x 15 replicates) and Experiment 2 (13 varieties x 10 replicates). Each tray contained 13 plants, one from each variety. Thus a total of 120 and 130 individual plants were maintained in Experiment 1 and Experiment 2, respectively. Plants in each tray rotated their position randomly every week to avoid any position effect. Leaf appearance interval of all varieties was monitored for two weeks and that was calculated around 10 days (90°C days). Plants were fed with following nutrient solution: 1mM NH_4NO_3 , 0.6mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.6mM $\text{MgCl}_2 \cdot \text{H}_2\text{O}$, 0.3mM K_2SO_4 , 0.3mM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 50 μM H_3BO_3 , 90 μM Fe-EDTA, 9 μM $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.7 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 μM $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ dissolved in tap water. The nutrient solution was refreshed weekly. All plants were under similar management until the destructive harvest.

In Experiment 1, destructive harvests were carried out at three different time points: i) on 50 days after sowing (DAS), a subset data was recorded at phytomer level for 3 replicates only for the variety *Shotabdi* for the following traits: number of root bearing phytomers (youngest root bearing phytomer, Pr 1, was the reference point (e.g., Yang *et al.*, 1998) & roots per phytomer; diameter and length of main axis, first order laterals, second order laterals and root hairs; densities of first & second order laterals and root hairs at their axis of origin, ii) on 60 DAS, subset of data were recorded at phytomer level with 4 replicates for the two varieties: *Shotabdi* and *Sonalika* for the same root traits as likes as the first harvest, iii) on 66 DAS, data were recorded for 8 varieties with 5 replicates only for the root bearing phytomer position 5 (Pr5, youngest roots were the reference point and numbered as phytomer 1) for all root traits similar to the first harvest along with number of live leaves and adventitious roots per main tiller.

Table 1. Description of the thirteen wheat varieties used for studying root growth and development hydroponically

| Variety | Year of release | Plant height | Growth duration (days) | Panicle initiation/ (days) | Yield, (t ha ⁻¹) | Special characteristics |
|-------------------------|---------------------|--------------|------------------------|----------------------------|------------------------------|--|
| Shotabdi | 2000 | 90 – 100 | 105 – 112 | 65 – 69 | 3.6 – 5.0 | Heat tolerant |
| Shourov | 1998 | 90 – 100 | 102 – 110 | 60 – 67 | 3.5 – 4.5 | Leaf rust tolerant |
| Sufi | 2005 | 90 – 102 | 100 – 110 | 58 – 62 | 3.6 – 4.8 | Leaf rust resistant, heat tolerant |
| Prodeep | 2005 | 95 – 100 | 102 – 110 | 64 – 66 | 3.5 – 5.1 | Leaf rust resistant, heat tolerant, good baking quality |
| Bijoy | 2005 | 95 – 105 | 103 – 112 | 60 – 65 | 4.3 – 5.0 | Heat tolerant |
| BARI gom25 | 2010 | 95 – 100 | 102 – 110 | 57 – 61 | 3.6 – 4.6 | Salt tolerance 6-8 dS cm ⁻¹ |
| BARI gom26 | 2010 | 92 – 96 | 104 – 110 | 60 – 63 | 3.5 – 4.5 | Leaf rust resistant, leaf spot tolerant |
| BARI gom27 ¹ | 2012 | 80-90 | 105-110 | 63-67 | 3.5 – 5.4 | Moderately heat tolerant, saline prone >8.0 dSm ⁻¹ |
| BARI gom28 ¹ | 2012 | 80-90 | 102-108 | 58-62 | 4.0 – 5.5 | Moderately terminal heat tolerant, saline prone >8.0 dSm ⁻¹ |
| Kheri ² | Indigenous cultivar | 113 | 110-115 | 65-69 | 2.5 – 3.0 | |
| Sonalika ² | | 92 | 100-110 | 58-62 | 30 – 3.5 | |
| Kanchan | 1983 | 90 – 100 | 106 – 112 | 60 – 68 | 3.5 – 4.6 | Leaf rust susceptible |
| Akbar | 1983 | 85 – 90 | 103 – 108 | 50 – 55 | 3.5 – 4.5 | Leaf rust tolerant |

Source: (Mondol *et al.*, 2011), ¹data collected from Wheat Research Centre of Bangladesh Agricultural Research Institute, ²collected by the authors from the field grown plants.

In Experiment 2, destructive harvests were carried out at two time points for all 13 varieties: the first one was on 40 DAS to record number of live leaves, adventitious roots, seminal roots, and dry weights of roots and shoots of the main tiller from 5 replicates. The second harvest was carried out on 57 DAS to record data on number of new roots formed at the Pr1, root bearing phytomer, adventitious roots, seminal roots, main axis length at Pr1-Pr4, maximum primary axis length at Pr3, root hair density with the diameter of the axis of origin, root hair length and diameter, root and shoot dry weights etc. All data were recorded for the main tiller only, with 3 replicates.

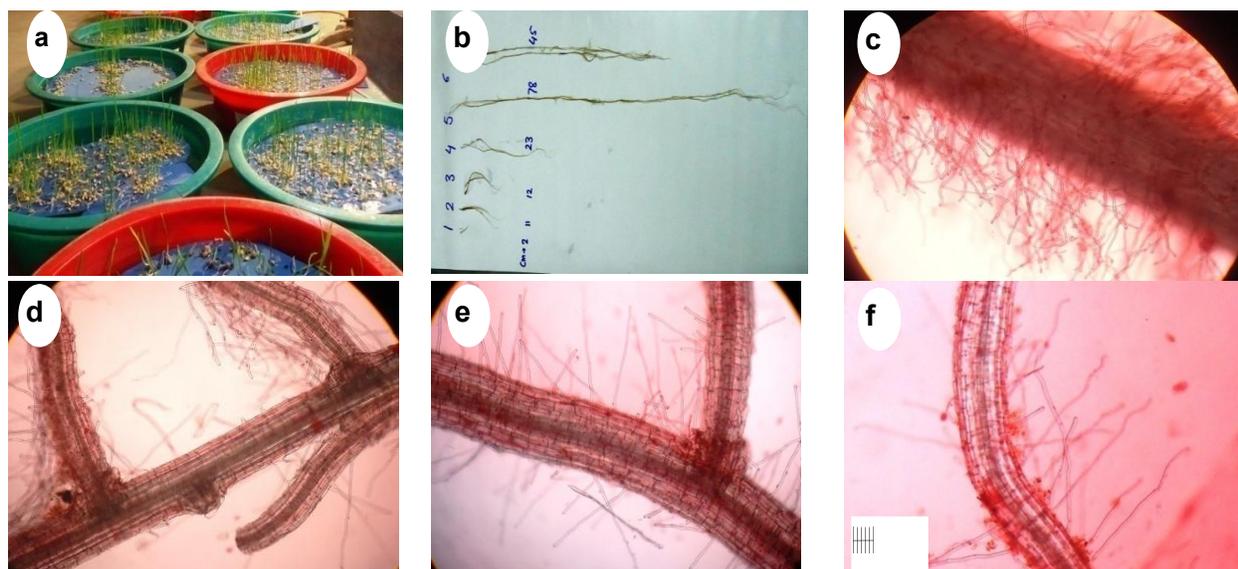


Fig. 1. Experimentation with the variable root traits of wheat at the phytomer level. a) Germination process of wheat seeds in tap water, b) Main axes collected from six different root bearing phytomers of wheat variety *Shotabdi*, c) numerous root hairs originated from a main axis of *Shotabdi*, d) a first order lateral with three second order laterals and root hairs of *Sonalika*, e) a primary branch with root hairs of *Sonalika* and f) a secondary branch of *BARI gom27* with root hairs. Root hair density and other measurements were carried out at 100x magnification. One small division at 100x is equivalent to 10 μ .

Diameter of the main axis, root hair density, length and diameter of root hair were measured at 100 x magnifications under a light microscope (Fig. 1). Safranin solution of 0.5% prepared in 50% alcohol was used for staining root hairs. Main axis elongation rate between Pr1 and Pr2 was measured from length gain at Pr2 from Pr1 per day (using data for phyllochron per day).

Data were analyzed using MINITAB 15 statistical software package to find out variations: i) among successively more developed phytomer of the same variety, *Shotabdi*, at the first harvest of Experiment 1 for different root treatments measured, ii) variation between two harvests, 50 DAS versus 60 DAS, of the same variety in the Experiment 1, iii) variations between two varieties, *Shotabdi* versus *Sonalika*, on 60 DAS for root development at phytomer level in Experiment 1, iv) variations among 8 varieties in Experiment 1 on 66 DAS, v) variations among 13 varieties on 40 and 57 DAS for the measured variables in Experiment 2. Analysis of variance was conducted using general linear model command. Principal component analysis, for the data obtained in Experiment 2, was carried for the selected traits, based on genotypic variations for root traits, of both experiments to find out the varietal differences associated with root traits.

Results

Experiment 1

Root hair diameter varied significantly among phytomer position (Fig. 2). Root hairs developed at Pr1 and Pr2 were greater in diameter than those originated from first and second order laterals measured at Pr3-4 and Pr5, respectively (Fig.1 & 2). Diameter of the root hairs had significant correlation with their axis of origin (Fig.1 & 2, $r = 0.515$, $p = 0.002$). Main axis length increased from Pr1 towards Pr5 for *Shotabdi*, measured on 50 and 60 DAS (Fig. 1b & 3a) and also for *Shotabdi* and *Sonalika*, measured on 60 DAS (Fig. 3b). Root hair density was initially recorded in per mm root length, decreased marginally from younger towards the older phytomer positions (Fig. 4, $p = 0.09$). As root hairs were not always measured at the similar axis of origin, e.g., either at main axis or at first order of laterals, therefore the density was converted to per mm^2 surface area (Fig. 4). Root hair densities in per mm^2 surface area were much higher at the main axes of Pr1-2 compared to that in per mm length (Fig.4).

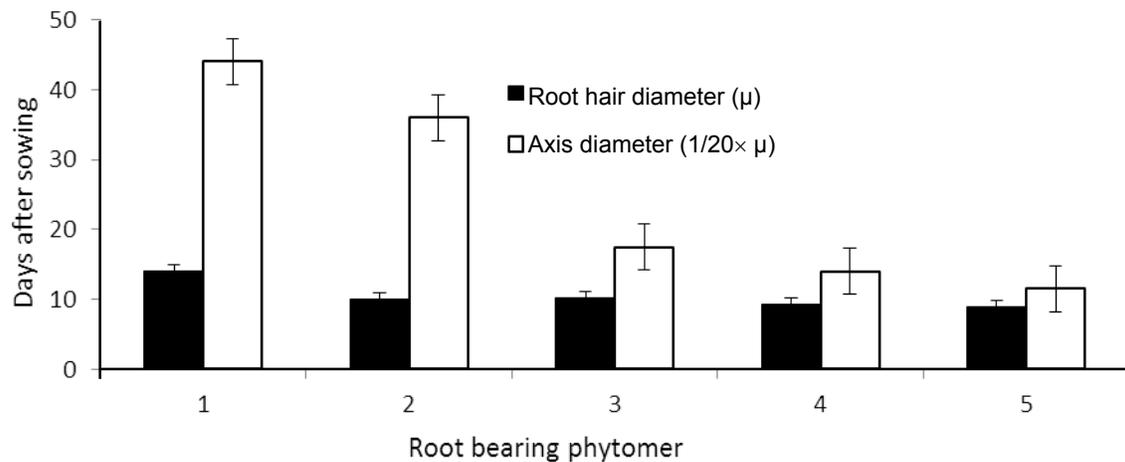


Fig. 2. Diameter of the root hairs (black bars) and their axis of origin (white bars) of wheat variety *Shotabdi* in Experiment 1 on 50 DAS at different root bearing phytomers. The axis of origin for root bearing phytomer (Pr) 1-2 refers main axis and those for Pr 3-5 refers first order laterals. Vertical bars indicate standard error mean. P values are 0.001 for black bars and <0.001 for white bars.

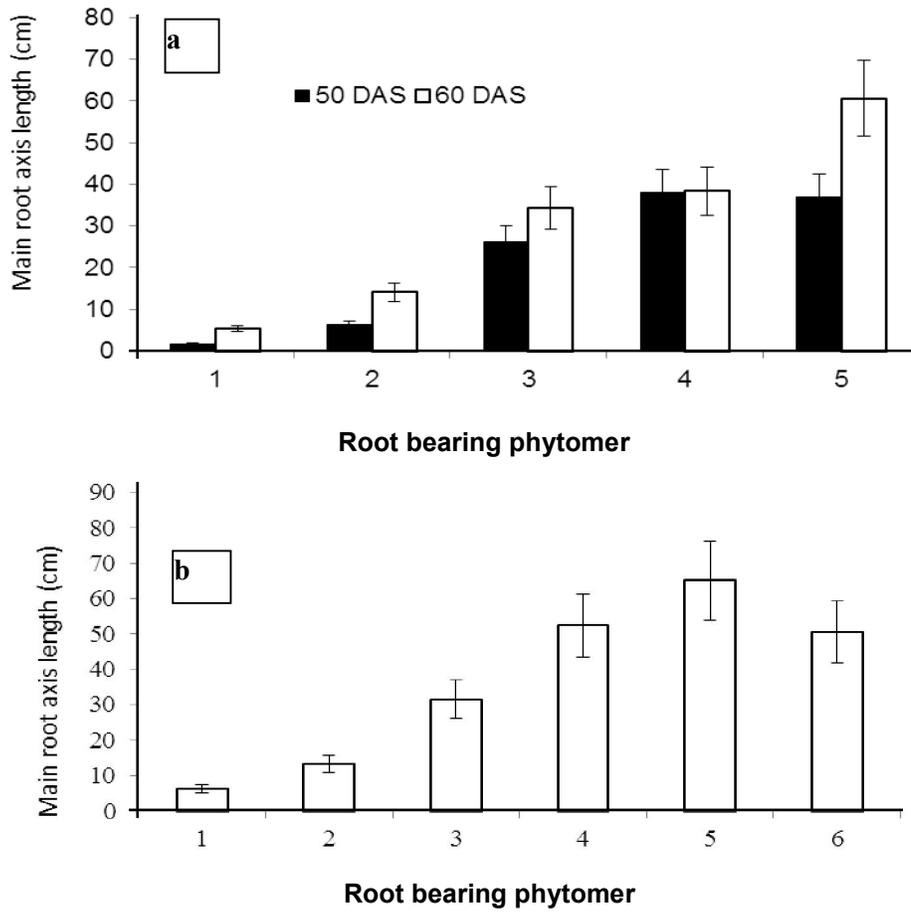


Fig. 3. Main axis length at different root bearing phytomer position of wheat in Experiment 1 for: a) variety *Shotabdi* on 50 and 60 DAS and b) two varieties *Shotabdi* and *Sonalika* 60 DAS. Vertical bars show back-transformed standard error of means in % of the log-transformed data. P < 0.001

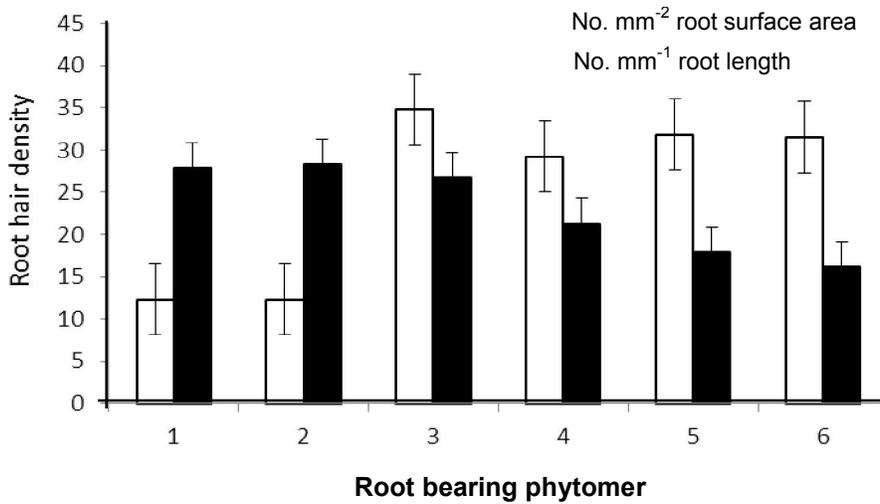


Fig. 4. Root hair density at different phytomer position (Pr) of *Shotabdi* and *Sonalika* in Experiment 1 accounted for 1 mm root length (black bars) and estimated for per mm² surface area (white bars) of main axis for Pr1-2, first order laterals Pr3-5 and second order laterals Pr 6. Vertical bars indicate standard error mean. P value is 0.001 for both white and black bars

Densities of the first order laterals originated per mm main axis were significantly different among varieties (Table 2, $p = 0.027$). *BARI gom27* accounted for the highest number of first order laterals per mm main axis (Table 2). Similar to density of first order laterals, dry weights of roots and shoots of the main tiller were also significantly differed among 8 varieties in Experiment 1 (Table 2). The variety *Shourov* accounted for the highest root and shoot dry weights followed by *BARI gom28* (Table 2). Even though both root and shoot dry weights differed significantly among varieties but the variation for root:shoot ratio was marginal (Table 2). Unlike both root and shoot dry weights, *Sonalika* accounted for the highest root:shoot ratio (Table 3).

Table 2. Density of first order laterals at the axis of origin, dry weights of roots and shoots and root:shoot ratio of 8 wheat varieties in Experiment 1. SEM, standard error or mean; P, probability of statistical significance

| Variety | Density of first order laterals (no. per mm main axis) | Root dry weight (g) | Shoot dry weight (g) | Root:shoot ratio |
|------------|--|---------------------|----------------------|------------------|
| Shotabdi | 4.8 | 0.22 | 0.80 | 0.29 |
| Shourov | 4.0 | 0.42 | 0.98 | 0.43 |
| BARI gom25 | 4.8 | 0.29 | 0.83 | 0.34 |
| BARI gom27 | 5.8 | 0.20 | 0.61 | 0.34 |
| BARI gom28 | 4.0 | 0.40 | 0.92 | 0.45 |
| Sonalika | 4.8 | 0.24 | 0.47 | 0.51 |
| Kanchan | 4.6 | 0.31 | 0.69 | 0.44 |
| Akbar | 5.0 | 0.29 | 0.72 | 0.38 |
| SEM | 0.8 | 0.051 | 0.09 | 0.05 |
| P value | 0.027 | 0.04 | 0.013 | 0.06 |

Table 3. Morphological description of root and shoot traits of 13 wheat varieties on 40 DAS in Experiment 2. SEM, standard error or mean; P, probability of statistical significance

| Variety | No. of adventitious roots | Root dry weight (mg) | Shoot dry weight (mg) | Root:shoot ratio |
|------------|---------------------------|----------------------|-----------------------|------------------|
| Shotabdi | 7.2 | 38 | 136 | 0.29 |
| Shourov | 9.4 | 54 | 128 | 0.41 |
| Sufi | 9.0 | 48 | 168 | 0.27 |
| Prodeep | 12.0 | 76 | 170 | 0.47 |
| Bijoy | 10.2 | 52 | 188 | 0.29 |
| BARI gom25 | 9.4 | 58 | 126 | 0.46 |
| BARI gom26 | 7.6 | 60 | 118 | 0.51 |
| BARI gom27 | 9.4 | 56 | 124 | 0.45 |
| BARI gom28 | 6.6 | 28 | 136 | 0.21 |
| Kheri | 6.6 | 26 | 100 | 0.26 |
| Sonalika | 8.6 | 66 | 168 | 0.39 |
| Kanchan | 8.0 | 40 | 108 | 0.36 |
| Akbar | 6.0 | 50 | 150 | 0.37 |
| SEM | 0.79 | 8.23 | 16.0 | 0.045 |
| P value | <0.001 | 0.003 | 0.006 | <0.001 |

Experiment 2

As it was reflected by the PC1 scores in Experiment 1 for varietal difference number of adventitious roots per plant was significantly different among 13 varieties in Experiment 2 both on 40 DAS (Table 3) and 57 DAS (Table 4). As like as Experiment 1, dry weights of roots and shoot and root:shoot ratio were also significantly differed among 13 varieties in Experiment 2 (Table 3). Root:shoot ratio was non-significant on 57 DAS for the same varieties, even though dry weights for both roots and shoots remained significant (Table 4). As PC1 in Experiment 1 signaled, main axis length at Pr1 and Pr2 differed significantly and length of first order laterals at Pr3 and root hair density per mm² surface area differed marginally among 13 varieties in Experiment 2 (Table 4). Number of root bearing phytomers was also differed highly significantly among 13 varieties as this trait was also appeared to show a significant contrast among 8 varieties in Experiment 1 (Table 4). Number of new roots appearance at Pr1 between 50 and 57 DAS had also significant variations for varieties (Table 4). Although number of live leaves did not produce any significant difference among varieties in Experiment 1 and on 40 DAS in Experiment 2, but showed significant variations on 57 DAS (Table 4). Other measured variables including main axis length at Pr4 & Pr5, main axis elongation rate between Pr1 and Pr2, root hair diameter and length ranged from 12.3 to 40.7 cm, 16.3 to 53.5 cm, 0.78 to 3.3 cm per day, 9 to 11 μ and 767 to 1300 μ , respectively, were non-significant for 13 varieties. PCA shows that PC1 and PC2 accounted for 42.4 and 14.2% data variation, respectively and both of the PC components resulted in significant variation among varieties for their respective PC scores (Table 5). PC1 had significant contrast for number of live leaves; new roots appeared between 50 and 57 days, root bearing phytomer, total adventitious roots and dry weights of roots and shoots for their negative co-efficient values for all of these traits (Table 5). That means, this PC separates varieties according to the 'plant size'. PC2 separates varieties significantly with positive co-efficients for number of live leaves, main axis length at Pr1 & Pr2, root hair diameter and negative co-efficients for new roots appeared between 50 and 57 days and root hair density (Table 5).

Table 4. Morphological description of root and shoot traits of 13 wheat varieties on 57 DAS in Experiment 2. Pr, root bearing phytomer position; SEM, standard error or mean; P, probability of statistical significance

| Variety | Live leaves | No. of new roots | No. of root bearing phytomer | No. of adventitious roots | Main axis length at Pr1 | Main axis length at Pr2 | Primary branch length at Pr3 | Root hair density (no. mm ⁻²) | Root dry weight (mg) | Shoot dry weight (mg) |
|------------|-------------|------------------|------------------------------|---------------------------|-------------------------|-------------------------|------------------------------|---|----------------------|-----------------------|
| Shotabdi | 2.7 | 1.0 | 4.3 | 9.0 | 11.7 | 27.8 | 2.33 | 16.5 | 20 | 180 |
| Shourov | 3.0 | 1.3 | 5.0 | 12.7 | 28.7 | 40.3 | 5.67 | 16.6 | 80 | 260 |
| Sufi | 2.3 | 0.7 | 4.0 | 8.0 | 12.7 | 15.0 | 3.17 | 11.9 | 40 | 180 |
| Prodeep | 5.3 | 5.3 | 7.0 | 17.7 | 5.5 | 15.8 | 1.50 | 27.1 | 160 | 490 |
| Bijoy | 3.0 | 2.0 | 4.7 | 9.0 | 5.7 | 11.3 | 5.17 | 20.7 | 50 | 230 |
| BARI gom25 | 3.3 | 2.0 | 5.7 | 12.7 | 6.0 | 20.7 | 3.83 | 22.1 | 70 | 220 |
| BARI gom26 | 2.3 | 3.3 | 4.3 | 10.0 | 3.0 | 10.8 | 4.00 | 26.9 | 50 | 170 |
| BARI gom27 | 2.3 | 2.0 | 4.3 | 10.0 | 9.5 | 24.5 | 5.83 | 35.0 | 40 | 200 |
| BARI gom28 | 2.0 | 1.3 | 5.3 | 10.0 | 8.7 | 41.7 | 2.50 | 22.9 | 50 | 280 |
| Kheri | 3.0 | 0.0 | 4.7 | 8.3 | 26.8 | 47.3 | 6.33 | 15.3 | 40 | 170 |
| Sonalika | 2.0 | 1.3 | 4.0 | 8.3 | 16.7 | 36.3 | 4.00 | 15.7 | 30 | 150 |
| Kanchan | 2.7 | 1.0 | 4.7 | 9.3 | 10.3 | 37.3 | 6.00 | 12.0 | 50 | 280 |
| Akbar | 2.0 | 1.0 | 4.7 | 9.7 | 10.5 | 34.0 | 4.33 | 28.5 | 60 | 320 |
| SEM | 0.49 | 0.64 | 0.36 | 1.44 | 5.15 | 7.22 | 1.08 | 1.6 | 17 | 52 |
| P value | 0.003 | 0.001 | <0.001 | 0.005 | 0.035 | 0.01 | 0.067 | 0.087 | 0.001 | 0.007 |

Table 5. Major principal components (PC) and their coefficients from principal component analysis of the root traits at the phytomer level along with shoot traits for 13 wheat varieties on 57 days after sowing. Pr, root bearing phytomer; p, probability of statistical significance of the PC (principal component) scores among varieties

| Traits | PC1 | PC2 |
|--|--------|-------|
| Number of live leaves | -0.34 | 0.34 |
| Number of new roots formed between 50 and 57 DAS | -0.38 | -0.31 |
| Number of root bearing phytomers | -0.40 | 0.18 |
| Total number of adventitious roots | -0.42 | 0.07 |
| Main axis length at Pr1 | 0.14 | 0.40 |
| Main axis length at Pr2 | 0.13 | 0.50 |
| Root hair density | -0.17 | -0.47 |
| Root hair diameter | 0.06 | 0.21 |
| Root hair length | -0.11 | -0.14 |
| Shoot dry weight | -0.38 | 0.18 |
| Root dry weight | -0.42 | 0.16 |
| % variation explained | 42 | 14 |
| P value (variety) | <0.001 | 0.009 |

Discussion

Phytomer difference

A shorter main axis length at Pr6 compared to Pr5 suggested that the root position perhaps achieved maximum possible axis length at Pr6 (Fig. 3b). The youngest root bearing phytomer receives most part of photoassimilate and that decreases gradually towards the older phytomer (Matthew and Kemball, 1997). Substrate supply usually diminishes below Pr6 in perennial ryegrass (Matthew and Kemball, 1997; Robin, 2011). Pr6 was developed at the very early stage, around 50 days before the day of destructive harvest when plant size was small. Root hair density was directly correlated with surface area of axis of origin (Fig. 4, $r = 0.365$, $p = 0.014$) suggesting that the more the epidermal cells available the higher the chance of root hair formation.

Varietal difference

Main axis length at Pr1 and Pr2 brought significant difference among 13 wheat varieties (Table 4) but at Pr3 and Pr4 that difference was not significant (data not presented). It is important to note couple of issues in comparing main axis length among varieties: i) main axis elongated at the Pr1 and Pr2 at a faster rate compared to the older root bearing phytomers (Robin, 2011), ii) main axis for a particular Pr at different replicates of same variety and different varieties did not appear at the same day, iii) destructive harvest process took 3-5 days across two experiments. Therefore in any further experimentation main axis elongation rate might be accounted in thermal time in rhizochron (root appearance interval) time scale for more validity. Significant difference in main axis elongation rate suggests that estimating main axis elongation would be regarded as an important selection criterion. PC2 indicated that main axis length at Pr1 and Pr2 is positively associated with number of live leaves present in main tiller (Table 5). Root growth and root elongation rate were also significantly different in some previous studies (see O'Toole and Bland, 1987; Shahzad *et al.*, 2012). First order laterals generally initiate at the Pr2 or Pr3 (Robin, 2011). Significant variation in length of first order laterals at Pr3 suggested that at Pr3 the laterals were at active elongation process, the same happens for main axis at Pr1 and Pr2.

PC1 showed that new root formation, number of root bearing phytomers, total number of adventitious roots per plant, and dry weights of roots and shoot are closely associated and hence directly dependent to number of live leaves per plant (Table 5, Robin, 2011). Dry weights of roots and shoots were consistently different among varieties across the experiments (Table 2 & 4, Chen *et al.*, 2005; Yumurtaci

and Uncuoglu, 2012). The results suggested these two traits should be always regarded as selection parameters. Root hair density was marginally different among 13 wheat varieties in Experiment 2 (Table 4) suggesting that this trait is important to consider as previous reports also indicated that under various stress condition root hair density was variable; e.g., Haling *et al.* (2010) under acidic and alkaline condition and Shabala *et al.* (2003) under various Na/Ca ionic ratios.

Conclusion

Based on the results of the two experiments the following root traits can be recommended for future breeding program: i) main axis length at the youngest 2-3 root bearing phytomer, ii) density and diameter of the root hairs along with root dry weight and shoot dry weight of the main tillers as all those traits were consistently significant across two experiments. The data can be also used for physiological growth modeling of roots, e.g., C budgeting of the wheat plants towards root construction.

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Author contributions

AHKR planned and designed experiments; analyzed data and written the manuscript. MJU, SA and PRP assisted AHKR managing plants and data collection.

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