



## Root architectural traits and yield stability in popular wheat (*Triticum aestivum*) varieties of India

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### ABSTRACT

The root system architecture has bearing on realizing the yield potential of genotypes. The 24 popular wheat (*Triticum aestivum* L.) varieties released during the last 50 years for North Western Plain Zone (NWPZ) of India were used to study root traits and yield contributing traits two years and three locations. Association between the deeper root angles and yield were significant and the number of tillers is also associated with the number and angular distribution of crown roots. There is a relative adjustment in yield attributing and root architectural traits of varieties. The varieties HD3086, PBW502, WH1124, DPW621-50, PBW550 and WH1105 have largely modulated the yield through root traits while, the varieties DBW17, WH1142, HD2967, HD2009 and HD2687 have modulated yield through TGW and tillers along with roots. The other old varieties were modulating yield through aboveground traits only. There were significant genotypic effects for deeper crown root angles, days to heading, and tillers per meter square and thousand-grain weight. WH1080 has the best combination of all the traits. The genotype  $\times$  environment and environmental effects were significant for other traits. Inclusion of crown root angles, mesocotyl length along with other above-ground traits in selection can help in designing better genotypes for future.

**Key words:** Root architecture, Spring wheat varieties, Stability, Yield

Wheat (*Triticum aestivum* L.) is the main cereal crop of India enabling food security. North-Western Plains Zone (NWPZ) of the country contributes largely to the national pool. The annual wheat production and productivity gradually improved over the last 50 years at the average rate of about one percent after the green revolution period (Gupta *et al.* 2016). The selection indices based on above-ground traits has been largely considered for development of most of the varieties released in India. The search for new and ideal plant type always remains a matter of pursuance for breeders especially to breakthrough the continuing slow progress in improvement in productivity (Bainsla *et al.* 2018a). More than 500 varieties have been released in India for cultivation in plains since the onset of the green revolution in the 1960s. However, only a few varieties could become mega varieties owing to their stable production across the years and locations. The competitive entries from the different centers in the zone are selected based upon the best combination of yield and disease resistance. The process of fine-tuning of the selection criteria based on above-ground traits are more challenging due to low explainable genetic variance among competing genotypes.

The relation between the number of tillers and the crown roots, which feed these tillers with nutrients and water in addition to giving anchoring support to sustain them, is of utmost importance. The yield attributes post-anthesis are also very important and are also dependent on roots. The complexity of interactions makes the realization of yield more subjective especially near the grain filling stage by terminal heat and lodging (Bainsla *et al.* 2018).

Scanty information is available on the root system architecture of Indian wheat varieties and their possible role in determining the expressed yield levels especially, under timely sown irrigated conditions. The present study is an attempt to understand the role of root architecture and the possibility for root-based selection along with above-ground parameters to realize a further gain in yield potential.

### MATERIALS AND METHODS

Twenty-four popular spring wheat varieties (Table 1), released between 1967 and 2015, well adapted to North Western Plain Zone (NWPZ) conditions were taken for study. The varieties were grown under timely sown irrigated conditions during *rabi* 2015-16 and *rabi* 2016-17 at three locations, viz. research farms of Indian Agricultural Research Institute, New Delhi, Chaudhary Charan Singh Haryana Agricultural University, Hisar and ICAR- Central Soil Salinity Research Institute, Karnal representing the

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Table 1 List of varieties, their origin and year of release

| Variety          | Cross/Designation                       | Year of release |
|------------------|-----------------------------------------|-----------------|
| Kalyan Sona (KS) | FN//K58/NTH//N10B/4/GB55                | 1967            |
| WL711            | NP/TOB'S'/3/8156//KAL/BB                | 1977            |
| WH157            | HD1981/RAJ821                           | 1978            |
| HD2329           | HD1962/E 4870/3/K 65/5/ HD1553/4/UP262  | 1985            |
| WH283            | HD1925/HD832//23584                     | 1985            |
| HD2009           | LR 64A / NAI 60                         | 1992            |
| WH542            | JUPATECO/BLUEJAY/URES                   | 1992            |
| UP2338           | UP368/VL421//UP262                      | 1995            |
| PBW343           | ND/VG1944//KAL//BB/3/YACO'S'/4/VEE#5'S' | 1996            |
| HD2687           | CPAN2009 / HD 2329                      | 1999            |
| WH711            | ALD'S'HUAC//HD2285/3/HFW17              | 2002            |
| HD2851           | CPAN 3004/WR 426//HW 2007               | 2004            |
| PBW502           | WH485/PBW343//RAJ1482                   | 2004            |
| DBW17            | CMH79A.95/3*CNO 79//RAJ3777             | 2007            |
| HD2894           | TURACO/PRINIA/DL 788-2/DW 871           | 2008            |
| PBW550           | WH 594/RAJ 3856//W 485                  | 2008            |
| WH1021           | NYOT95/SONAK                            | 2008            |
| HD2967           | ALD/COC//URES/HD2160M/HD2278            | 2010            |
| DPW621-50        | KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES | 2011            |
| WH1080           | 21 SAWSN151                             | 2011            |
| WH1105           | MILAN/S87230//BABAX                     | 2013            |
| HD3086           | DBW14/HD2733//HUW468                    | 2014            |
| WH1124           | MUNIA/CHTO/AMSEL                        | 2014            |
| WH1142           | CHEN AE.SQU. (TAUS)//FCT/3/2*WEAVER     | 2015            |

productive environments of the zone. Thus, the evaluation was done at a total of six environments, viz. Delhi1 (Delhi 2015-16), Delhi2 (Delhi 2016-17), Hisar1 (Hisar 2015-16), Hisar2 (Hisar 2016-17) Karnal1 (Karnal 2015-16), Karnal2 (Karnal 2016-17) keeping two replications and plot size of 6×1.2m<sup>2</sup>. Row to row spacing was kept at 22cm and recommended agronomic practices were followed to raise a healthy crop.

## SAMPLING AND OBSERVATIONS

### Root traits

The observations were recorded at two locations, i.e. Delhi and Hisar for two years. Five plants were randomly chosen from a random sample of 20 (10 plants from each replication) at Zadok's stage 37 (Zadok *et al.* 1974) wherein all the crown roots and tillers have already been developed and flag leaf is just visible.

The plants were gently excised from the soil without harming the number and natural orientation of crown roots and were kept soaked in water to maintain the turgidity. The roots were gently washed and the root hairs were removed with the help of forceps leaving crown roots and mesocotyl intact. The angle of crown roots was recorded in natural form as per the in-house method (Sakhare *et al.* 2019).

The distribution pattern of roots was observed by counting the number of crown roots in the interval of 10° by keeping the tillers and the node of origin of the crown roots perpendicular to the plane of the paper and the primary angles were measured by standard arch protractor. The observation on the number of crown roots per plant and number of tillers per plant were made by counting the same. The mesocotyl is the primary root originating from the seed and seminal roots are originated from the same which provide the basic anchorage to wheat plant. The length of the mesocotyl was measured in cm.

### Above-ground traits

The observations were recorded at three locations for two years on tillers per plant (TPP), tillers per square meter (TMS), days to heading (DH), days to maturity (DM), plant height in cm (PHT), thousand-grain weight in grams (TGW) and plot yield (Yield) in grams.

### Data analysis

The comparative and combined analysis of root traits and yield attributing above-ground traits was done using different command lines of the R-software which is an open-source statistical tool (RStudio Team 2016). The correlations chart was drawn using the function Chart. Correlation' in the R package "Performance Analytics" (Peterson *et al.* 2018). Cluster analysis was done using the R package "cluster" using K means (Martin *et al.* 2018). The heatmap of two way cluster diagram was visualized by using the R- package "pheatmap" using inbuilt commands for visualizing the heat map and with hierarchical clustering method using Euclidean distances (Raivo Kolde 2018). META-R (Multi-Environment Trait analysis with R for windows Version 6.01 (Alvarado *et al.* 2017) was used for the analysis of variance for G× E significance and the heritability estimates.) Version 6.01. GGE-Biplot analysis was done to estimate the nature and extent of Genotype× Environment interaction by using R package GGEbiplotGUI (Frutos *et al.* 2014).

## RESULTS AND DISCUSSION

Root system architecture (RSA), the spatial configuration of a root system in the soil, is used to describe the shape and structure of root systems. The RSA is an important factor in understanding the phenomena contributing to the overall performance of a genotype. The current study involves the angular distribution of seminal roots and mesocotyl. The genetic basis of the angular distribution of seminal roots has been reported to be governed by quantitative loci (Hamada *et al.* 2012).

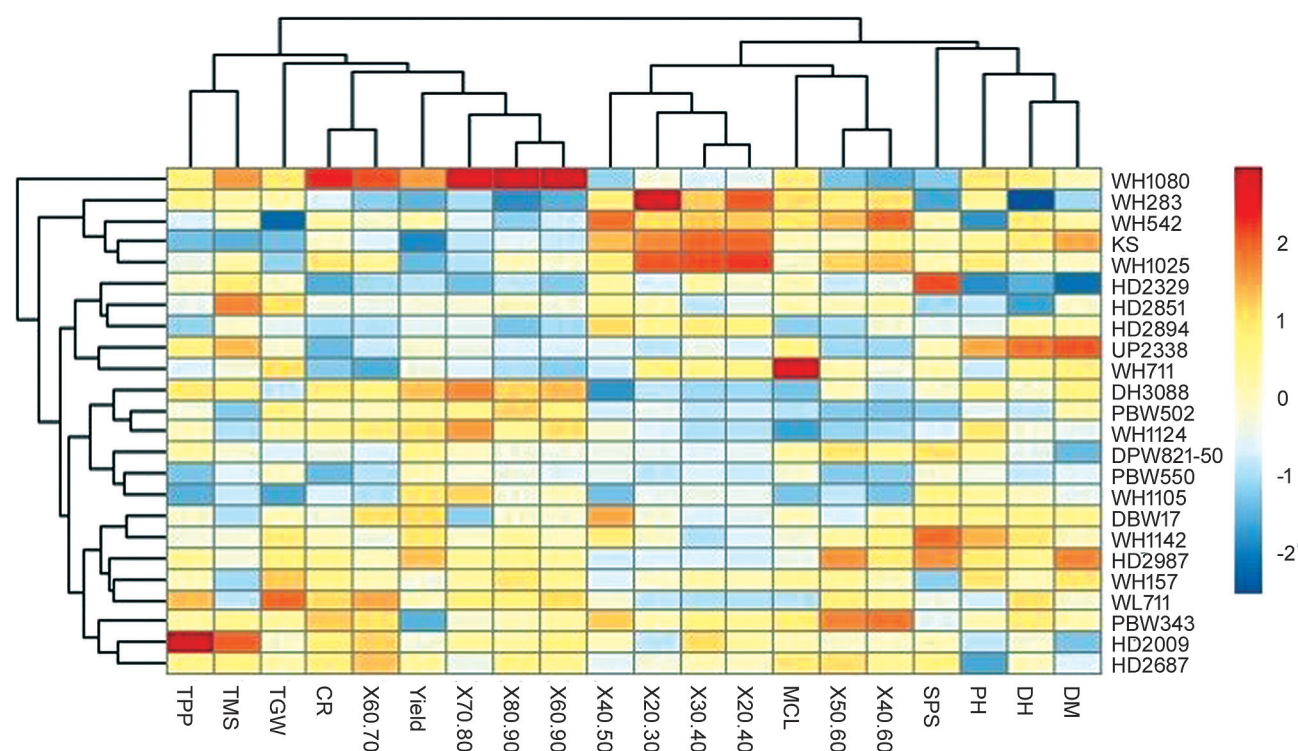


Fig 1 Heat map based on hierarchical clustering of normalized variables of root and aboveground traits. CR-Number of crown roots, x20.40 - crown root angle between 20 to 40°, x40.60- crown root angle 40-60°, x60.90- crown root angle 60-90°, MCL- mesocotyl length, DH- days to heading, PH- plant height, TPP- tillers per plant, TMS- Tillers per meter square, SPS- number of spikelets per spike, TGW- thousand grain weight

Although, the selection based upon above-ground traits should have a larger bearing on the root system architecture of plants, yet the use of the correlated response could not be realized. The gravitropic response of the developmental roots and shoots is under genetic control (Roychoudhry and Kepinski 2015). The latest studies on crown roots in rice give some insight into genes and QTLs cereal root architecture. Crown roots and their angular distribution (Supplementary Fig 1), therefore, have a larger bearing on the yield and overall development of wheat plant and analogy can be drawn from the information on other cereal crops including rice. The root angles have not been studied in wheat, however, a major QTL named *DEEPER ROOTING 1 (DRO1)* identified in deep-rooting wild rice variety Kinan- dang Patong (KP) is an important regulator of root growth angle in rice (Roychoudhry and Kepinski 2015; Uga *et al.* 2013).

### Correlations

The average data overall environment and all genotypes were near to normal for crown root numbers, crown roots between 60°-90° angle, TPS, TMS, SPS, MCL, DM, TGW and Yield with little or no skewness. Lower angle roots (20°-40°) were negatively skewed with while categories, i.e. 40°-60° and plant height were binomially distributed with two peaks. The correlations were estimated based on an average of all genotypes across all the environments

(Supplementary Fig 2).

The correlation between the number of productive tillers and angular distribution of crown roots was found to be significantly positive with deeper angle roots 60°-70° (0.58), 80°-90° (0.37) and 60°-90° (0.46). Mesocotyl length (MCL) was having a significant positive association with shallow root angles such as 20°-40° (0.41) although it is negatively correlated with deeper root angles 60°-90° (-0.31). The significant and positive interclass correlations were found among deeper root angles 60°-70°, 70°-80° and 80°-90° as well as among shallow root angles, i.e. 20°-30°, 30°-40° and 40°-50°. The yield showed a positive correlation with TPS, TMS, and DH among the attributing traits when analyzed separately. But after combining the root traits with the yield and attributing traits a different picture comes wherein yield is significantly and positively and significantly correlated with deeper root angles (60°-90°), and a weaker positive correlation with number of crown roots, DH, DM, PH, TPP, TMS, SPS, and TGW was realized. On the contrary significant negative correlation between yield and shallow root angles (20°-40°) while the traits 40°-60° and MCL had a weaker negative correlation with yield. There are hardly any studies done on interclass correlations among root architectural traits vis a vis aboveground traits in wheat, therefore, this study shall open scope for utilizing this information in future (Sakhare *et al.* 2019).

### Clustering

The heat map of two-way cluster analysis based on the normalized data of different traits classified 24 varieties into five clusters the first cluster from bottom side comprising HD2687, HD2009, PBW343, WL711, HD2967, WH1142 and DBW17 wherein the characters associated with yield is better as per the depiction in heat map owing to its positive coefficient between 1 and 2 on a colour scheme (Fig 1). The second cluster had WH1105, PBW550, DPW621-50, WH1124, PBW502, and HD3086 which are relatively newer varieties are having the favourable root system of deeper roots along with high SPS and DH and DM. The third cluster comprising WH711, UP2338, HD2894, HD2851, and HD2329 are shallow-rooted but having fairly good TGW, TPP and TMS. The fourth cluster comprising WH1025, KS, WH542, and WH283 is high in shallow angled roots, intermediate roots, and MCL. The fifth cluster is having single entry WH1080 with the maximum number of favourable traits together and reaching an ideal genotype. The cluster plot analysis based on the K means based on agronomic traits classified all the genotypes into three major clusters capturing only 47.81% of the point variability by the two principal components While when we included the root traits the drastic increase in the variability captured was observed to be 71.43% of point variability with more discriminating resolution (Supplementary Fig 3). There was a clear correspondence between the two clustering methods with some of the rearrangements wherein the variety WH1080 grouped with, HD3086, WH1124 and PBW502 based on the hierarchical two-way clustering. The varieties PBW343, HD2967, WL711, HD2009, HD2687 and WH157 are grouped in cluster 3 and corresponding cluster 1 in hierarchical two-way clustering. The varieties WH1025, WH542, WH283 and KS grouped in cluster 4 are there in the corresponding fourth cluster. The first cluster comprising remaining genotypes is divided into three sub-clusters with corresponding clusters in the heat map. There is a significant increase in the explainable point variability based on the cluster analysis of the varieties when we included root traits along with the yield attributing traits. The two-way clustering using the heat map based upon the hierarchical clustering provides an elucidation of different varieties and the relative contribution of different traits in their performance (Appels *et al.* 2018; Raivo Kolde 2018). The yield is strongly influenced by the root architecture of plant Kazemi *et al.* (1979). The partitioning of assimilates between roots and shoots appears highly dependent on the wheat genotype (Sadhu and Bhaduri 1984).

### Genotype $\times$ environment interaction

The analysis of variance using the Best linear unbiased prediction (BLUP) of means of traits over all the genotypes and all the environments comprising of two locations and two years for root traits and two years and three locations for shoot traits revealed significant interaction component for almost all the traits under study (Supplementary Table 1). The genotypic effects were found significant for 60°-70°

and 60°-90° for root angles, while days to heading, plant height, tillers per m<sup>2</sup> and 1000 grain weight were significant at one percent probability. Genotype  $\times$  Environment effects were non-significant only for crown roots at angles 40°-50° and 60°-70° while significant for crown roots spreading at 30°-40° at five percent probability, while rest of the traits were highly significant. Environmental effects were non-significant for shallow root angles ranging from 20°-50° while significantly influencing the deeper root angles and all the yield contributing traits. Lowest heritability estimates for shallow root angles and while approaching moderate heritability (40-50%) for deeper root angles and crown root numbers. The above-ground traits showed moderate heritability value (>50%) except days to maturity and yield which were having low heritability. The heritability estimates of different traits were found to be of moderate range therefore, combining of favourable traits in the selection cycle is likely to provide a better response to selection rather than individual traits. Moderate heritability values for the total root length and root branching had also been reported by (Monyo and Whittington 1970). The control of root systems has been reported to be largely influenced by additive genetic systems, which allow progress to be made by selection on root quantity and depth of penetration (Kazemi *et al.* 1979; Monyo and Whittington 1970). Although the additive portion of the total variance was not very high, it was suggested that lines with favourable root to shoot ratios may be obtained from the direct selection in early generations of wheat crossings (Waisel *et al.* 2002).

**Stability and adaptability:** The GGE model-based principal components PC1 (x-axis) and PC2 (y-axis) explained maximum variability of 51.83 % and 22.74% respectively, present among genotypes and environments (Supplementary Fig 4). The model applied for yield in different environments revealed concurrence among Delhi2, Karnal1 and Karnal2 environments which were average production environments. While Delhi 1 and Hisar2 were slightly unfavourable environments and the most favourable environment was the Hisar1. The varieties performing well under favourable environment include DBW17, DPW621-50, WH1142 and HD2967. The varieties like WH1105, WH1080, WH1124 and HD3086 were performing under a slightly unfavourable environment while PBW502 and WH711 were performing better under unfavourable environment. The varieties PBW343, HD2894, UP2328 are a low performer in a good environment. If we compare two varieties HD2967 and HD3086 the performance is better for HD2967 being more responsive in the favourable environment while varieties like WH1080, WH1105, HD3086, WH1142 and WH1124 are consistent performers with slight fluctuations in the production environment. The multi-environment studies are the backbone of the breeding program as the success of variety lies in the performance across the locations and years. Grain yield is a function of genotype, environment, and genotype  $\times$  environment interaction (GEI) (Hamam and Khaled 2009; Memon *et al.* 2007, Trethowan and Crossa 2007).

The concept of mega variety and mega-environment are in a place wherein the genetic constitution of a mega variety is so optimized to buffer the effect of variables of the environment over a larger area and vice-versa (Rajaram *et al.* 1995). India being a union of different states representing a variety of climatic conditions and management regimes (zones) and wheat being grown on a very large area (31m ha) has the requisite of a combination of different adaptive traits along with the yielding ability. Significant genotypic effects could only be realized in a few traits while the genotype  $\times$  environment and environment being largely significant to indicate that the mega variety is largely exploiting favourable genotype  $\times$  environment. Mega variety HD2967 which replaced another mega variety PBW343 is an example of that which is most promising as per GGE-biplot analysis falling in the first quadrant that is combining a good environment and good genotype. The fact that HD2967 breaking all the records is largely realizable due to well-understood management regime of this particular cultivar and a great degree of plasticity of the performance. HD3086 another popular variety is growing towards achieving the status of mega variety on the contrary, is a good performer under slightly compromised environment. Therefore, the recent trend indicates that this variety over-performs all the varieties including HD2967 whenever there is sudden stress of terminal temperature or lodging due to sudden rainy and stormy conditions. WH1080 which is a selection from CIMMYT lines perhaps the variety with optimum recombination of favourable traits but not being picked up by farmers due to unknown factors of preference and a similar fate was also experienced by WH542 in past (Yadav *et al.* 2010). Therefore, there are chances of further improvement in varietal developmental approaches keeping root traits in mind.

### Conclusion

More than 500 wheat varieties have been released since the onset of green revolution mainly through selection for desirable above-ground traits. A combination of adequate root architecture along with an excellent response to above-ground traits has resulted in increased plasticity of only a few varieties which were able to consistently perform making them popular enough to be called as mega varieties. The significant increase in the total of variance explained on principal components with the inclusion of roots traits along with above-ground traits is a testimony of the importance of the hidden part of the plant. The association of yield and number of tillers with deep angular distribution 60°- 90° of crown roots were found significant in the presented study. Surprisingly the significant negative association of shallow angled crown roots with yield is a trade-off with the number of deeper angled roots. Crown roots falling at 60°-70° were found to be least affected by the environment while there were significant genotypic effects. Therefore, the varieties with better root distribution in 60°- 70° angles perhaps could perform better through modulation of the number of tillers and increased plasticity. Therefore, along with aboveground

traits, utilizing crown root angles and mesocotyl length which are easy to measure and also have a response to selection will be a win-win situation for breeders.

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