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# Screening Genetic Variation in Maize for Deep Root Mass in Greenhouse and Its Association with Grain Yield Under Water-Stressed Field Conditions

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## Abstract

Plant roots have been recognized to play an important adaptive role in drought prone environments. There have been many efforts to improve root traits in order to develop drought tolerant cereal crops including maize but significant progress has not yet to be made. Twelve maize hybrids and their corresponding 12 female inbred parents were evaluated for genetic variation in deep root mass and other root traits in PVC tubes. The hybrids were selected based on their grain yield performance under water-stressed conditions in the field. Plants were grown in three different growing media, and a mixture of sand, vermiculite, perlite and soil was found to be the best growing medium to study root growth. Significant phenotypic variation was observed among inbred lines and among hybrids for deep root mass (DRDW) and other related root traits under well-watered and water-stressed conditions. Based on individual hybrid comparisons and correlation analysis, deep root mass estimated in well-watered and water-stressed conditions in the greenhouse was found to be associated with grain yield under water-stressed conditions in the field. Hybrids with higher grain yield under water-stress showed considerable higher DRDW than the hybrids with lower grain yield. A conservation of the trait DRDW was observed between inbreds and hybrids as both groups exhibited similar patterns of variation. The current screening system for root traits is simple and inexpensive, making it useful for evaluating large number of inbred lines or hybrids for root traits under well-watered or water-stressed conditions for drought tolerance.

**Keywords**: maize, deep root mass, total root mass, root length, drought tolerance

## Introduction

Water deficit has been recognized as the as biggest abiotic stress causing substantial crop losses around the world. During the last two decades, the impacts of drought in the United States have increased significantly with an increased number of droughts or an increase in their severity (Wilhite and Hayes, 1998; Changnon et al, 2000). The 1988 drought in the Midwestern US resulted in a 30% reduction in US corn production (Rosenzweig et al, 2001) and cost about $30 billion (Easterling and Karl, 2000). The 2012 drought in the US was the worst in 60 years, causing maize production to be the lowest since 1995 (USDA-NASS, 2013; USDA-ERS, 2013). More frequent occurrences of water shortages are expected due to climate projection and increasing competition for water among urban industrial and agricultural demand (IPCC, 2012; Haro von Mogel, 2013). In the western Corn Belt, 57 percent of the maize area is rainfed while in the central and eastern Corn Belt maize is grown almost entirely under rainfed conditions (Grassini et al, 2009) where crops are always threatened by drought. Growing drought tolerant varieties is one way to mitigate the negative consequences of drought (Passiora, 2007; Comas et al, 2013). Drought tolerance is a complex trait (Ludlow and Muchow, 1990; Quarrie, 1996) involving a number of morpho-physiological traits, including root characters. It can be achieved in a number of ways, including drought avoidance or desiccation prevention, or combination of both, or through effective use of limited water supply, or through recovery of growth following rehydration after drought stress (Chaves et al, 2003; Passiora, 2012). A deep root system with thick roots and extensive branching ability is considered a major component of drought avoidance, enabling the plants to extract water from deep soil layers (Fukai and Cooper, 1995; Gowda et al, 2011). Root characteristics, particularly root depth, are likely to increase plant water uptake, and therefore dehydration avoidance mechanisms and crop resistance to drought effects (Passiora, 1983; Serraj et al, 2009). Root traits associated with maintaining plant productivity under drought include roots with small fine root diameters, long specific (main/laterals) root length, and considerable root length density, especially at soil depths with available water (Comas, 2013). Crop plants with deep, bushy root ecosystems could simultaneously improve both soil structure and its steady-state carbon; water and nutrient retention; as

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well as sustainable yields (Kell, 2011). Kiregaard et al (2007) showed that an extra 10.5 mm of additional subsoil water used in the 1.35–1.85 m layer after anthesis increased grain yield by 0.62 t ha$^{-1}$ in wheat. Landraces of upland rice, adapted to drought, exhibited substantially larger root systems with some large diameter roots able to colonize the deep soil layers even in the presence of plough pans (Ekanayake et al, 1985). Good drought tolerance of rice is also positively related to (32)P uptake (Reyniers et al, 1982), an estimator of root length density, as well as water uptake (Mambani and Lal, 1983a; 1983b; Puckridge and O'Toole, 1981) from soil layers one meter deep. Some drought-tolerant genotypes of sorghum have deeper roots (Ludlow et al, 1990; Santamaria et al, 1990) and higher yields. In the root system of maize, lateral roots are of major importance for the efficient short-distance exploitation of water and nutrients (Eissenstat, 1992; McCully, 1999), and they make up about eight times the surface area of their parental axile root and take up about eight times as much water. Water uptake of a maize root, i.e. the axis and its associated laterals, is maximal at 30 to 60 cm from the main root tip and decreases to about 25% of the maximum in older regions (Varney and Canny, 1993). Manschadi et al (2006) observed that a drought tolerant wheat genotype had more compact root architecture and a greater root length at depth than sensitive genotype. Henry et al (2011) reported significant variation in root length density at a depth of 30-45 cm among 20 rice genotypes under drought stress and found the genotype «Dular» with deep root growth had greater drought resistance and highest drought resistance index. A correlation of root density at 35 cm depth with indicators of drought avoidance in rice was reported (Cairns et al, 2009). Field testing of upland rice in India with four introgressed QTLs was found to produce longer root lengths and a yield advantage of 1 t ha$^{-1}$ compared to controls (Stele et al, 2006). Uga et al (2013) incorporated a QTL allele conferring deep (steep) root growth angle into a drought sensitive rice variety with shallow roots. The resulting rice line displayed greater root distribution at deeper soil layers and better yield under drought conditions. Genetic variability studies in maize for root architecture are challenging due to highly heterogeneous nature of root architecture within and among different cultivars as a response to a complex field and soil matrix (Lynch, 1995; Bohn et al, 2006; Clark et al, 2011). Burton et al (2013) reported that maize landraces have greater variation in root architectural traits and have longer nodal roots and larger xylem than related wild Zea species. Longer roots were shown to assist in the capture of mobile resources in the soil and are considered to be a primary determinant of drought tolerance in maize (Ribaut et al, 2009; Zhu et al, 2010). Hund et al (2009) observed greater rooting depth in the drought tolerant tropical maize inbred lines than the sensitive lines. Genetic variation among maize hybrids in primary root length, number of lateral roots and root dry weight at early seedling stage under drought stress condition in the field was also recorded (Qayyum et al, 2012). In response to evapotranspiration demands, shoots drive water uptake through a root system (Comas et al, 2013) and amount of water uptake is determined by root architecture, i.e., root angles, rooting depth, root diameter, number of root branches and length of root hairs (Lynch, 2013). Wasson et al (2012) proposed selection on the traits to improve root systems and water uptake in water-limited wheat crops, which includes deep roots, greater root branching at median and deeper soil layers, reduced root length density near the surface, and longer root hairs with increased xylem diameter for decreased resistance to water movement from soil to roots. Information on the genetic control of root traits in the field and their relationship with grain yield is limited mainly due to great difficulty in extracting intact root system from soil. While there are a number of reports on deep rooting and its association with drought tolerance, there is a lack of information on the extent of variation in root mass at deeper soil lay-

Table 1 - List of the hybrids with their yield performance in well-watered and water-stressed conditions in field trial at Brule, Nebraska in 2012 and their corresponding female inbred parents.

| Hybrid Id # | Genotype (Hybrids)† | Grain yield (Mg ha$^{-1}$) under well-watered condition | Grain yield (Mg ha$^{-1}$) under water-stressed condition‡ | Performance in water-stressed condition | Inbred Id # | Genotype (Inbreds) |
|---|---|---|---|---|---|---|
| | Set 1 | | | | | |
| 1 | LH156/MBS2747† | 10.8c | 8.6a | High | 1 | LH156 |
| 2 | LH82/MBS2747 | 14.7a | 7.4ab | Medium high | 2 | LH82 |
| 3 | NC364/MBS2747 | 11.2c | 4.6dc | Low | 3 | NC364 |
| 4 | PHG72/MBS2747 | 12.0bc | 6.8abc | Medium low | 4 | PHG72 |
| 5 | PHK42/MBS2747 | 12.5bc | 8.4a | High | 5 | PHK42 |
| 6 | Pa91/MBS2747 | 10.9c | 5.3bcd | Low | 6 | Pa91 |
| | Set 2 | | | | | |
| 7 | 2369/SGI071† | 11.2c | 8.2a | High | 7 | 2369 |
| 8 | F42/SGI071 | 11.9bc | 7.1ab | Medium low | 8 | F42 |
| 9 | LH193/SGI071 | 13.4ab | 8.8a | High | 9 | LH193 |
| 10 | LH194/SGI071 | 12.2bc | 8.5a | High | 10 | LH194 |
| 11 | N552/SGI071 | 11.2c | 5.6bcd | Low | 11 | N552 |
| 12 | PHG86/SGI071 | 12.1bc | 3.7d | Low | 12 | PHG86 |

†MBS2747 and SGI071 were commercial testers used in making topcrosses (hybrids); ‡field watered with 40% of full irrigation given in well-watered field.

ers and its association with yield performance under drought conditions in maize. Root traits could be evaluated in greenhouse under controlled environment at ease in long pots or PVC tubes but a growing medium that support good root growth, allow good evaluation of root traits and better root extraction, has yet to be identified. To our knowledge, to study corn root growth, information is available only on two different growing media (Manavalan et al, 2011; Roots Lab, 2013). In 2012, we evaluated 98 maize topcrosses (hereafter referred to as hybrids) derived from crosses between a set of diverse inbred lines and two commercial testers for grain yield and other traits in well-watered (WW) and water-stressed (WS) conditions at Water Resources Field Laboratory near Brule, Nebraska. From this panel of 98 hybrids, we selected hybrids differing for grain yield under WS: five hybrids that displayed high yield, three hybrids that displayed intermediate yield, and four hybrids that displayed low yield (Table 1). Both hybrids and their female parental inbred lines were evaluated for root traits in the greenhouse using 1 m tall PVC tubes and three types of growing media. The objectives of the research were to: i) identify a growing medium that support good root growth and evaluation of root traits under greenhouse condition, ii) observe genetic variation among inbred lines and among hybrids for root traits, and iii) compare grain yield performance of hybrids under water-stressed condition in field with deep root mass and other root traits under greenhouse conditions.

## Materials and Methods

### *Plant materials*

Twelve hybrids were selected from a panel of 98 hybrids (topcrosses) derived from crosses between a set of diverse inbreds obtained from the North Central Regional Plant Introduction Station (NCRPIS, Ames, IA, www.panzea.org) and two commercial testers, MBS2747 and SGI07. Selections were made on the basis of grain yield under water stress during a 2012 field trial near Brule, NE. The 12 corresponding female inbred parents were also included in greenhouse evaluations (Table 1). Hybrids and inbreds were grouped into two sets. Set one (ID no. 1 to 6) included the six hybrids with MBS2747 as the common tester parent. The corresponding six female inbred lines were also included in set one. Set two (ID no. 7 to 12) included the six hybrids with SGI071 as a common tester parent and the corresponding female inbred lines.

### *Field evaluation*

Ninety-eight hybrids were in evaluated under well-watered and water-stressed conditions at the Water Resources Field Laboratory near Brule, Nebraska. The water-stressed plots were watered with 40% of full irrigation given to the well-watered plots. The experiment was laid out in randomized complete block design with three replications for both water treatments. Field plots consisted of two rows 0.76 m apart and 6.08 m long planted to a density of 71,630 plants $ha^{-1}$. Fertilizer rate was 200-18-0 lbs of NPK per acre. Nitrogen fertilizer was sprayed during V8 stage while phosphorus was applied at planting time. The soil type of the experimental site was silt loam. Soil moisture in both water regimes was monitored using Watermark soil moisture sensors (The Irrometer Company, Inc, Riverside, CA) through measuring soil water tension at 4 soil depths (1ft, 2ft, 3ft, and 4ft). During the growing season (May 15 to October 15, 2012) total precipitation was 4.5 inches, the average daily day (high) temperature was 87.7°F while night (low) temperature was 53°F, and average relative humidity was 43.4%. The average solar radiation was 516.9 langleys per day. Machine-harvestable grain yield data was collected and adjusted to 155 g $kg^{-1}$ moisture. The field trial was completely balanced. An analysis of variance using Proc GLM was used to partition variation to hybrid, block, and error sources of variation, and the genotypes showed significant variation for grain yield and other agronomic triats (data not shown). Soil moisture was lower in the water-stressed block than the well-watered block throughout the growing season as revealed by the Watermark soil moisture monitors. Average grain yield in the water-stress block was 40% lower than average grain yield of the well-watered block, indicating plants grown under reduced irrigation experienced water stress.

### *Evaluation of root traits*

Hybrids and inbreds were evaluated for root growth in four experiments that involved growing plants in 1 m tall PVC tubes in the greenhouse. Tube diameter was 10 cm. The first three experiments were conducted in a temperature-controlled greenhouse located on the University of Nebraska-Lincoln campus. The fourth experiment was conducted in the greenhouse at the West Central Research and Extension Center (North Plate, NE) with no temperature control. The bottom end of the PVC tube was sealed with fiberglass mesh screen so the sand-soil mix was held in place while allowing good drainage. The PVC tubes were placed inside a wooden frame to keep them upright. Tubes were filled with a growing media mixture of several selected components. While filling, the tubes were tapped gently to pack down the soil mix. The tubes were soaked with water one day before planting. Three seeds were planted directly in each tube at about 2 cm depth and thinned to one plant eight days after planting (DAP). In well-watered set, plants were watered almost every day and fertilizer was applied generally at 10 or 11 DAP with a solution of 200 ppm of 20-20-20 of NPK.

Experiment 1: Set 1 inbreds and hybrids (Table 1) were included in this trial and were grown in two different growing media mixtures during April, 2013.

Growing media 1 was a mixture of turface and sand (TS) with a ratio of 2:1 (v/v) (Manavalan, 2011) while the other media was a mixture of sand, vermiculite, perlite and sand (SVPS) with ratio of 5:3:1:1 (v/v) (Roots Lab, Penn State university, PA, 2013). The turface used was Turface Atheletics produced by Profile Products LLC, Buffalo Grove, IL, and the sand used was washed sand. The experiment was laid out in a randomized complete block design with three replications and watered every day during the entire growing period. The greenhouse temperature was set as 70-78°F during the day and 68-74°F during the night. Plants of the TS set were harvested 28 DAP while the plants of SVPS set were harvested 30 DAP. The whole plant with intact roots was pulled out of the tubes and removed carefully from the soils and washed twice.

Experiment 2: Set 2 inbreds and hybrids (Table 1) were included in this trial grown in May and June, 2013 with greenhouse temperature set to 70-78°F during the day and 68-74°F during the night. Plants were grown in SVPS media and laid out in a randomized complete block design with five replications. Plants were watered almost every day. The plants were harvested 27 DAP and roots were washed twice. The relevant shoot and root data were recorded on the same day.

Experiment 3: Set 1 inbreds and hybrids were grown in the months of July and August, 2013 with greenhouse temperature set at 70-78°F during the day and 67-72°F during the night. Plants were grown in SVPS growing media following randomized complete block design with three replications. Plants were watered nearly every day for first 20 days after planting, and watering was suspended from 21 DAP until the root harvesting at 44 DAP.

Experiment 4: The experiment was conducted in the greenhouse of West Central Research and Extension Center, North Platte, NE during September and October, 2013. Two selected inbreds (LH156 and NC364) and their two corresponding hybrids were grown in sand (washed) only in three replications and were harvested at 24 DAP. In previous two experiments (Expt. 1 and Expt. 3), inbred line LH156 and its corresponding hybrids produced longer roots and greater total root weight than NC364 and its related hybrid, and these contrasting root traits were the reasons for their selection for this test to observe root growth in sand.

On the day of harvesting, data were recorded on the following morphological characteristics: (1) Shoot length (SL): Plant height from stem base to the tip of the longest leaf, (2) Leaf length (LL): Length from collar to leaf blade tip, (3) Leaf width (LW): Width at the

Table 2 - Analysis of variance of shoot and root traits of six inbreds and six hybrids (set 1) grown for 30 days in SVPS and 28 days in TS growing media in well-watered condition in experiment 1.

| Source | df | SL[§] MS cm | LL MS cm | LW MS cm | RL MS cm | SDW MS g | TRDW MS g |
|---|---|---|---|---|---|---|---|
| Inbreds_TS | | | | | | | |
| Rep | 2 | 27.1 | 1.7 | 0.02 | 226.2 | 1.00 | 0.07 |
| Genotype | 5 | 96.6** | 47.0 | 0.24 | 344.5** | 0.18* | 0.13** |
| Error | 10 | 13.0 | 34.7 | 0.16 | 46.8 | 0.06 | 0.03 |
| Mean | | 45.9 | 28.3 | 2.36 | 44.8 | 0.85 | 0.56 |
| Hybrids_TS | | | | | | | |
| Rep | 2 | 40.9 | 44.1 | 0.41 | 113.5 | 0.15 | 0.08 |
| Genotype | 5 | 61.6** | 32.2 | 0.03 | 53.6 | 0.03 | 0.01 |
| Error | 10 | 5.9 | 20.9 | 0.06 | 61.2 | 0.03 | 0.01 |
| Mean | | 50.0 | 29.3 | 2.26 | 49.2 | 0.94 | 0.69 |
| Inbreds_SVPS | | | | | | | |
| Rep | 2 | 59.9 | 23.8 | 0.19 | 529.9 | 0.14 | 0.02 |
| Genotype | 5 | 241.2** | 91.6 | 0.19 | 596.7** | 0.50** | 0.26** |
| Error | 10 | 16.6 | 36.2 | 0.12 | 119.2 | 0.04 | 0.02 |
| Mean | | 50.2 | 35.2 | 2.95 | 96.9 | 1.23 | 0.76 |
| Hybrids_SVPS | | | | | | | |
| Rep | 2 | 53.2 | 57.3 | 0.42 | 30.1 | 0.39 | 0.12 |
| Genotype | 5 | 61.8 | 19.9 | 0.10 | 543.4 | 0.10 | 0.15* |
| Error | 10 | 21.2 | 29.2 | 0.04 | 423.2 | 0.10 | 0.03 |
| Mean | 1 | 57.0 | 39.8 | 3.19 | 118.0 | 1.58 | 1.12 |
| Growing Media (for Inbreds) | 1 | 164.6** | 426.4** | 3.13** | 24426.7** | 1.334** | 0.342** |
| Media × Inbred | 5 | 31.80 | 30.5 | 0.079 | 162.7 | 0.074 | 0.0262 |
| Growing Media (for Hybrids) | 1 | 434.0** | 993.3** | 7.72** | 43822** | 3.672** | 1.707** |
| Media × Hybrid | 5 | 38.2 | 14.04 | 0.038 | 321.2 | 0.078 | 0.069* |

SVPS = sand, vermiculite, perlite and soil mix;TS=Turface and sand mix.
[§]SL - shoot length, LL - leaf length, LW - Leaf width, RL - root length, SDW - shoot dry weight, TRDW - total root dry weight.

midsection of the last fully expanded leaf, (4) Root length (RL): Length from stem base (i.e., root base) to tip of the longest root. Roots were cut at 45 cm from the stem base (i.e., root base), and divided into upper root (UR) portion and deep root (DR) portion. All tissues were dried in oven at 70°C for five days. Dried samples were weighed and data was recorded for shoot dry weight (SDW), upper root dry weight (URDW), and deep root dry weight (DRDW) and total root dry weight (TRDW). The DRDW and TRDW was used to compute deep root ratio (DRR) as the ratio of the deep root mass to the total root mass while deep root to shoot ratio (DRSR) was computed as the ratio of deep root mass to the total shoot mass and expressed in percentage.

### *Data analysis*

Analysis of variance was performed using Proc GLM of SAS (SAS Institute, Inc, Cary, NC) to test for significant differences among inbreds and hybrids. Using ANOVA procedure, significant differences between pairs of genotypes for several important shoot and root related traits were identified by conducting a t-test and estimating least-significant difference values at 0.05 level of probability. Simple phenotypic correlation coefficients (based on mean values) among shoot and root traits and with grain yield in water-stressed field were calculated using PROC CORR statement.

## Results

### *Root and shoot growth in three different growing media in well-watered condition in experiment 1*

The mean root length of inbreds and hybrids in TS growing media in 28 days was 44.8 and 49.2 cm, respectively, while it was 96.9 and 118 cm, respectively, in 30 days in SVPS growing media (Table 2). The mean root length of the two inbreds and two hybrids was 44.4 and 48.5 cm, respectively, in sand grown for 24 days in experiment 4 (data in table format not presented). The mean TRDW of inbreds and hybrids was 0.56 and 0.69 g, respectively, in TS medium while it was 0.76 and 1.12 g, respectively, in SVPS medium (Table 2). The mean TRDW for two inbreds and two hybrids in sand media was 0.38 and 0.54

Table 3 - Mean shoot and root trait values of the inbreds and hybrids (set 1) evaluated in SVPS and TS growing media under well-watered condition in greenhouse and grain yield of the hybrids in water-stressed condition in field.

| Genotype | SL | LL | LW | RL | SDW | TRDW |
|---|---|---|---|---|---|---|
| | cm | cm | cm | cm | g | g |
| A. Genotypes were grown in TS mix for 28 days in experiment 1 | | | | | | |
| Inbreds | | | | | | |
| LH156 | 53.5 | 29.8 | 2.42 | 56.7a | 1.26a | 0.94a |
| LH82 | 39.5 | 23.2 | 2.22 | 31.5c | 0.57b | 0.34b |
| NC364 | 41.5 | 24.5 | 2.90 | 34.5bc | 1.00ab | 0.59b |
| PHG72 | 44.7 | 29.3 | 2.22 | 47.2ab | 0.69b | 0.40b |
| PHK42 | 44.1 | 29.0 | 2.11 | 42.1abc | 0.72b | 0.48b |
| Pa91 | 52.1 | 34.1 | 2.29 | 56.5a | 0.88ab | 0.61ab |
| LSD0.05 | | | | 15.6 | 0.45 | 0.33 |
| Hybrids | | | | | | |
| LH156/MBS2747 | 48.5 | 28.3 | 2.29 | 50.1a | 0.92a | 0.71a |
| LH82/MBS2747 | 44.1 | 28.7 | 2.38 | 42.3a | 0.81a | 0.61a |
| NC364/MBS2747 | 45.8 | 24.0 | 2.38 | 50.4a | 0.85a | 0.62a |
| PHG72/MBS2747 | 53.0 | 31.6 | 2.16 | 47.4a | 0.97a | 0.66a |
| PHK42/MBS2747 | 54.3 | 29.5 | 2.20 | 55.2a | 1.08a | 0.77a |
| Pa91/MBS2747 | 54.5 | 33.7 | 2.20 | 49.5a | 1.04a | 0.73a |
| $LSD_{0.05}$ | | | | 14.9 | 0.39 | 0.26 |
| B. Genotypes were grown in SVPS mix for 30 days in experiment 1 | | | | | | |
| Inbreds | | | | | | |
| LH156 | 61.9 | 38.8 | 3.23 | 101.3ab | 1.97a | 1.33a |
| LH82 | 44.8 | 36.7 | 3.07 | 95.3b | 1.02bc | 0.62bc |
| NC364 | 40.9 | 28.6 | 3.15 | 80.4b | 1.23b | 0.63bc |
| PHG72 | 53.0 | 34.2 | 2.78 | 87.2b | 1.06bc | 0.60bc |
| PHK42 | 41.9 | 29.8 | 2.55 | 95.5b | 0.78c | 0.55c |
| Pa91 | 58.6 | 43.2 | 2.93 | 121.5a | 1.37b | 0.82b |
| $LSD_{0.05}$ | | | | 24.40 | 0.43 | 0.23 |
| Hybrids | | | | | | |
| LH156/MBS2747 | 61.8 | 43.4 | 3.44 | 131.2a | 1.845a | 1.37a |
| LH82/MBS2747 | 57.3 | 39.1 | 3.23 | 122.3a | 1.73a | 1.29ab |
| NC364/MBS2747 | 48.7 | 36.2 | 3.29 | 102.0a | 1.37a | 0.79c |
| PHG72/MBS2747 | 56.7 | 38.0 | 3.23 | 132.5a | 1.47a | 1.08ac |
| PHK42/MBS2747 | 60.2 | 40.4 | 2.92 | 122.7a | 1.65a | 1.27ab |
| Pa91/MBS2747 | 57.2 | 41.7 | 3.04 | 102.8a | 1.43a | 0.95bc |
| $LSD_{0.05}$ | | | | 33.60 | 0.690 | 0.38 |

g, respectively, obtained in experiment 4. The mean shoot length of inbreds and hybrids in TS mix was 45.9 and 50.0 cm, respectively, while it was 50.2 and 57.0 cm in SVPS mix (Table 2). Similarly, in sand media, it was 26.5 and 30.2 cm for inbreds and hybrids, respectively. Shoot growth was relatively much lower in sand media than both SVPS and TS media.

Significant phenotypic variation for SL, RL, SDW and TRDW was observed among set 1 inbreds while among hybrids of the same set (1), significant variation was observed only for SL and none for any root trait grown in TS media in Experiment 1 (Table 2). In SVPS media, significant variation was observed for SL, RL, SDW and TRDW among inbreds whereas significant variation was observed only for TRDW among hybrids. Genotype growing media interaction (for TS and SVPS media only) was not significant for inbred group but it was significant only for TRDW for hybrid group (Table 2). Effect of media was highly significant for all shoot and root traits. Among the six inbreds, Pa91 had the longest roots followed by LH156 while Inbred NC364 had the shortest in SVPS media (Table 3). In TS media, LH156 produced longest roots whereas LH82 and NC364 had smallest (Table 3). Inbred LH156 yielded highest TRDW in both SVPS and TS media as opposed to PHK42 that yielded lowest in SVPS media, and LH82 which yielded lowest in TS media (Table 3). Among the hybrids, LH156/MBS2747 had the highest TRDW while NC364/MBS2747 had the lowest in SVPS media (Table 3).

***Variation in shoot and root traits evaluated in well-watered conditions in experiment 2***

Among set 2 inbreds, significant phenotypic variation was observed for SL, LL, LW, RL (Supplementary figure 1A), SDW, URDW, DRDW, TRDW, DRR and DRSR while among hybrids (set 2) significant variation was observed for SL, LL, LW, DRDW, TRDW, DRR and DRSR evaluated in SVPS media under well-watered condition in experiment 2 (Table 4A). Variation for URDW was not significant among the hybrids. Out of six inbreds, 2369 (0.07 g) showed highest DRDW followed by LH194 (0.06 g) whereas N552 (0.003 g) showed lowest followed by PHG86 (0.02 g) (Table 5A). Inbreds 2369 and LH194 yielded higher TRDW while inbreds N552 and PHG86 yielded lower TRDW. Higher yield producing hybrids, LH194/SGI071 (0.09 g), 2369/SGI071 (0.08 g) and LH193/SGI071 (0.07 g) yielded higher DRDW while lower yield producing hybrids N552/SGI071 (0.02 g) and PHG86/SGI071 (0.03 g) yielded much lower DRDW (Table 5A). These hybrids exhibited similar performance for DRR and DRSR.

***Variation in shoot and root traits evaluated in water-stressed conditions in experiment 3***

Under WS condition (SVPS media) significant variation for different shoot and root traits including RL (Supplementary figure 1B), DRDW and TRDW was observed among set 1 inbreds and hybrids (Table 4B). Among the inbreds, LH156 and PHK42 had longer roots and higher DRDW, DRR and DRSR while NC364 had smallest roots and lowest DRDW, TRDW, DRR, and DRSR (Table 5B). Among the hybrids, LH156/MBS2747, LH82/MBS2747 performed better across the traits, RL, DRDW, TRDW, DRR and DRSR while NC364/MBS2747 performed poor. Other hybrids showed variable response for these traits in response to water-stress (Table 5B). It may be mentioned here that hybrid LH156/MBS2747 had highest TRDW while hybrid NC364/MBS2747 had lowest TRDW in well-watered condition too (experiment 1)

Table 4 - Analysis of variance of shoot and root traits for six inbreds and corresponding six hybrids in each set.

| Source | DF | SL<br>MS<br>cm | LL<br>MS<br>cm | LW<br>MS<br>cm | RL<br>MS<br>cm | SDW<br>MS<br>g | URDW†<br>MS<br>g | DRDW<br>MS<br>g | TRDW<br>MS<br>g | DRR<br>MS<br>(%) | DRSR<br>MS<br>(%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A. Set 2 genotypes grown in well-watered condition for 27 days in experiment 2 | | | | | | | | | | | |
| Inbreds | | | | | | | | | | | |
| Rep | 4 | 52.5** | 14.6** | 0.04 | 276.8 | 0.08** | 0.004 | 0.001** | 0.01 | 46.2** | 6.3** |
| Genotype | 5 | 52.5*** | 84.9*** | 0.42*** | 714.8** | 0.06** | 0.007* | 0.002*** | 0.02* | 112.6** | 13.5** |
| Error | 20 | 14.1 | 1.47 | 0.02 | 112.5 | 0.02 | 0.003 | 0.0001 | 0.01 | 9.4 | 3.6 |
| Mean | | 52.5 | 34.0 | 2.28 | 78.6 | 0.73 | 0.335 | 0.03 | 0.38 | 8.4 | 4.8 |
| Hybrids | | | | | | | | | | | |
| Rep | 4 | 282.8** | 28.5 | 0.03 | 184.9 | 0.36** | 0.010 | 0.001 | 0.01 | 13.3 | 20.2 |
| Genotype | 5 | 66.3* | 42.3* | 0.17** | 162.5 | 0.03 | 0.006 | 0.002* | 0.01* | 86.4* | 26.9* |
| Error | 20 | 22.0 | 15.1 | 0.03 | 183.5 | 0.05 | 0.006 | 0.001 | 0.003 | 26.2 | 42.9 |
| Mean | | 64.6 | 41.3 | 2.52 | 79.2 | 1.03 | 0.360 | 0.05 | 0.41 | 13.2 | 6.2 |
| B. Set 1 genotypes grown for 44 days with no-water from 21st day after planting in experiment 3 | | | | | | | | | | | |
| Inbreds | | | | | | | | | | | |
| Rep | 2 | 132 | 31.6 | 0.11 | 70.02 | 0.58 | 0.02 | 0.00 | 0.02 | 14.3 | 0.8 |
| Genotype | 5 | 221.4 | 134.4* | 0.23 | 1323.1* | 0.31 | 0.04 | 0.08*** | 0.21* | 685.9** | 153.6** |
| Error | 10 | 79.0 | 33.1 | 0.24 | 305.9 | 0.28 | 0.02 | 0.00 | 0.04 | 31.9 | 18.7 |
| Mean | | 69.9 | 48.5 | 2.93 | 106.4 | 1.71 | 0.51 | 0.25 | 0.77 | 29.4 | 13.1 |
| Hybrids | | | | | | | | | | | |
| Rep | 2 | 67.0 | 17.6 | 0.36* | 266.9 | 0.90 | 0.21** | 0.01 | 0.28** | 33.0 | 2.0 |
| Genotype | 5 | 114.2* | 71.7** | 0.01 | 1002.7* | 0.28 | 0.02 | 0.06** | 0.12* | 241.5* | 98.1 |
| Error | 10 | 34.7 | 11.7 | 0.08 | 278.1 | 0.27 | 0.02 | 0.01 | 0.03 | 49.3 | 35.8 |
| Mean | | 77.4 | 49.6 | 3.21 | 112.6 | 2.17 | 0.77 | 0.34 | 1.09 | 29.3 | 15.5 |

†URDW - upper root dry weight, DRDW - deep root dry weight, TRDW - total root dry weight, DRR - deep root ratio, DRSR - deep root to shoot ratio. significant at probability level of *0.05, **0.01, and ***0.001, respectively.

Table 5 - Mean shoot and root trait values of the inbreds and hybrids (set 1 and set 2) evaluated in SVPS mix in well-watered and water-stressed conditions in two separate experiments in greenhouse and grain yield of hybrids under water-stressed condition in field.

| Pedigree | SL cm | LL cm | LW cm | RL cm | SDW g | URDW g | DRDW g | TRDW g | DRR (%) | DRSR (%) | GY† Mg ha$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A. Genotypes (set 2) were grown in well-watered condition for 27 days in experiment 2 | | | | | | | | | | | |
| Inbreds | | | | | | | | | | | |
| 2369 | 56.6 | 39.5 | 2.54 | 95.5a | 0.83 | 0.39 | 0.07a | 0.45a | 15.3a | 8.6a | - |
| F42 | 57.0 | 34.8 | 2.41 | 79.2ab | 0.86 | 0.38 | 0.03bc | 0.41ab | 7.0b | 4.1b | - |
| LH193 | 53.7 | 31.6 | 2.36 | 73.9bc | 0.79 | 0.35 | 0.03bc | 0.37ab | 7.5b | 3.9b | - |
| LH194 | 41.9 | 27.7 | 2.51 | 92.a | 0.64 | 0.33 | 0.06ab | 0.40ab | 14.8a | 10.1a | - |
| N552 | 52.8 | 33.5 | 1.99 | 58.5c | 0.65 | 0.29 | 0.003c | 0.30b | 1.09b | 0.5b | - |
| PHG86 | 53.1 | 37.0 | 1.84 | 74.8bc | 0.60 | 0.29 | 0.02c | 0.29b | 6.5b | 3.7b | - |
| $LSD_{0.05}$ | | | | 16.7 | | | 0.03 | 0.12 | 6.3 | 4.2 | - |
| Hybrids | | | | | | | | | | | |
| 2369/SGI071 | 66.1 | 43.3 | 2.63 | 82.9ab | 1.16 | 0.40 | 0.08ab | 0.46a | 17.1a | 8.7a | 8.2a |
| F42/SGI071 | 62.8 | 37.4 | 2.31 | 78.9ab | 0.93 | 0.34 | 0.06bc | 0.40ab | 13.6ab | 7.3ab | 7.1ab |
| LH193/SGI071 | 66.2 | 42.9 | 2.60 | 89.4a | 1.06 | 0.41 | 0.07ab | 0.47a | 14.06ab | 6.9ab | 8.8a |
| LH194/SGI071 | 58.3 | 39.0 | 2.79 | 78.7ab | 1.02 | 0.34 | 0.09a | 0.44ab | 21.2a | 9.0a | 8.5a |
| N552/SGI071 | 65.3 | 40.0 | 2.31 | 75.2ab | 0.99 | 0.36 | 0.02d | 0.35b | 6.5b | 2.7b | 5.6bc |
| PHG86/SGI071 | 68.9 | 45.1 | 2.52 | 71.9b | 1.06 | 0.32 | 0.03cd | 0.34b | 8.8b | 2.7b | 3.7c |
| $LSD_{0.05}$ | | | | 17.10 | | | 0.03 | 0.11 | 7.60 | 5.10 | 2.37 |
| B. Genotypes (set 1) were grown for 44 days with no-water from 21st day after planting in experiment 3 | | | | | | | | | | | |
| Inbreds | | | | | | | | | | | |
| LH156 | 80.0 | 54.6 | 3.40 | 121.3ab | 2.19 | 0.57 | 0.58a | 1.22a | 49.4a | 22.9a | - |
| LH82 | 60.8 | 40.1 | 2.63 | 95.7bc | 1.40 | 0.50 | 0.10de | 0.60bc | 16.7d | 7.6cd | - |
| NC364 | 54.2 | 37.5 | 2.72 | 65.2c | 1.22 | 0.40 | 0.007e | 0.41c | 1.1e | 0.5d | - |
| PHG72 | 71.4 | 49.5 | 2.87 | 106.7b | 1.98 | 0.40 | 0.21cd | 0.61bc | 33.7bc | 11.4c | - |
| PHK42 | 72.4 | 54.2 | 2.77 | 143.9a | 1.76 | 0.48 | 0.35b | 0.83ab | 42.3ab | 20.3ab | - |
| Pa91 | 74.3 | 51.5 | 3.07 | 105.3b | 1.75 | 0.71 | 0.30bc | 1.09a | 28.9c | 14.5bc | - |
| $LSD_{0.05}$ | | | | 31.6 | | | 0.12 | 0.38 | 10.9 | 8.2 | - |
| Hybrids | | | | | | | | | | | |
| LH156/MBS2747 | 80.1 | 52.4 | 3.27 | 130.6a | 2.41 | 0.81 | 0.50a | 1.31a | 38.5a | 21.2ab | 8.6a |
| LH82/MBS2747 | 74.0 | 42.8 | 3.23 | 120.0a | 2.09 | 0.83 | 0.43ab | 1.27ab | 34.1a | 21.9a | 7.4ab |
| NC364/MBS2747 | 68.8 | 44.0 | 3.20 | 78.6b | 1.71 | 0.62 | 0.12d | 0.74b | 14.3c | 6.6c | 4.6c |
| PHG72/MBS2747 | 85.6 | 54.4 | 3.20 | 121.0a | 2.60 | 0.78 | 0.40ab | 1.17ab | 34.1a | 15.6abc | 6.8abc |
| PHK42/MBS2747 | 74.0 | 51.9 | 3.23 | 118.3a | 2.15 | 0.75 | 0.32bc | 1.07ab | 30.7ab | 15.1abc | 8.4a |
| Pa91/MBS2747 | 81.7 | 52.0 | 3.10 | 106.6ab | 2.05 | 0.86 | 0.21cd | 1.07ab | 20.7bc | 11.5bc | 5.3bc |
| $LSD_{0.05}$ | | | | 29.5 | | | 0.17 | 0.51 | 12.7 | 10.2 | 2.82 |

†GY - Grain yield obtained under water-stressed condition in a field trial at Brule, NE in 2012.

(Table 3).

***Correlation among shoot and root traits***

Correlation analysis was performed using the combined data of all inbreds and hybrids separately for experiment 2 and experiment 3, and the correlation co-efficient values are given in Table 6. LW showed significant relationship with RL, SDW, URDW, DRDW, TRDW, DRR and DRSR in experiment 2 (Table 6A), and with similar traits but RL and DRR in experiment 3 (Table 6B). SDW showed moderate to stronger relationship with SL, LL, LW, DRDW, TRDW, and DRR in both experiments. RL exhibited moderate to stronger relationship with DRDW, TRDW, DRR and DRSR in both experiments (Table 6A,B). RL showed weak relation with URDW ($r = 0.65$, $P < 0.05$) in experiment 2 while no relation in experiment 3. DRDW showed stronger relation with TRDW, DRR and DRSR in both experiments. Correlation analysis was also performed to observe relations among root traits based on the data of hybrids only. DRDW showed significantly high relation with RL ($r = 0.93$, $P = 0.01$) and with TRDW ($r = 0.93$, $P = 0.03$) for set 1 while for set 2 it showed significant correlation with TRDW ( $r= 0.90$, $P = 0.01$) but not with RL (data not provided in table format).

***Relationship of root traits with grain yield under water-stress in field***

To evaluate the association between root traits evaluated in greenhouse with grain yield under water-stress in the field, a visual comparison of root trait values of individual hybrids with their grain yield values under water-stress in field was made based on the relative performance among the hybrids and results of the t-tests. In experiment 2, set 2 high yielding hybrid LH193/SGI071 (82.9 cm) had significantly longer RL than low yielding hybrid PHG86/SGI071 (71.9 cm) but other hybrids did not show pairwise significant differences (Table 5A). In experiment 3, all three higher yielding hybrids LH156/MBS2747 (130.6 cm), LH82/MBS2747 (120 cm) and PHK42/MBS2747 (118.3 cm) had longer root length than low yielding hybrid NC364/MBS2747 (78.6 cm). Lower yielding hybrid PHG72/MBS2747 (121 cm) also had relatively longer roots (Table 5B). In experiment 2, set 2 hybrids 2369/SGI071, LH193/SGI071 and LH194/SGI071 had higher DRDW (0.08, 0.07 and 0.09 g, respectively) and TRDW (0.46, 0.47 and 0.44 g, respectively) and also had higher GY (8.2, 8.8, and 8.5 Mg ha$^{-1}$, respectively) (Table 5A). On the other hand, hybrids N552/SGI071 and PHG86/SGI071 had lower DRDW of 0.02 and 0.03 g, respectively and lower TRDW of 0.35 and 0.34 g, respectively, as well as lower GY of 5.6 and 3.7 Mg $ha^{-1}$ (Table 5A). In experiment 3, Hybrids LH156/MBS2747 and LH82/MBS2747 produced higher DRDW (0.50 and 0.43 g, respectively) and higher TRDW (1.31 and 1.27 g, respectively) and also had higher GY (8.6 and 7.4 Mg $ha^{-1}$, respectively) under water-stress as compared to hybrid NC364/MBS2747 which yielded lower DRDW of 0.12 g, and lower TRDW of 0.74 g, and also had lower GY of 4.6 Mg $ha^{-1}$ (Table 5B). Aside from this, hybrid Pa91/MBS2747 had relatively low DRDW and low grain yield.

Although sample size was small, a correlation analysis was also performed to assess phenotypic relationships between root traits evaluated under greenhouse conditions with GY under water-stress conditions in field. For set 2 hybrids, GY in water-stressed condition in field showed significant relationships with RL ($r = 0.86$, $P = 0.03$), DRDW ($r = 0.86$, $P = 0.03$), TRDW ($r = 0.95$, $P = 0.003$) and DRSR ($r = 0.88$, $P = 0.02$) (data in table format not presented). For set 1 hybrids (evaluated in partial water-stress in greenhouse), GY showed significant relationship with RL ($r = 0.86$, $P = 0.03$), DRDW ($r = 0.83$, $P = 0.04$), DRR ($r = 0.88$, $P = 0.02$), and DRSR ($r = 0.82$, $P = 0.04$) but did not show significant relation with TRDW.

### *Comparison between inbreds and hybrids for variation pattern for deep root dry weight*

Deep root dry weights for the 12 inbreds and their corresponding hybrids were similar in terms of their relative values (Supplementary figure 1C), suggesting a relevance on variations for this trait between inbreds and hybrids. DRDW trait values of inbreds and hybrids of set 1 from experiment 3 and the values of set 2 from experiment 2 were used to evaluate the trait conservation between inbreds and their corresponding hybrids. DRDW value of LH156 (0.58 g) of set 1 from experiment 3 ranked 1 among 6 inbreds and DRDW value of the related hybrid LH156/MBS2757 (0.50 g) ranked 1 among the hybrids, while inbred NC364 (0.007 g) ranked 6 and the related hybrid NC364/MBS2747 (0.12 g) ranked 6 (Table 5B). In experiment 2, inbred 2369 (0.07 g) of set 2 ranked 1 among the 6 inbreds while its corresponding hybrid 2369/SGI07 ranked 2 among hybrids (Table 5A). Inbred LH194 (0.06 g) ranked 2 among inbreds while its related hybrid LH194/SGI071 (0.09 g) ranked 1. Inbred PHG86 (0.024) ranked 5 and its related hybrid PHR86/SGI071 (0.03 g) ranked 5 among the hybrids too. Similarly, Inbred N552 (0.003 g) ranked 6 among inbreds while corresponding hybrid N552/SGI071 (0.02 g) ranked 6. We also performed phenotypic correlation analysis between inbreds and hybrids for DRDW for set 1 and set 2. The set 2 inbreds and hybrids showed significant positive correlation for DRDW ($r = 0.88$, $P = 0.02$) whereas set 1 hybrids did not show significant relationship for DRDW.

## Discussion

### *Effect of growing media on root growth in well-watered condition*

Plants were grown in three different growing media, TS (turface and sand mix), SVPS (sand, vermiculite, perlite and soil mix) and sand only to evaluate root growth in order to identify a medium which supports the best root growth for our future studies. We grew the plants in TS and SVPS media in experiment 1 and observed SVPS media facilitated better root growth. This medium was selected for the two subsequent experiments reported herein. After this point, we became curious to grow roots in sand only with a view

Table 6 - Pearson's correlation matrix showing relationships among shoot and root traits.

| | LL | LW | RL | SDW | URDW | DRDW | TRDW | DRR | DRSR |
|---|---|---|---|---|---|---|---|---|---|
| A. Based on combined data of set 2 inbreds and hybrids evaluated in well-watered condition in experiment 2 | | | | | | | | | |
| SL | 0.91*** | 0.30 | -0.10 | 0.86** | 0.42 | 0.15 | -0.05 | 0.12 | -0.16 |
| LL | | 0.33 | 0.05 | 0.80** | 0.39 | 0.28 | 0.01 | 0.26 | -0.02 |
| LW | | | 0.57* | 0.70** | 0.66* | 0.80** | 0.79** | 0.80** | 0.66* |
| RL | | | | 0.17 | 0.65* | 0.72** | 0.85*** | 0.72** | 0.84*** |
| SDW | | | | | 0.63* | 0.51 | 0.36 | 0.49 | 0.23 |
| URDW | | | | | | 0.58* | 0.71** | 0.50 | 0.47 |
| DRDW | | | | | | | 0.86*** | 0.98*** | 0.92** |
| TRDW | | | | | | | | 0.83*** | 0.90*** |
| DRR | | | | | | | | | 0.92*** |
| B. Based on combined data of set 1 inbreds and hybrids evaluated in partial water-stressed condition in experiment 3 | | | | | | | | | |
| SL | 0.85*** | 0.73** | 0.69** | 0.92*** | 0.70** | 0.76** | 0.85*** | 0.71** | 0.71** |
| LL | | 0.51 | 0.76** | 0.74** | 0.36 | 0.69** | 0.63* | 0.78** | 0.68* |
| LW | | | 0.34 | 0.76** | 0.69** | 0.72** | 0.83*** | 0.50 | 0.58* |
| RL | | | | 0.67** | 0.3635 | 0.81*** | 0.68** | 0.90*** | 0.91*** |
| SDW | | | | | 0.66* | 0.79** | 0.82*** | 0.70** | 0.70** |
| URDW | | | | | | 0.48 | 0.83*** | 0.22835 | 0.45227 |
| DRDW | | | | | | | 0.87*** | 0.91*** | 0.95** |
| TRDW | | | | | | | | 0.68** | 0.83*** |
| DRR | | | | | | | | | 0.93*** |

significant at a probability level of *0.05, **0.01, and ***0.001, respectively.

to grow roots on a more homogenous growing medium. However, upon comparison, among the three growing media, SVPS mix supported relatively much better rooting depth and total root mass for both inbreds and hybrids despite differences in growth duration. The mean root length we observed in SVPS media was higher than a reported a median root length of corn plants grown in TS media for similar growth duration (Manavalan et al, 2011). The SVPS growing mix is being used for root growth studies in the Roots Lab of Penn State University, PA (2012). The better root growth in SVPS media was probably due to the presence of vermiculite and perlite which had loosened the soil compaction, provided better aeration, and allowed adequate drainage of water than TS and only sand media. The turface particles are calcined clay prepared after baking at very high temperature; they are relatively harder than vermiculite and perlite which probably offered some mechanical impedance that led to the restricted root growth unlike SVPS medium.

***Evaluation of root traits variation in PVC tubes***

Studying root architecture extensively under field condition is still limited due to the expenditure of time and labor involved in destructive techniques like the core method and the likelihood of under-estimation of root depth and density with alternative method like mini-rhizotron (Wiesler and Horst, 1994; Pages and Bengough, 1997; Vamerali et al, 2012). To circumvent these constraints, we used a simple and inexpensive system including soil media that allowed root growth with minimum impedance and soil strength variations, uniform moisture, and easy extraction of intact roots. We are in agreement to the suggestions by Salekdeh et al (2009) who stated that PVC tubes are preferable to pots when testing deep root growth and the ability of roots to access water in the soil profile, and reproducible levels of stress can be applied at specific developmental stages. Pierre (2012) opined that studies on soil moisture dynamics relative to root growth can be conducted using PVC tubes which provide a soil depth that is more representative of the field conditions, and root access to deep soil water. Using deep pots (76.2 cm height), Monovalan et al (2011) observed phenotypic variation among maize inbred lines for root length, root weight and shoot weight. Similarly, using PVC tubes or long plastic pots in greenhouse, phenotypic variations in rice root traits, such as root length, root thickness, total root mass, deep root mass, deep root ration and deep root-to-shoot ratio were evaluated (Thanh et al, 1999; Toorchi et al, 2002; Kamoshita et al, 2002). Huang et al (1997) estimated root growth of turfgrass species at different soil layers after imposing water stress in PVC tubes in order to evaluate drought tolerance. Bonos et al (2004) evaluated tall fescue and rye grass populations in flexible polyethylene and PVC tubes for selection of increased deep root production. Maize root traits could be studied using hydroponics during early developmental stages (Tuberosa et al, 2002), but one of our objectives was to impose partial water stress and its effects on root traits variation.

We observed phenotypic variation for root length, total root dry weight, deep root dry weight, deep-root ratio and deep root-to-shoot ratio among inbreds in all experiments, indicating constitutive nature of genetic control for these traits. Extent of variation for «deep root dry weight» among inbreds in experiment 2 (WW) ($P = 0.001$) and in experiment 3 (WS) ($P = 0.001$) was observed to be higher than the related trait «total root dry weight» ($P = 0.05$). This supports Azhiri-Sigari et al (2000) and Kamoshita et al (2000), who demonstrated genetic variation in constitutive root traits, and also indicated adaptive responses of root traits, especially in deeper soil layers. Inbred LH156 produced consistently highest total root dry weight in all experiments under well-watered and water-stressed conditions while NC364 consistently performed poor. In most analyses, variation for deep-root ratio and deep root-to-shoot ratio was found significant and highly dependent on variation on deep root mass. Values for both these traits for a particular genotype could be improved by increasing deep root mass.

***Relationship of deep root mass to drought tolerance***

We estimated deep root mass as the dry weight of the root section below 45 cm from the root base, (i.e., soil surface) which could be relatively closer to the expected deep root mass in actual field condition for similar (early) growth stages of the corn plants. This approach is in support of the studies by Thanh et al (1999), Kamoshita et al (2002), and Courtois et al (2013), who estimated deep root mass using root weight of the root section below 30 cm depth in rice and observed significant phenotypic variation for this trait. Kamoshita et al (2002) repeatedly observed higher deep root mass in the rice line IR58821, a parent of a mapping population than the other parent IR52561 in greenhouse studies which is in consistence with the actual field performance. Similarly, Henry et al (2011) reported significant variation in root length density (an indirect estimate of root mass) at a depth of 30-45 cm among 20 rice genotypes under drought stress condition, and found genotype 'Dular' with deep root growth had greater drought resistance. A correlation of root density at 35 cm depth with indicators of drought avoidance in upland rice was also reported (Cairns et al, 2009). Bonos et al (2004) made selection for deep root mass in tall fescue and rye grass populations by measuring deep root mass in the lower 30 cm root section with a view to improve drought tolerance. Hund et al (2009) estimated rooting depth under field condition at a point above which 95% of the all roots were located rather than based on the maximum root length of a few roots, and found to be greater in the tropical maize inbred lines known to have drought tolerance than those were drought sensitive. Their approach is fundamentally in agreement with the approach we have undertaken to designate deep root mass in lower section of the roots (deeper soil layers in PVC tubes) and relate this to the drought tolerance rather than associating the root length alone with drought tolerance based on mere maximum root length.

To demonstrate a relationship between root traits including deep root dry weight measured in greenhouse trials and grain yield achieved in water-stress condition in field, correlation analyses were performed, although a small number of genotypes were included in each set and trial. Grain yield showed significant positive relationship with root length, deep root dry weight and total. Aside from the correlations, based on t-tests, we compared the deep root dry weight with grain yield for individual hybrids based on relative performances among hybrids. For set 2, hybrids 2369/SGI071, LH193/SGI071 and LH194/SGI071 had relatively higher deep root dry weight and grain yield than the hybrids N552/SGI071 and PHG86/SGI071 (Table 5A). For set 1, LH156/MBS2747, LH82/MBS2747 and PHK42/MBS2747 had relatively higher deep root dry weight as well as grain yield than the hybrids NC364/MBS2747 and Pa91/MBS2747 (Table 5B). Similar ranking patterns of hybrids for deep root dry weight and grain yield again suggests an association between deep root mass and drought tolerance. For root length and total dry weight, similar ranking patterns are not as clear as seen for deep root dry weight and thus, less comparable with grain yield (Table 5).

Based on the data of hybrids only, root length showed significant relation with deep root dry weight for set 1 but not for set 2 whereas total root dry weight showed significant relation (with deep root mass) for both set 1 and 2. Variation or pairwise differences for root length among set 2 hybrids was not as pronounced as seen for deep root dry weight (Table 4A, Table 5A) was the reason for lacking significant relation between root length and deep root mass (in experiment 2). But based on the combined data of inbreds and hybrids, both root length and total dry weight showed high correlations with deep root mass. Genotypes with very long roots may not always yield very high deep root mass, for example, inbred PHK42 had longer roots than LH156 but it yielded lower deep root mass than LH156 (Table 5B). Similarly, hybrid LH194/SGI071 and N552/SGI071 had similar root length but LH194/SGI071 yielded much more deep root mass than N552 (Table 5A). We observed visually, not quantified, that the genotypes with high deep root mass had relatively higher number and longer main roots than the genotypes with low deep root mass in the lower sections of the roots. We also observed that the high deep root mass producing genotypes had more longer and relatively thicker lateral roots than the low deep root mass producing genotypes. We did not notice variation with regard to number and length of root hairs probably because of their minute structures.

Deep rooting has been implicated as a mechanism to avoid water stress by extracting water from deep soil layers (Yoshida and Hasegawa, 1982; Fukai and Cooper, 1995; Gowda, 2011). Uga et al (2013) demonstrated the maintenance of high yield under drought conditions by a rice variety with increased root distribution in the deeper soil layers after introgression with a quantitative trait loci controlling root growth angle. The introgression line exhibited steeper root growth angles but had the similar total root and shoot biomass as the recipient variety which had shallower root system. Here, we have implicated deep root mass, instead of only deep rooting or total root mass, with drought tolerance. We considered deep root mass as a combination of root length, root number, lateral branches, and root thickness in lower soil horizons, and we also agree in published reports/opinions that a genotype with higher number of deep roots and many longer lateral branches and many long root hairs will extract water efficiently from lower soil layers (Herder et al, 2010) than a genotype with fewer smaller main and lateral roots in deeper soil layers. Difference in rice genotypes with respect to root growth in deeper soil layers was reported by Samson and Wade (1998). Manske and Vlek (2002) reported that most drought tolerant semi dwarf bread wheat genotypes had higher root length density in deeper soil layers (i.e., higher deep root mass) than non-tolerant controls. However, overall, the results suggest that root length, deep root mass and total root mass are related and are implicated with drought tolerance but deep root mass could be used as a more reliable trait for selection for drought tolerance in maize. Further studies are required using a larger panel of genotypes to validate this kind of association.

We assumed that the genetic variation observed in the greenhouse PVC tube setup in early growth stages of hybrids was also maintained in the later growth stage under field condition (Comas et al, 2013) as the similar pattern of variation was exhibited in the yield performance of the hybrids under water-stress. A positive correlation between root traits of maize seedlings and those of mature plants has been reported by Nass and Zuber (1971). Tuberosa et al (2002) identified the genomic regions co-located with QTLs (quantitative trait loci) controlling the weight of adventitious seminal roots of maize grown in hydroponics with the QTLs controlling grain yield in well-watered and water-stressed conditions in the field, suggesting a possible maintenance of greenhouse root weight trait variation àobserved in early vegetative stage up to the late growth stages in the field.

***Conservation of deep root mass between inbreds and hybrids***

The pattern of variations in DRDW we observed in the inbred lines were tended to be reflected in the same manner in the hybrids indicating this trait to be highly heritable but a study with a large population is needed to verify this. The higher trait values of DRDW observed in the inbreds and in their corresponding hybrids and similarly, lower trait values observed in the inbreds and their corresponding hybrids are in similar relative ranking manner. This trait conservation will allow the scientists to use inbreds to evaluate the variation of root traits with a good a prediction that the identified trait values will be inherited to the crosses when the same studied inbred lines are used but, of course, there is a chance of deviation due to specific combining ability effects. Kamoshita et al (2002) reported high broad sense heritability for deep root mass in rice. Several diverse inbred lines with high deep root mass and low deep root mass have been identified and these could be used as parents for developing bi-parental mapping populations to study the genetic basis of deep root mass and its response to water stress.

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