

Contribution of root system architecture and function in the performance of tropical maize (*Zea mays* L.) genotypes under different moisture regimes

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Abstract

Understanding how roots respond to increasing rate of evapotranspiration in warmer days and exposure to dry spells is crucial for saving productivity of rainfed crops, including maize, grown in Asian tropics. In a semi-automatic root phenotyping facility (lysimetric system) a set of 100 elite and diverse tropical maize inbred lines were phenotyped under managed drought stress (DT) and well-watered (WW) conditions. Plants were grown in PVC (Polyvinyl chloride) cylinder of 30.0 cm diameter and 150.0 cm length. In drought experiment, last irrigation was applied based accumulated growing degree days (Σ GDD) criteria to achieved reproductive stress DT, whereas optimal moisture was maintained in WW trials. Data recorded on various root structural and function traits in both DT and WW trials. Significant phenotypic variability was observed for various root traits, including both structural and functional traits, under both the moisture regimes. Correlation studies showed that grain yield of early maturity group of genotypes was positively and significantly associated with all the root structural traits under drought, whereas, in case of medium and late maturity group of entries root structural traits showed either weak positive or significant negative correlation with grain yield under drought. Though, root functional traits of all the maturity group of genotypes showed positive and significant correlations with both grain yield and total biomass under both well-watered drought stress. Regression analysis showed that water uptake had significant positive relationship with total biomass in all the three-maturity group of genotypes. However, grain yield seems to be less dependent directly on the total amount of water uptake. We conclude that contribution of various traits in root system architecture under drought or well-watered conditions vary with maturity of genotypes. However, root functional traits, such as water uptake and transpiration efficiency are equally important across maturity groups and water availability regimes.

Keywords: Drought stress; Maize, Root system architecture; Water-deficit; *Zea mays* L.

Abbreviations: CAAM_CIMMYT Asia Association Mapping panel; GDD_Growing degree days; DT_drought stress; VPD_Vapour Pressure Deficit; WW_Well watered;

Introduction

Climate change is threatening global crop productivity, especially for the rainfed crops, such as - maize, grown in the lowland tropics. Rainfed systems, which represents major (~80%) part of maize mega-environments in Asian tropics, are largely dependent on monsoon rains, and other prevailing weather conditions and therefore extremely vulnerable to climate change effects (Zaidi et al., 2020). Studies suggested sharper increase in both day and night temperatures in near future, which could adversely affect maize production in the tropical regions (Lobell et al., 2011; Cairns et al., 2012). Such impacts have already been experienced in the Asian region in several recognizable ways, such as shifting seasons and significant inter-annual variation in rainfall pattern with increased frequency of extreme weather events during main cropping season (Prasanna et al., 2021). Heat stress in combination with drought, has emerged as one of the major constraints to maize production in tropics (Cairns et al., 2013). Frequent episodes of high temperatures (often above 35°C) along with prolonged dry spells are a common phenomenon in most of

the semi-arid tropical maize growing areas, causing irreversible damage on maize crop and eventually resulting in considerable yield penalties (Zaidi et al., 2020).

With progressive climate change effects plants have a greater demand for water in warmer environments due to increased water loss through evapotranspiration (Heckathorn et al., 2013). Crop breeding programs have traditionally focused on the aboveground plant parts for development of new stress-resilient crop varieties suitable for target population of environments (TPEs) to minimise losses due to various abiotic and biotic stresses. While shoot traits drive water and nutrient through plant, root traits ultimately determine access to water and nutrients, and thus, set limits for shoot functions. Being the first plant part exposed to various soil related stresses, including drought, roots play a pivotal role in adaptation to such stress conditions and govern the overall performance of plants. Despite well-known vital roles of plant roots in anchorage, absorption of water and nutrients (Lynch, 1995) most often this important hidden-half is ignored due to complexity

involved in studying root traits due to technical and/or logistic challenges in phenotyping roots as a whole system and their interaction with the soil. However, availability of non-destructive methods such as lysimetric system, X-ray tomography etc. allow study related root growth and functional dynamics during crop cycle.

Roots that allow access to water with minimum use of carbon holds great promise for plant adaptation to drought stress conditions (Hund et al., 2009). Water uptake takes place in the root either through aquaporins or membrane channels that facilitates water transport inside the cells, or by diffusion through plasmatic membrane (Maurel et al., 2015). Studies on the maize crop showed that warmer temperatures decrease aquaporin quantity and increases membrane fluidity. Membranes become rigid that reduces water uptake (Iglesias-Acosta et al., 2010; Ionenko et al., 2010). Negative effects of water deficit on roots results in severely compromised water and nutrient uptake, which eventually results in significant reduction in crop yields. Therefore, new maize cultivars better adapted to climate-change induced weather extremes, such as severe drought stress at critical growth stage(s), will have to have root system with improved water and nutrient uptake as well as use efficiency to ensure yield stability under stress condition. In the present study we aimed to (i) identify available genotypic variability in root traits in tropical maize, including both structural and functional traits, (ii) analyze changes in root system architecture and functions with moisture depletion in root zone and (iii) assess contribution of various root traits in performance of different maturity group of genotypes under optimal moisture conditions and drought stress.

Results

Genotypic variation for root and shoot traits

While main targeted traits for the study were root structural and functional traits under drought and well-watered conditions, observations were also recorded on key agronomic traits, including grain yield and total above-ground biomass under DT and WW conditions. Combined analysis for two years data within each water regimes showed that genotypic variability was significant for various root and shoot traits under both DT and WW, except PH, ASI, RD and RDW under WW conditions (Table 1). Compared to WW, mean values for most of the root and shoot traits decreased under drought stress, except ASI and TE where the values increased, and AD did not register any significant change between the two treatments. Also, the variation in most of the traits increased substantially under drought stress, especially in case of root traits - RW, RV, WU, and also shoot traits - ASI, TB and GY. Drought stress severely affected root traits; though the effect was more pronounced on root functional traits where mean WU decreased by 54.5% and mean TE increased by 60.0%. Effect of drought stress was also severe on various shoot traits, In comparison to WW condition, mean ASI increased by 5.0 days under DT, whereas mean GY and TB reduced by 54.7 and 43.9%, respectively. Broad-sense heritability was high (≥ 0.5) for almost all the root and shoot traits under both WW and DT conditions, except WU, TE and TB under WW and RV under drought stress. It was also observed that heritability of most of the traits increased under drought stress, except for RV, ASI and PH.

Maturity group effect on root traits under drought stress

Maturity group-wise dissection of various root traits showed substantial variation in structural traits under drought stress (Fig. 1). Compared to late maturity most of the early maturity group of genotypes showed relatively shallow RD, RV and RW. Rooting depth of most of the early maturity genotypes (72%) ranged between 50 to 100cm, whereas in case of late maturity 54.2% genotypes had RD more than 100 to 150cm and 10.4% genotypes had >150cm. In medium maturity group 45.6% genotypes had RD >100cm, whereas 50% entries had RD between 50-100cm. Root volume and RW also followed similar trends with maturity group of genotypes. In case of WU most of the early maturity genotypes (74%) grouped into relatively low (≤ 12 litres) water using entries during the reproductive phase of crop growth, whereas most of the late maturity entries (65%) extracted relatively more water (≥ 12 to >16 litres) during same period. Majority of the medium maturity entries (82%) were grouped in the range of 9-16 litres water using entries during reproductive phase. Irrespective of water uptake during reproductive phase, maximum number of entries across all the maturity groups (>50%) showed relatively similar rate of TE (11-15 g litre⁻¹), followed by >30% with 5-10 g litre⁻¹ during the same period of crop growth. These finding showed that distinct variation due to maturity groups was less apparent in case of root functional traits, especially TE.

Phenotypic correlations between root traits and grain yield or total biomass

Correlation analysis between various root traits with GY showed that under well-watered conditions, except RW in early maturity and RV in medium maturity genotypes, all other structural traits had non-significant relationship (Fig. 2). Most of these structural traits showed positive and significant correlation with TB under well-watered conditions, except RD in all the maturity groups and RLD in early maturity genotypes. However, under drought stress all the root structural traits showed positive and significant correlations with both GY and TB in early as well as medium maturity genotypes, except RLD with TB in both maturity and RV with GY of medium maturity. In case of late maturity group of genotypes, the correlation of RV and RW was significantly negative with GY under drought stress. Rooting depth also showed negative, though non-significant, correlation with GY of late maturity entries under drought stress. Total biomass also showed significantly negative correlation with RW, whereas RLD, RD and RV had non-significant correlation with TB of late maturity entries under drought stress. On the other hand, irrespective of maturity group of genotypes root functional traits, both WU and TE, showed positive and significant correlations with GY as well as TB under drought stress and also under well-watered conditions (Fig. 3). Under optimal moisture conditions there was no major variation in this relationship due maturity group of genotypes. However, under drought stress the correlation between WU or TE with GY as well as TB relatively strong in early maturity, followed by medium and late maturity genotypes. In case of late maturity, the correlation between WU and GY was non-significant.

Relationship between root functional traits and plant performance

Regression analysis showed that, irrespective of maturity group of genotypes, the relationship between WU and TB

was linear and statistically significant ($P < 0.01$) under both optimal moisture and drought stress conditions (Fig. 4). Though, under drought stress the amount of water uptake during stress period made relatively more contributions in early and medium maturity group of genotypes. Also, under optimal moisture GY of all the three maturity groups of genotypes was also significantly ($P < 0.05$) associated with amount of water uptake during reproductive period. Under drought stress late maturity groups of genotypes showed weak and non-significant relationship with water uptake; though its effects on GY of early and medium maturity genotypes was statistically significant ($P < 0.01$). Regression analysis of GY and TB over TE of different maturity groups showed that under both well-watered and drought stress conditions TE significantly ($P < 0.01$) contributed to both GY and TB of all three maturity groups of genotypes (Fig. 5). Though, the contribution of TE was relatively more for TB than GY, and GY of early maturity group genotypes showed strong relationship with TE, followed by medium and late genotypes under both optimal moisture and drought stress.

Discussion

The questions that we set out to answer through this study were (i) how the variability in root architecture and function contributes to the performance of genotypes under WW and DT stress, and (ii) whether contributions of various structural and functional traits vary in different maturity group of genotypes. In the present study, we observed significant variability for all the root and shoot traits under drought stress (Table 1). The variation was significant in almost all the traits under optimal moisture as well, except for couple of roots (RD and RW) and shoot (PH and ASI) traits. This is explained by the fact that the set of inbred lines was carefully selected for the study, and they represented good genetic diversity of the lowland tropical maize. Previous studies on improved maize or maize landraces also reported significant genotypic variability for various root structural, anatomical, and functional traits under different moisture regimes (Burton et al., 2013; Zaidi et al., 2016; Qiao et al., 2018; Zhao et al., 2018). The available genotypic variation in root traits is considered an asset for breeding programs aimed at producing cultivars with improved adaptation to water-limited environments (Manavalan et al., 2011; Wasaya et al., 2018; Klein et al., 2020). While comparing mean values and range for the traits under WW and DT it was observed that mean values of most of the root and shoot traits, except AD, ASI and TE, decreased, whereas range of these traits substantially increased under drought stress. The increased variation observed under drought stress suggests that root system architecture is plastic and dynamic, allowing plants to respond to their environment to optimize acquisition of soil resources such as water and nutrients (Vamerali et al., 2003; Zhu et al., 2011).

Maturity group wise analysis showed a significant variation in root traits of three different maturity group of genotypes under drought stress, especially in case of structural traits. Early maturity group of genotypes had relatively smaller root architecture system (RSA) with shallow root depth, and relatively low RW and RV compared to late and medium maturity group of genotypes (Fig. 1). Variability in RD of early maturing genotypes was non-significant under optimal soil moisture, while it was significant under drought, which suggested that smaller root system is one of the stress avoidance mechanisms for early maturity genotypes to optimize biomass partitioning and use of available water

under limited water availability (Zhao et al., 2018). In the study on tropical maize inbred lines with distinct response to drought stress Hund et al. (2009) also found that combination of high-water use efficiency (WUE) and sufficient water acquisition by a root system with fewer lateral roots in the topsoil but with thicker, deep axile roots can increase drought tolerance. These findings suggest that root allocation and distribution is one of the plant growth strategies and their general response to water deficits and distribution of available soil water. This agrees with the findings in present study as distinct variation in root functional traits due to maturity group was less apparent, especially in case of TE and also WU (Fig. 1). Vamerali et al. (2003) reported that a drought tolerant hybrid maintained high transpiration at very low values of the fraction of transpirable soil water, whereas drought susceptible hybrid showed a higher response to soil drying. The latter reduced the rate of transpiration starting from a higher level of transpirable soil water. The study suggested that more conservative strategy towards limited water and nutrient resources in drought susceptible hybrid, and a greater tendency towards stress avoidance in drought tolerant one. Based on the study on a set maize hybrids spanning a century of breeding Messina et al. (2021) showed that root system architecture and grain yield have changed with decades of maize breeding, but not the crop water uptake. They showed that single cross hybrids have smaller root systems than double-cross hybrids, but soil water extraction measured under field conditions were not significantly different between two types of hybrids.

Phenotypic correlations between various root traits and plant performance in terms of TB and GY showed that across maturity group of genotypes most of the root structural traits, except RD, showed significantly positive correlation with TB under DT as well as under WW conditions (Fig. 2). Previous studies on maize also reported positive correlation between root traits with leaf area, biomass, and grain yield under optimal moisture conditions (Junying et al., 1989). Grain yield of early maturity group of genotypes was positively and significantly correlated with all the root structural traits under DT and with RW under optimal moisture. However, in case of medium and late maturity group of entries most of the traits, except few traits of medium maturity genotypes such as- RV under optimal and RD and RLD of under drought, showed either weak positive or significant negative correlation with GY under DT or WW (Fig. 2). Tuberosa et al. (2002) also found positive and significant relation between seminal root traits and grain yield, but the relationship was found negative between primary rooting and the grain yield under water stress. Correlation studies in dry season sorghum genotypes under receding moisture indicated significant and positive correlation between grain yield and root traits viz.; root weight, root number and root length (Shinde et al., 2017). Findings of our study and also previous studies (Rangarajan et al., 2018; Strock et al., 2019) suggested that there is no single root phenotype that is ideal across all environments and maturity group of genotypes to maximize grain yield under drought stress, as access to resources, e.g., water, may be influenced by several other aspects of the environment, such as soil texture etc.

Correlation analysis between root functional traits and TB or GY showed that across maturity groups the relationship was positive and significant under both WW and DT, except between WU and GY in late maturity under DT (Fig. 3). It is

Table 1. Genotypic variability for various root and shoot traits of maize inbred lines evaluated under well-water condition and drought stress.

	Rooting depth (cm)	Root dry weight (g plant ⁻¹)	Root volume (cm ³ plant ⁻¹)	Root length density (cm cm ⁻³)	Water uptake during flowering (liters)	Transpiration efficiency during flowering (g liter ⁻¹)	Anthesis (days)	Anthesis-silking interval (days)	Plant height (cm)	Grain yield (g plant ⁻¹)	Total biomass (g plant ⁻¹)
Well-watered condition											
Mean	158.2	16.0	100.2	2.4	22.3	9.4	53.9	2.2	196.6	123.7	272.3
Min	109.0	12.9	76.7	1.3	17.6	6.7	51.1	-2.7	120.9	84.8	164.2
Max	229.0	28.3	209.3	3.4	28.1	16.8	64.2	4.6	256.7	204.0	426.8
LSD	26.16	3.04	32.50	0.23	5.62	29.92	4.32	3.55	29.24	61.80	135.77
Significance	ns	ns	*	**	**	*	*	ns	ns	**	*
Vg	143.73	12.48	2210.27	0.02	3.96	145.15	20.68	1.82	423.35	891.00	3152.00
H	0.53	0.71	0.90	0.57	0.36	0.42	0.83	0.52	0.69	0.50	0.43
Drought stress											
Mean	133.9	11.6	74.8	1.6	10.1	15.0	52.2	7.2	152.7	58.5	152.7
Min	88.3	6.3	15.7	0.8	2.9	9.4	50.1	3.2	102.5	0.6	23.1
Max	244.0	33.8	326.6	2.2	17.5	23.1	63.8	17.3	227.5	145.2	315.1
LSD	18.42	3.99	87.14	0.28	2.81	5.98	4.26	3.90	32.80	35.12	67.33
Significance	**	*	**	**	***	**	**	**	*	***	**
Vg	1643.54	22.76	1336.33	0.10	4.16	7.91	8.80	4.88	478.00	379.68	1927.00
H	0.96	0.86	0.44	0.85	0.70	0.57	0.59	0.59	0.52	0.58	0.65

*, **, *** indicates statistical significance at P<0.05, 0.01 and 0.001, respectively; ns = non-significant

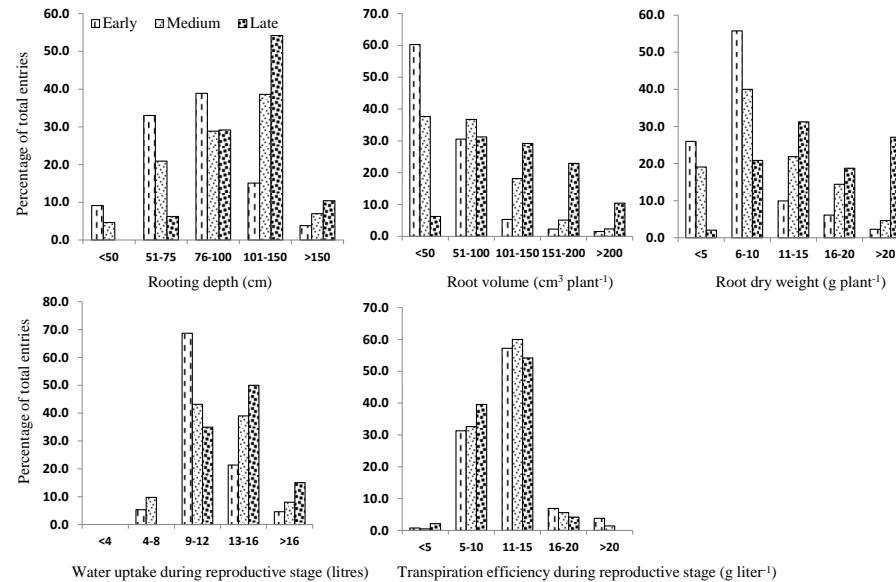


Fig 1. Frequency distribution of various root traits in different maturity group of maize genotypes under reproductive stage drought stress.

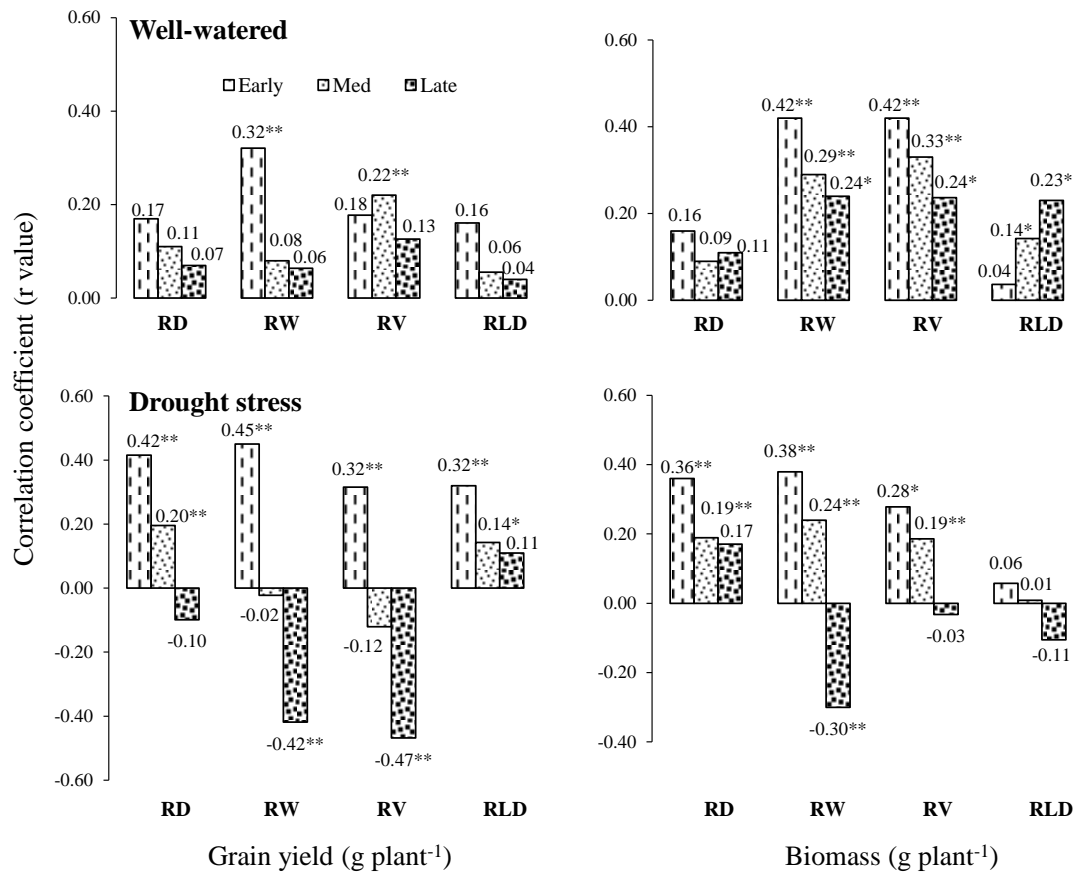


Fig 2. Phenotypic correlation of grain yield and plant biomass with root depth (RD), root dry weight (RW), root volume (RV) and root length density (RLD) under drought and well-watered conditions in various maturity group of maize genotypes. (* and ** indicates statistical significance at $P < 0.05$ and 0.01 , respectively).

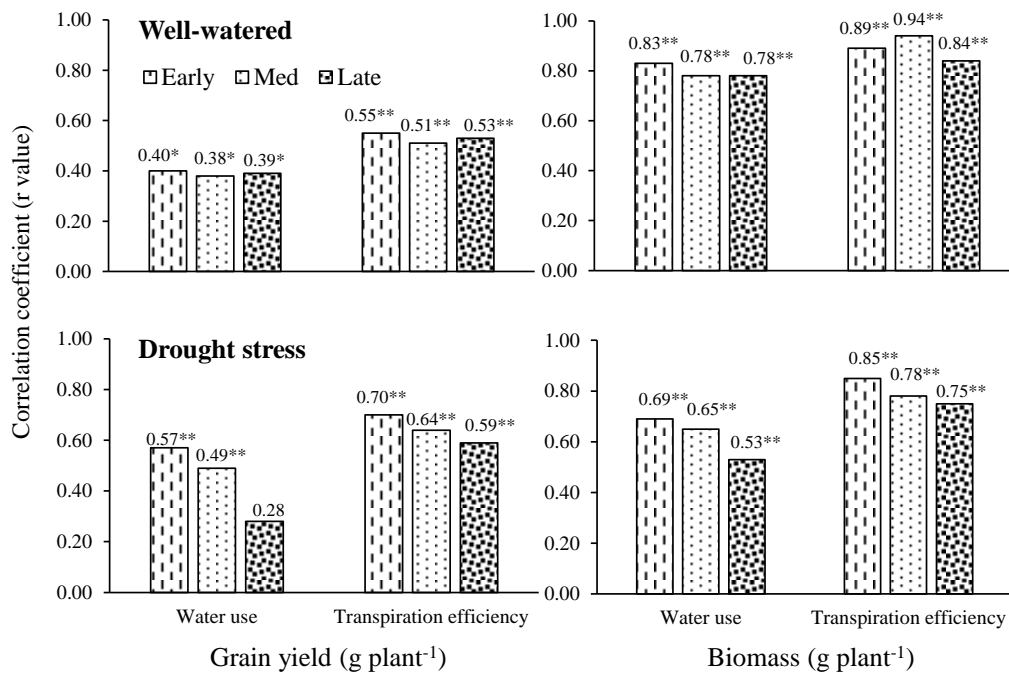


Fig 3. Phenotypic correlation of grain yield and plant biomass with water-used during reproductive phase and transpiration efficiency under drought and well-watered conditions in different maturity group of maize genotypes. (* and ** indicates statistical significance at $P < 0.05$ and 0.01 , respectively).

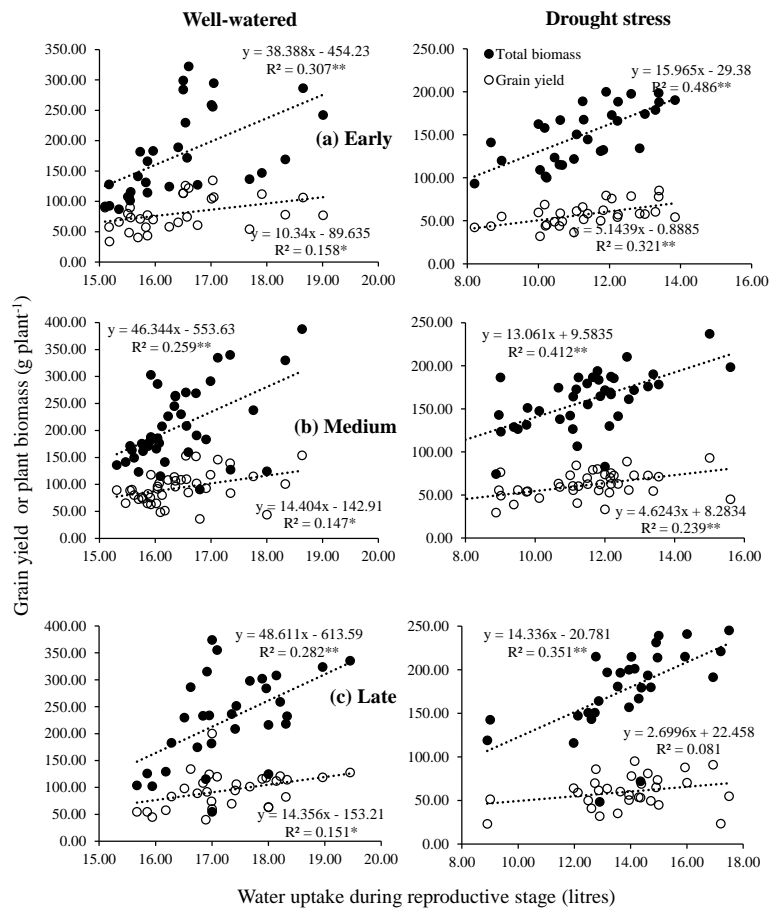


Fig 4. Relationship between grain yield or plant biomass with water-uptake under drought and well-watered conditions in different maturity group of maize genotypes. (* and ** indicates statistical significance at P<0.05 and 0.01, respectively).

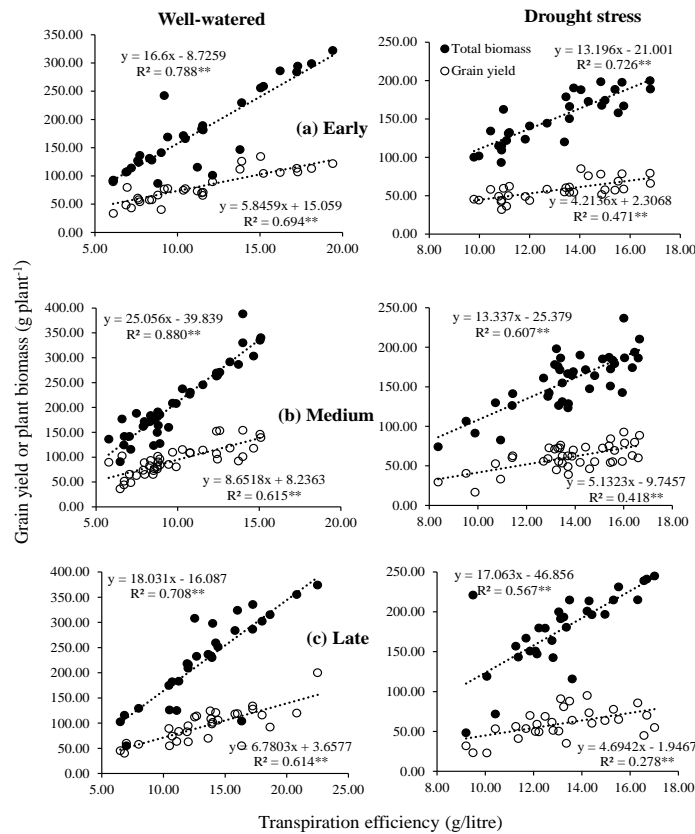


Fig 5. Relationship between grain yield or plant biomass with transpiration efficiency under drought and well-watered conditions in different maturity group of maize genotypes. (* and ** indicates statistical significance at P<0.05 and 0.01, respectively).

also observed that the relationship of both WU and TE with GY was relatively strong under drought stress. Root water uptake capacity under DT seems to be crucial when coping with water deficit events (Matsuo et al., 2009) but genotypic variation in water uptake capacity under drought conditions might not always be related to a higher investment in root biomass, but with specific hydraulic characteristics of roots (Kondo, et al. 2000). Across maturity groups, the relationship of TE was relatively strong with both TB and GY, under both WW and DT conditions (Fig. 3). Ratnakumar et al. (2009) found that under an intermittent drought regime, TE and water extraction from the soil profile were the most important components during a period corresponding to pod filling, a key determinant for grain yield in peanut. Maize leaves often operate at lower TE than potentially achievable for a species with C_4 metabolism (Bruce, 2010). The study concluded that significant genotypic variation in leaf TE exists in maize, and that TE could be improved without reducing photosynthesis.

Regression analysis showed that WU had significant positive relationship with TB of all the three-maturity group of genotypes under both WW and DT stress (Fig. 4). Though GY also showed significant relationship with WU ($P < 0.05$) but under WW conditions the dependency of GY seems to be relatively less on total water uptake, as expressed by relatively low R^2 values under WW compared to DT (Fig. 4). Under DT dependence of GY of late maturity genotypes to total WU was non-significant ($P < 0.13$), which suggested that WU contributed more to TB than GY of late maturity entries. However, the dependency of GY on WU under DT increased in early and medium maturity genotypes as indicated by highly significant R^2 values ($P < 0.01$). Water uptake is perhaps the most important component of a simple crop growth model defined by Passioura (1977). Water uptake should be the prime target (McIntyre et al., 1995; Dardanelli et al., 2004) and as such water uptake is unlikely to be dependent on differences of axial resistance to water flow. While measuring the volume of water taken up by roots is certainly an important factor, understanding the kinetics of water uptake, and how this kinetics relates to phenological stage of a plant in different maturity groups are equally important. Maize is known for its high susceptibility to drought stress during the four weeks bracketing flowering (Shaw, 1977), and therefore the key stage to monitor water-uptake and its kinetics would be the reproductive stages, starting from tassel emergence till early grain filling stage. Enough water at key crop stage, such as reproductive phase in this study, is more important than across the whole cycle (Boote et al., 1982). In a comparison of soil water extraction between an old versus a modern Pioneer hybrid Campos et al. (2004) found that the older hybrid extracted more soil water, especially in the upper soil layers when tested under the same imposed water deficit regime. The old hybrid suffered much greater yield loss, in part because it had extracted most of the plant-available water in the rooting zone before it entered the critical flowering period.

Irrespective of maturity group of genotypes, both TB and GY were highly dependent to TE under both WW and DT conditions (Fig. 5). In general, maize plants have a conservative hydraulic conductance, and by decreasing transpiration they maintain carbon assimilation during drought or during wilting (Comas et al., 2013), and thereby maintain high TE in tolerant genotypes that contribute to maintain high biomass and eventually in improved yields under water-deficit conditions. In a study on rice crop under

the three irrigation patterns Zhou et al. (2020) found highest correlation between water use efficiency and dry matter under controlled conditions. Blum (2009) argued that as long as the biochemistry of photosynthesis cannot be improved genetically, greater genotypic transpiration efficiency is driven mainly by plant traits that reduce transpiration and crop water-use. Since biomass production is tightly linked to transpiration, breeding for maximized soil moisture capture for transpiration is the most important target for yield improvement under drought stress. Genotypic diversity among maize hybrids in transpiration response to vapor pressure deficit (VPD) indicated that a limited-transpiration trait may contribute to improved drought tolerance and yield in maize (Messina et al., 2015). Limiting transpiration at VPD above a threshold can increase both daily transpiration efficiency and water availability for late season use. However, the impact of this trait on maize productivity may vary with geography, environment type, expression of the trait, and plant density.

Materials and Methods

Plant germplasm

The study was conducted on 100 tropical maize inbred lines, including 30 each from early and late maturity groups, and 40 from medium maturity group (Supplementary Table-1), selected from an association mapping panel, the CIMMYT Asia Association Mapping (CAAM) panel, constituted with 396 diverse lines. The CAAM panel included advanced stage maize inbred lines derived from CIMMYT's tropical and sub-tropical pools and populations from Latin America, sub-Saharan Africa, and Asia maize program. The 396 lines were identified for the constitution of association mapping panel after evaluating line trials with over 1000 lines at CIMMYT maize farm, International Crop Research Institute for Semi-Arid Tropics (ICRISAT) campus, Hyderabad, India (Geo coordinates - 17.3850°N, 78.4867°E and 542 masl) under well-watered conditions. The lines with reasonably good adaptation in Asian tropics were selected for constituting the CAAM panel, avoiding sister lines or over-representation of lines derived from the same pools or populations. Apart from lines from CIMMYT-Asia maize program, the panel also included lines derived from CIMMYT's drought tolerant populations, including *Tuxpeno Sequia-C6* (Tropical late white-dent), *La Posta Sequia-C7* (Tropical late white-dent), DTPY-C9 (Tropical medium yellow-flint), Pool-26 Sequia (Tropical late yellow-flint), DTPW-C9 (Tropical medium white-flint), G18 Sequia C5 (Tropical early yellow-dent) and Pool16 BN Sequia-C5 (Tropical medium white-dent), which were systematically developed and improved for drought tolerance through full-sib or S1 recurrent selection scheme (Edmeades and Deutsch, 1994). The sub-set of 100 lines from the panel was selected based on performance of lines under managed drought stress at flowering stage. They were grouped into three maturity groups based on days to anthesis, i.e.- early maturity = ≤ 50 days, medium maturity = 51-55 days, and late maturity = ≥ 55 days. The lines in each maturity group in the CAAM panel were ranked in descending order for grain yield under drought stress and selection was made from the range of top to bottom ranking lines after a regular interval to select 30 lines each from early and late maturity and 40 lines from medium maturity that represent diverse performance of these maturity group lines under stress.

Root phenotyping system

The experiments were conducted in a root phenotyping facility at the ICRISAT campus, Hyderabad, India. The facility was built based on lysimetric system that provides opportunity to directly assess and quantify root traits and their dynamics under various growing conditions and allows high-precision phenotyping of various root traits. The root study in this system is based on a real-time measurement of water uptake and measure root structural traits under different growing conditions, such as optimal moisture and water deficit conditions. The facility was built under a semi-automated rainout shelter that helps in avoiding unintended moisture in trials due to rains during the experimentation. Plants were grown in mini-rhizotrons, which are made-up of PVC (Polyvinyl chloride) cylinder of 30.0 cm diameter and 150.0 cm length, filled with a mixture of Vertisol + Alfisol + sterilized farmyard manure in the ratio of 15:5:1 by volume. In each PVC cylinder an end plate was fixed at 3.0 cm from the bottom with four screws in a way to retain the soil at the bottom but to freely allow water drainage. The soil in cylinders had a very similar bulk density to field conditions, and cylinders weighed a mean weight of 185 kg including PVC tube and soil. The PVC cylinders were arranged in eight trenches (1.5 m deep, 1.6 m wide and 30.0m long) in a way to match levels of cylinder and outside soil surfaces, and separated from one another by approximately 20.0 cm. In this way maize plants in rhizotrons were placed at a density of 6.25 plants m², matching with plant population close to a field planting density (row-to-row distance of 60.0 cm and plant-to-plant spacing of 20.0 cm). Border effects were managed by placing a row of potted maize plants on all sides of the trenches.

Crop management and stress treatment

The experiments were conducted during the dry season of 2015 and 2016 at root phenotyping facility at ICRISAT campus, Hyderabad, India. The off-season is identified as the best time of the year for managed drought stress trials as it is usually rain-free, and the temperature regimes is also congenial, i.e., T_{min} ranged from 10-15°C and T_{max} from 28-37°C during most part of the experimentation starting from planting in last week of November until grain-filling stage which occurs by the end of February or early March. Soil that was used to fill the cylinders was thoroughly incorporated with ammonium sulphate, urea, muriate of potash and zinc sulphate at the rate of 800, 174, 320 and 53 mg kg⁻¹ soil. Top-dressing of 3.0 g urea cylinder⁻¹ was done at V₃₋₄ stage, V₇₋₈ stage (or just before applying last irrigation in drought set) and at early grain filling (after resuming irrigation in drought set). Two set of trials, one each under well-watered (WW) and drought (DT) stress conditions, were planted in rhizotrons placed in separate pits. Planting of the three-maturity group of lines was staggered in such a way that all the lines reached flowering stage at almost the same period. In both WW and DT trials each entry was planted in eight replications (one cylinder considered as one replication). In each cylinder three seeds were sown and a measured amount of water (5.0 litres cylinder⁻¹) was applied as first irrigation for germination. Extra plants were thinned on 10th day after sowing to maintain one seedling per cylinder. Growing degree days (GDD) was recorded starting from date of first irrigation until a cumulative GDD (\sum GDD) value reached to 450, 550 and 650°C, respectively for early, medium and late maturity entries trial, when last irrigation is planned to be applied in three maturity group of entries. Till

that stage a measured amount of water was applied to maintain the same level of water in each cylinder in both the WW and DT trials. At the time applying last irrigation each cylinder in DT and WW trial was saturated with 25.0 litres of water, and there after irrigation was stopped in the DT trial for imposing drought stress. However, the irrigation with measured amount of water was continued in cylinders under WW conditions. In both DT and WW trials, the soil surface of the cylinders was covered with polyethylene beads immediately after saturation to prevent evaporative loss of moisture from the soil in cylinders. In this way it was ensured that water loss from the cylinder was largely (~90%, calculated based on calibrations in the past) through the transpiration process by the plants. After 24 hours once the excess water out-flow stopped upon saturation each cylinder was weighed in both the treatments to record the initial weight of the cylinders. Weighing of the cylinders was done by lifting each cylinder one at a time using the metal-collar fixed at the top of the cylinder, with a block-chained pulley. A S-type load cell (Mettler-Toledo, Geneva, Switzerland) was hooked between the collar of the cylinder and the pulley. A scale of 200 kg capacity allowed repeated measurements with accuracy of about \pm 20.0 g. In the WW treatment the optimal moisture was maintained by weighing the cylinders on regular intervals and the amount of water lost from the initial weighing was compensated by adding the loss of water through transpiration process. In drought stress trials no irrigation was applied until two weeks after anthesis. This was followed on individual genotype basis, i.e., after completion of 50% anthesis a final weight of all the cylinders for that entry was taken and then stress was terminated by applying 20 litres water in two instalments: 10 litres each with a gap of five hours. Simultaneously, final weight of the cylinder for that respective genotype under WW conditions was also recorded. The difference in initial weight and final weight were used in calculating the amount of water uptake during reproductive stage under drought stress and under well-watered conditions during same period.

Phenotyping root traits

The set of 100 maize inbred lines were phenotyped for various root traits, including a range of root structural and functional traits under managed DT and WW conditions. In both DT and WW trials data was recorded on root functional traits including water uptake (WU) during reproductive growth stage and transpiration efficiency (TE), and structural traits, including rooting depth (RD), root dry weight (RW), root volume (RV), and root length-density (RLD). Apart from root traits, data on key shoot traits, including plant height (PH), days to 50% anthesis (AD), 50% silking (SD) and anthesis-silking interval (ASI) was also recorded. At harvest, grain yield (GY) and total above-ground biomass (TB) was recorded for each entry under both DT and WW conditions. Total biomass was recorded after oven drying the whole plant (excluding roots) at 65°C for 72hrs, whereas GY per plant was calculated after adjusting the ear weight for average shelling percentage - 80%, and grain moisture content - 12.5%.

Reproductive period water uptake (WU) in DT was calculated based on initial and final weight of cylinder using formula mentioned below, whereas in case of WW treatment this was estimated as the cumulative amount of water added in cylinders that were lost due to evapotranspiration during same period:

Water uptake (L) = Initial weight of cylinder (Kg)– Final weight of cylinder (Kg)

Transpiration efficiency (TE) was calculated based on the total amount of water transpired from the time of last irrigation for imposing drought stress (and same time in well-watered trial) until physiological maturity and the total plant biomass that was recorded by harvesting the whole plants (excluding roots). In both the trials, the amount of water that transpired before the last irrigation for imposing drought stress was not accounted. It is assumed that since up to that stage all the entries in both the trials were maintained at the same level of optimal moisture the relative genotypic variability for TE would vary due to water uptake and biomass produced during the stage with drought stress. Transpiration efficiency was calculated using the formula given by Richard (1991) as follows:

$$\text{Transpiration efficiency (g L}^{-1}\text{)} = \frac{\text{Shoot dry weight (g)}}{\text{Water transpired (L)}}$$

To measure the root structural traits, cylinders were shifted to a designated root washing area. Soil adhering to the roots were carefully removed by passing a fine jet of water through the cylinder. After removing the soil, the intact root was taken out of the cylinder and washed again with clean water to remove small solid clods that adhered the roots. Rooting depth (RD) was measured as the length of root from stem collar to tip of the root. Roots were scanned and digitalized images were used for measuring the root volume (RV) and root length density (RLD) using WinRhizo software, Regent Instruments Inc., Canada (https://regent.qc.ca/assets/winrhizo_about.html). Roots were then dried in hot-air oven at 70°C for three days and root dry weight (RW) was recorded.

Statistical analysis of phenotype data and association analysis

Data on root structural and functional traits along with other agronomical traits were subjected to analysis of variance (ANOVA), separately for two water regimes, i.e., DT and WW condition. A combined analysis for over the years within each management was done and mean values of various traits under DT and WW conditions were used to estimate the phenotypic correlation between various root traits and grain yield or total biomass. Regression analysis was conducted between root functional traits and grain yield or biomass to assess the relationship between root function traits with overall performance of genotypes under DT or WW conditions.

Conclusions

From the finding of the present study, we conclude that significant genotypic variability exists in lowland tropical maize for various traits in root system architecture and root function. Contribution of most of the root structural traits vary with the rate of phenological growth and development, whereas, root functional traits, such as ability to extract water and transpiration efficiency under different moisture regimes, contributes equally to crop performance across maturity group of genotypes. Available genotypic variability within tropical maize can be identified using the available non-invasive and non-destructive methods for root phenotyping. Suitable root traits for TPEs can be identified and mainstreamed in a maize breeding program, along with

known drought-adaptive shoot traits for targeted breeding for improved-stable yields in environments with increasing deficit for water caused by frequent dry spells and/or exposure to warmer days and nights.

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References

- Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* 112(2-3):119-123. doi:10.1016/j.fcr.2009.03.009.
- Boote KJ, Stansell JR, Schubert AM and Stone JF (1982) Irrigation, Water use, and Water relations. 164–205 *In* H.E. Pattee and C.T. Young (ed.). *Peanut Science and Technology*. Am. Peanut Res. Educ. Soc. Yoakum, TX.
- Bruce JA (2010) Leaf Transpiration Efficiency of Some Drought-Resistant Maize Lines. *Crop Sci.* 50(4), 1409-1413.
- Burton AL, Kathleen MB, Jonathan PL (2013) Phenotypic Diversity of Root Anatomical and Architectural Traits in *Zea* Species. *Crop Sci.* 53: 1042-1055.
- Cairns JE, Sonder K, Zaidi PH, Verhulst N, Mahuku G, Babu R, Nair SK, Das B, Govaerts B, Vinayan MT, Rashid Z, Noor JJ, Devi P, Vicente FS, Prasanna BM (2012) Maize Production in a Changing Climate: Impacts, Adaptation and Mitigation Strategies. *Adv Agron.* 114: 1-58.
- Cairns JE, Crossa J, Zaidi PH, Grudloyma P, Sanchez C, Araus JL, Thaitad S, Makumbi D, Magrokosho C, Banziger M, Menkir A, Hearne S, Atlin GA (2013) Identification of drought, heat, and combined drought and heat tolerant donors in maize. *Crop Sci.* 53:1335–1346. <https://doi.org/10.2135/cropsci2012.09.0545>
- Campos H, Cooper M, Habben JE, Edmeades GO, Schussler JR (2004) Improving drought tolerance in maize: a view from industry. *Field Crops Res.* 90:19–34
- Comas LH, Steven RB, VonMark VC, Patrick FB, David AD (2013) Root traits contributing to plant productivity under drought. *Front Plant Sci.* 41:442. doi: 10.3389/fpls.2013.00442
- Dardanelli JL, Ritchie JT, Calmon M, Andriani JM, Collino DJ (2004) An empirical model for root water uptake. *Field Crops Res.* 87:59-71.
- Edmeades GO, Deutsch JA (1994) Development of new maize genetic resources. Technical Publication, CIMMYT, El Batan, Mexico.
- Heckathorn SA, Giri A, Mishra S, Bista D (2013) “Heat stress and roots,” in *Climate Change and Plant Abiotic Stress Tolerance*, eds N. Tuteja, and S. S. Gill (Weinheim: Wiley-VCH Verlag GmbH & Co), 109–136. doi:10.1002/9783527675265.ch05
- Hund A, Nathinee R, Markus L (2009) Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. *Plant Soil* 318:311–325.
- Iglesias-Acosta M, Martínez-Ballesta, MC, Teruel, JA, Carvajal M (2010) The response of broccoli plants to high temperature and possible role of root aquaporins. *Environ Exp Bot.* 68:83–90. doi: 10.1016/j.envexpbot.2009.10.007

- Ionenko IF, Anisimov AV, Dautova NR (2010) Effect of temperature on water transport through aquaporins. *Biol. Plant.* 54:488–494. doi: 10.1007/s10535-010-0086-z
- Junying D, Yujiang E, Weilian G (1989) Studies on the relationship between root growth and yield in maize II. The interaction of root system and leaves of maize and its relation with yield. *Acta AgronSinica (China)* 14(4):310-314.
- Klein, SP, Hannah MS, Alden CP, Kathleen MB, Jonathan PL (2020) Multiple Integrated Root Phenotypes Are Associated with Improved Drought Tolerance. *Plant Physiol.* 183:1011–1025
- Kondo M, Murty MVR, Aragones DV (2000) Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. *Soil Sci Plant Nutr.* 46:721–732.
- Lobell DB, Bänziger M, Magorokosho C, Vivek B (2011) Nonlinear heat effects on African maize as evidenced by historical yield trials. *Nature Climate Change* 1:42–45. <https://doi.org/10.1038/nclimate1043>
- Lynch J (1995) Root architecture and plant productivity. *Plant Physiol.* 109:7
- Manavalan LP, Theresa M, Henry TN (2011) Natural genetic variation for root traits among diversity lines of maize (*Zea mays* L.). *Maydica* 56(1)-1707.
- Matsuo N, Ozawa K, Mochizuki T (2009) Genotypic differences in root hydraulic conductance of rice (*Oryza sativa* L.) in response to water regimes. *Plant Soil* 316:25–34.
- Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L (2015) Aquaporins in plants. *Physiol Rev* 95:1321–1358. doi: 10.1152/physrev. 00008.2015
- McIntyre BD, Riha SJ, Flower DJ (1995) Water Uptake by Pearl Millet in a semi-arid environment. *Field Crops Res.* 43:67-76.
- Messina C, Dan M, Hanna P, , Randy C, Andrea S, Yinan F, Carla G, Tom T, Geoff G, Graeme LH, Mark C (2021) Reproductive resilience but not root architecture underpins yield improvement under drought in maize. *J Exp Bot.* 72:5235–5245.
- Messina C, Thomas RS, Graeme LH, Dian C, Jason T, Zac O, Carla G, Mark C (2015) Limited-Transpiration Trait May Increase Maize Drought Tolerance in the US Corn Belt. *Agron J.* 107(6):1978-1986.
- Passioura JB (1977) Grain yield, harvest index and water use of wheat. *J Aust Inst Agric Sci.* 43:117-121.
- Prasanna BM, Jill EC, Zaidi PH, Beyene Y, Makumbi D, Gowda M, Magorokosho C, Zaman-Allah M, Olsen M, Das A, Worku M, Gethi J, Vivek BS, Nair SK, Mir Z, Vinayan MT, Issa AB, Vicente FS, Dhliwayo T, Zhang X (2021) Beat the stress: breeding for climate resilience in maize for the tropical rainfed environments. *Theo. Appl Gen.* 134:729–1752. <https://doi.org/10.1007/s00122-021-03773-7>
- Qiao S, F Y, Wu A, Xu B, Zhang S, Deng X, Djalovic I, Siddique KHM, Chen Y (2018) Dissecting root trait variability in maize genotypes using the semi-hydroponic phenotyping platform. *Plant Soil* 439: 79-90. <https://doi.org/10.1007/11104-018-3803-6>
- Rangarajan H, Postma JA, Lynch JP (2018) Co-optimization of axial root phenotypes for nitrogen and phosphorus acquisition in common bean. *Ann Bot.* 122: 485–499.
- Ratnakumar P, Vadez V, Nigam SN, Krishnamurthy L (2009) Assessment of transpiration efficiency in peanut (*Arachis hypogaea* L.) under drought using a lysimetric system. *Plant Biol (Stuttg).* 2009 Nov;11 Suppl 1:124-30. doi: 10.1111/j.1438-8677.2009.00260.x
- Richards RA (1991) Crop improvement for temperate Australia-future opportunities. *Field Crop Res.* 26:141–169. doi:10.1016/0378-4290(91)90033-R
- Shaw RH (1977) Water use and requirements of maize — a review. In WMO, *Agronomy of the Maize (corn) Crop*, World Meteorological Organization, Geneva; 119–134.
- Shinde MS, Awari VR, Patil VR, Gadakh SR, Nirmal SV, Dalvi US, Andhale LV (2017) Root Traits and Its Correlation with Grain Yield of Rabi Sorghum Genotypes in Phule Root Box Structure under Receding Soil Moisture Condition. *Int J Curr Microbiol App Sci.* 6(3): 977-981. <https://doi.org/10.20546/ijcmas.2017.603.116>.
- Strock CF, Burrridge J, Massas ASF, Beaver J, Beebe S, Camilo SA, Fourie D, Jochua C, Miguel M, Miklas PN (2019) Seedling root architecture and its relationship with seed yield across diverse environments in *Phaseolus vulgaris*. *Field Crops Res.* 237: 53–64
- Tuberosa R, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Mol Biol* 48: 697-712.
- Vamerali T, Saccomani M, Bona1 S, Mosca1 G, Guarise1 M, Ganis A (2003) A comparison of root characteristics in relation to nutrient and water stress in two maize hybrids. *Plant Soil* 255: 157–167.
- Wasaya A, Zhang X, Fang Q, Yan Z (2018). Root Phenotyping for Drought Tolerance: A Rev Agron, 8, 241; doi:10.3390/agronomy8110241
- Zaidi PH, Seetharam K, Krishna G, Krishnamurthy L, Gajanan S, Babu R (2016) Genomic Regions Associated with Root Traits under Drought Stress in Tropical Maize (*Zea mays* L.). *PLoS ONE* 11(10): e0164340. doi:10.1371/journal.pone.0164340.
- Zaidi PH; Nguyen T, Ha DN, Thaitad S, Ahmed S, Arshad M, Koirala KB, Rijal TR, Kuchanur PH, Patil AM, Mandal SS, Kumar R, Singh SB, Kumar B, Shahi JP, Patel MB, Gumma MK, Pandey K, Chaurasia R, Haque A, Seetharam K, Das RR, Vinayan MT, Mir Z, Nair SK, Vivek BS (2020) Stress-resilient maize for climate-vulnerable ecologies in the Asian tropics. *Aust J Crop Sci.* 14(08):1264-1274. doi:10.21475/ajcs.20.14.08.p2405.
- Zhao JJ, Qing-wu X, Jessup KE, Xiao-bo H, Bao-zhen H, Marek TH, Wenwei, Evett SR, O’Shaughnessy SA, Brauer DK (2018) Shoot and root traits in drought tolerant maize (*Zea mays* L.) hybrids. *J Integ Agric* 17(5): 1093–1105.
- Zhou L, Meng X, Zhang Z (2020) Association Analysis of Growth Characteristics, WUE, and RUE of Rice in Cold Region Under Different Irrigation Patterns. *J Inst Eng India Ser A* 101:421–431. <https://doi.org/10.1007/s40030-020-00452-6>
- Zhu J, Ingram PA, Benfey PA, Elich T (2011) From lab to field, new approaches to phenotyping root system architecture. *Current Opin Plant Biol.* 14:310–317.