Original Paper Open Access

Natural genetic variation for root traits among diversity lines of maize (*Zea Mays* L.)

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Abstract

Maize (*Z. mays* L.) is the third most important food grain for humankind after rice and wheat. Maize is mostly grown under rain-fed conditions and among the cereals, it is the second most susceptible to drought next to rice. Constitutive variation for root traits is an important adaptation under drought prone conditions. The objective of this study is to screen the twenty five diverse parental lines used in the maize nested association mapping panel along with the common parental line, B73, for constitutive root traits (including rooting depth and root biomass) and shoot traits. All the lines were grown with five replications in 72 cm deep pots containing a turface:sand mixture (2:1 v/v) for 30 days under well-watered conditions in a temperature and humidity controlled green house. Significant variation existed among the diverse lines for root length, root biomass, shoot length, and leaf area. The average root length ranged from 17.5 to 106 cm. The genotypes with a deep root system also recorded greater root biomass and leaf area. The natural genetic variation exhibited by these lines could be exploited to identify potential quantitative trait loci controlling root architecture. Using the nested association mapping populations that were developed from these diverse lines, would allow for in-depth analysis and fine-mapping of prospective candidate genes for root architecture in maize.

Keywords: maize, diversity lines, root length, leaf area, correlation

Introduction

The natural genetic variation for root traits especially rooting depth and distribution is essential for plants to adapt to adverse soil conditions including water deficit, flooding tolerance and nutrient acquisition. Considerable variation for root architecture exists among and between crop species, allowing for soil exploration in dynamic soil conditions (Fitter, 2002). In any given year, approximately 20-25% of global maize area is affected by drought (Banziger and Araus, 2007). The value of root traits in maize for adverse abiotic stress conditions has been well documented (Sharp and Davies, 1985; Schroder et al, 1996; Richner et al, 1997; Zhu et al, 2007; Hochholdinger and Tuberosa, 2009; Hund, 2010; Zaidi et al, 2010). An inverse relationship between rooting depth and available soil water was reported in maize under field conditions (Dwyer et al, 1988). However, due to the difficulties in extracting intact root system from soil, time and manpower constraints, it is not easy to screen large numbers of germplasm under field conditions to capture the natural genetic variation for root traits. In addition, as plants grow older, the complexity of their root system increases (Iyer-Pascuzzi et al, 2010).

Identification of quantitative trait loci (QTL) is the first step towards understanding genetic components contributing to root development. In most crop plants, QTL studies used a parent with less breeding value to magnify the phenotypic variation for traits of interest. However, this limited the utility of the detected QTLs in the Marker Assisted Breeding program (Tsonev et al, 2009). In addition, identification of the gene(s) underlying a specific QTL after the initial genetic mapping (linkage mapping) is not possible due to the poor resolution of the analysis itself (Salvi and Tuberosa, 2005). The QTL supporting interval mapped by primary analysis normally range from 10-30 cM which on an average correspond to 2.1 Mb genomic region that constitutes around 310 genes in maize (Salvi and Tuberosa, 2005). Alternatively, QTLs can be detected through association mapping based on linkage disequilibrium (Yu and Buckler, 2006). However association mapping is powerful only when the relevant alleles are present in high frequency and it does not detect rare alleles with good confidence, unless their effect is very large (Rafalski, 2010). To overcome the difficulties associated with bi-parental linkage mapping and association mapping, Mcmullen et al (2009) combined the advantages of both approaches by devising a nested association mapping (NAM) approach in maize. This NAM population is based on 25 diversity inbred lines which could be useful to identify genetic architecture of complex traits (Yu et al, 2008).

With the availability of this highly valuable NAM population resource, rapid identification of QTL underlying root morphology would be achieved in less time, provided the root morphology of the parental lines is understood. The objective of this

 RECEIVED 25/03/2011

Table 1- Details of the diverse inbred lines used in the study.

Diversity inbred lines	Climate Origin*	Average seed weight range (mg ± 1)	Average plant height range at 30 days after sowing (cm)
CML103, CML228, CML247, CML277, CML322, CML333, CML52, CML69, Ki11, Ki3, NC350, NC358, Tzi8	Tropical/Subtropical	143-394	14-61
B97, HP301, IL14H, Ky21, M162W, M37W, Mo18W, MS71, Oh43, Oh7B, P39, Tx303	Temperate	113-324	34-65
B73	Temperate	276	50.8

* based on Flint-Garcia et al. 2005

study is to screen the 25 diverse inbred lines (DLs) along with the common parent, B73, for constitutive root architectural traits under non-stressed conditions.

Materials and Methods

Plant growth and materials

Plants were grown in a temperature and humidity controlled greenhouse at the University of Missouri, Columbia, MO. The conditions of growth were set up as 29/21°C (day/night) temperature, 12h photoperiod and light intensity of ~1,620 μmolm^{-2}s^{-1} at the canopy.

The 25 diverse parental lines along with B73 were obtained from the North Central Regional Plant Introduction Station (NCRPIS), Ames, IA, USA (Table 1). The plants were grown in deep pots (TPOT1L Long One, 76.2 cm height and 10 cm diameter from Stuwe & Sons Inc. Oregon, USA) filled with a mixture of turface and sand (2:1 v/v). This media is suitable for root studies as it has cation exchange capacity and facilitates the root removal without damage (Manavalan et al, 2010). All tubes were mounted in wooden racks in a completely randomized design with five replications per cultivar. Two weighed seeds (±1 mg) were planted 3 cm deep into wetted media. After germination, plants were thinned to one per tube.

The plants were watered daily and 15 days after sowing, they were supplied with 5 grams of water soluble all-purpose fertilizer containing micronutrients (Peters Professional 20-20-20 from Scotts Company, LLC). In a preliminary trial, B73 plants were grown with ten replications (data not shown) in the same conditions to determine length of time for roots to reach the bottom of the tube. Most of the plants reached the bottom of the tube around 29-30 days after sowing. So in this study, plants were grown for 30 days. Plant height, leaf number, and leaf area were recorded non-destructively on the 30th day. For leaf area, the length and width (mid section) of all the leaves in a plant was measured. The total length and width were multiplied to obtain leaf area (cm^2). During the growth cycle, it was noticed that some of the inbred lines showed broader leaves. So on 30th day, the width of the first fully expanded leaves was recorded by taking three width measurements in the mid section of the leaf, and average was recorded. The tubes were cut longitudinally along the edge and the turface:sand media was carefully removed. The intact root system was lifted and washed thoroughly with tap water by dipping the roots in three trays of water to remove any adhering sand particles. Length, fresh weight, and dry weights of shoot and root were recorded. The length of the primary root was recorded as root length, and the shoot length was measured from the soil surface to the tip of the longest leaf. After fresh weight measurements, the shoot portion and root portion were wrapped in separate bags and dried in a gravity flow convection oven (Isotemp standard lab oven 5.0 ft^3 from Thermo Fisher Scientific Inc) at 65 °C for 48 hours. After brief cooling, the root and shoots were weighed to record the dry weight.

Data Analysis

Data were analyzed using SAS (version 9.2, by SAS Institute, Inc., Cary, N.C, USA). The Proc GLM (General Linear Model) procedure was used to estimate the differences between genotypes. Genotypic means were tested for differences based on least significant difference (LSD) at a probability level of 0.05. The genotypes were ranked using the 't' test. Analysis of covariance was performed for all traits using shoot length as a covariate to identify the influence of seedling vigor. Simple phenotypic correlation coefficients (based on mean values) among the nine traits were calculated using the PROC CORR statement. A cluster analysis was also conducted to identify discrete groups of genotypes with similar root and shoot length. The clustering was performed in SAS using centroid cluster method with three levels of clustering

Table 2 - Leaf traits of the diverse lines at 30 days after sowing: values are presented as mean ± SE of the mean (n=5). Means followed by same letters are not significantly different at p <0.05 level based on Tukey's studentised range (HSD) test.

Genotype	Leaf number	Leaf area (cm^2)	Width of first fully expanded leaf (cm)
B73	4.8 ± 0.2 ^{fg}	923.2 ± 88 ^{efg}	2.2 ± 0.2 ^{efg}
B97	5.0 ± 0 ^{ef}	858.3 ± 60 ^{fg}	2.5 ± 0.2 ^{de}
CML103	3.2 ± 0.2 ^{kl}	279.4 ± 13 ^{jkl}	1.4 ± 0.0 ^{ij}
CML228	4.0± 0 ^{hi}	517.1 ± 29 ^{ijk}	1.9 ± 0.0 ^{gh}
CML247	3.3 ± 0.3 ^{jkl}	120.1 ± 15 ^{l}	1.1 ± 0.1 ^{jk}
CML277	3.0 ± 0 ^{l}	79.5 ± 12 ^{l}	0.95 ± 0.1 ^{k}
CML322	6.2 ± 0.4 ^{b}	1317.9 ± 151 ^{abcd}	2.5 ± 0 ^{de}
CML333	5.8 ± 0.3 ^{bcd}	1472.0 ± 162 ^{abc}	2.4 ± 0.1 ^{ef}
CML52	3.7 ± 0.3 ^{ijk}	218.1 ± 53 ^{kl}	1.0 ± 0.0 ^{jk}
CML69	5.3 ± 0.3 ^{def}	1135.3 ± 159 ^{def}	2.6 ± 0.3 ^{bcde}
HP301	5.0 ± 0 ^{ef}	734.7 ± 88 ^{ghi}	2.0 ± 0 ^{fgh}
IL14H	5.4 ± 0.2 ^{de}	930.2 ± 78 ^{efg}	2.0 ± 0 ^{fgh}
Ki11	3.8 ± 0.2 ^{ij}	553.5 ± 100 ^{hij}	2.0 ± 0.2 ^{fgh}
KY21	6.0 ± 0 ^{bc}	1587.8 ± 89 ^{ab}	3.1 ± 0.0 ^{a}
Ki3	5.2 ± 0.2 ^{def}	828.1 ± 105 ^{gh}	2.3 ± 0.2 ^{ef}
M162W	5.4 ± 0.2 ^{de}	818.2 ± 180 ^{gh}	2.3 ± 0.2 ^{efg}
M37W	5.6 ± 0.2 ^{cd}	1160.8 ± 99 ^{de}	2.4 ± 0.1 ^{de}
Mo18W	6.0 ± 0 ^{bc}	1509.2 ± 97 ^{abc}	2.8 ± 0.1 ^{abcd}
MS71	6.0 ± 0 ^{bc}	1309.8 ± 112 ^{abcd}	2.5 ± 0 ^{cde}
NC350	6.0± 0 ^{bc}	1289.7 ± 56 ^{bcd}	2.0 ± 0 ^{fgh}
NC358	7.0 ± 0 ^{a}	1487.1 ± 153 ^{abc}	3.0 ± 0.3 ^{ab}
Oh43	3.8 ± 0.4 ^{ij}	242.8 ± 42 ^{kl}	1.3 ± 0.1 ^{ijk}
Oh7B	4.4 ± 0.2 ^{gh}	245.0 ± 5 ^{kl}	1.2 ± 0.1 ^{jk}
P39	6.0 ± 0 ^{bc}	1263.0 ± 114 ^{cd}	2.0 ± 0.2 ^{fgh}
Tx303	4.4 ± 0.2 ^{gh}	440.0 ± 32 ^{ijk}	1.7 ± 0.1 ^{hi}
Tzi8	6.0± 0 ^{bc}	1590.9 ± 159 ^{a}	2.9 ± 0.3 ^{abc}
$LSD_{0.05}$	0.591	299.49	0.423

based on squared euclidean distance.

Results

Total root length and shoot length

The diversity lines (DLs) exhibited significant variation for root length at 30 days after sowing (Figure 1A). Among the lines Mo18W showed the deepest root length (106 cm) and CML277 recorded the smallest root length (17.5 cm). The reference line, B73, recorded a root length higher than the median (77.4 cm). Though significant differences existed between the DLs, several lines were comparable for root length. Cluster analysis of root length indicated that the DLs formed three discrete groups (Figure 1B). Group 1 included two genotypes, Mo18W and MS71, which had the highest root length. Group 2 had four cultivars (CML277, CML247, CML52 and Oh43) which showed the lowest root length. The remaining 20 lines formed group three which had medium root length and were not significantly different from each other (p <0.05). The coefficient of variation within the replications for root length was 15.6%. Analysis of variance of various root and shoot traits using shoot length as a covariate was performed (Table 4A). The results indicated that when shoot length was taken as a covariate, there was a significant difference between the genotypes and a non-significant difference for shoot length was noticed. Test of between subjects effects for dependent variable root length with shoot length as covariate, clearly indicated that there was a significant difference between genotypes (R^2 = 0.425) (Table 4B).Though shoot length has an interaction with genotype (R^2 = 0.420), the R^2 value for shoot length indicate that this is not applicable to all genotypes.

Many of the lines showed uniform shoot length and less diversity for shoot length was observed between the DLs (Figure 2A). Shoot length ranged from 14.75 cm (CML277) to 65.5 cm (Mo18W). Some of the deep rooting lines also showed high shoot length. Cluster analysis of shoot length based on median distance classified the genotypes into three clusters with CML247, CML277 showing the least shoot length (Figure 2B). Most of the genotypes were grouped into a single cluster with a shoot length median around 20.

Leaf traits

The observations on leaf traits, namely: leaf area (LA); leaf number/plant (LN); and width of the first fully expanded leaf (LW), is presented in Table 2. The leaf number at the same age varied among the DLs. NC358 had the highest leaf number, 7, which was

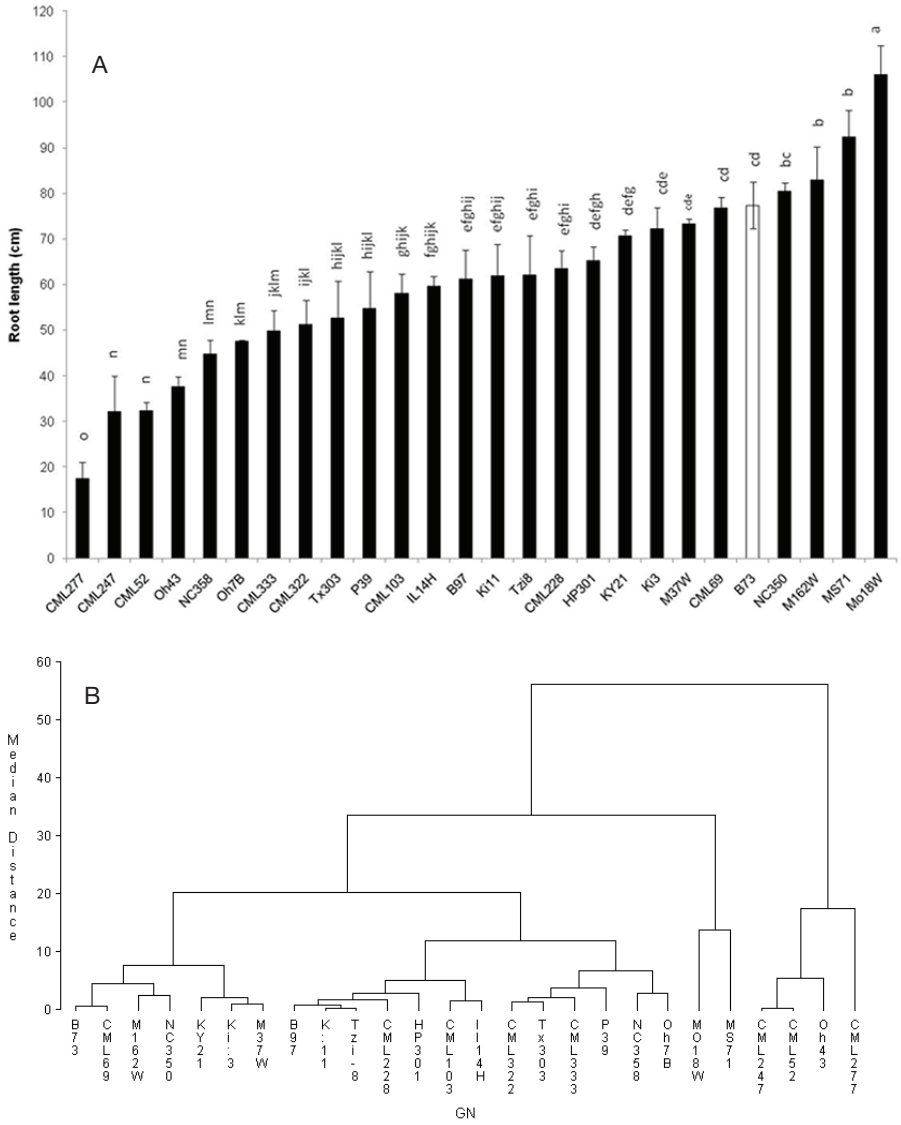

Figure 1- A) Root length of 26 diverse inbred lines thirty days after sowing. Reference line B73 is represented by an open bar. Bars followed by different letters are significantly different according to t- test ($p < 0.001$ level). B) Clustering of root length of 26 diverse maize lines based on median distance.

significantly higher than other lines ($p < 0.05$ level). Most of the DLs had 5-6 leaves per plant; except CML247, CML277, and CML103, which had only three leaves. The leaf area of the DLs was significantly diverse that ranged from 79.5 cm^2 (CML277) to 1590 cm^2 (Tzi-8). Line Mo18W which had the deepest root, also showed greater leaf area. Significant differences among the DLs were noticed for width of the first fully expanded leaf which ranged from 0.95 cm-3.00 cm. Covariance analyses showed that all leaf traits except leaf width had a significant interaction with shoot length (Table 4B).

Root and shoot root biomass

There was a significant difference between DLs for biomass traits, namely root and shoot fresh weight (RFW, SFW), root and shoot dry weight (RDW, SDW) (Table 3). MS71 had the highest root dry weight (3.00 g) followed by M37W, CML322, and B73, whereas lowest root dry weight was recorded by Oh43 (2.13 g). CML333 recorded the highest shoot dry weight (3.31 g) and CML52 recorded the lowest root dry weight (2.10 g). The difference between most of the DLs for shoot dry weight was negligible. A similar trend was maintained for shoot and root fresh weight for germplasm ranking. No significant difference for RS ratio (root-shoot ratio) was observed among the DLs except CML247, which exhibited a high root:shoot ratio (Table 3). The RS ranged from 0.84-1.0 in other DLs.

Phenotypic correlation

Relatively a close correlation was exhibited be-

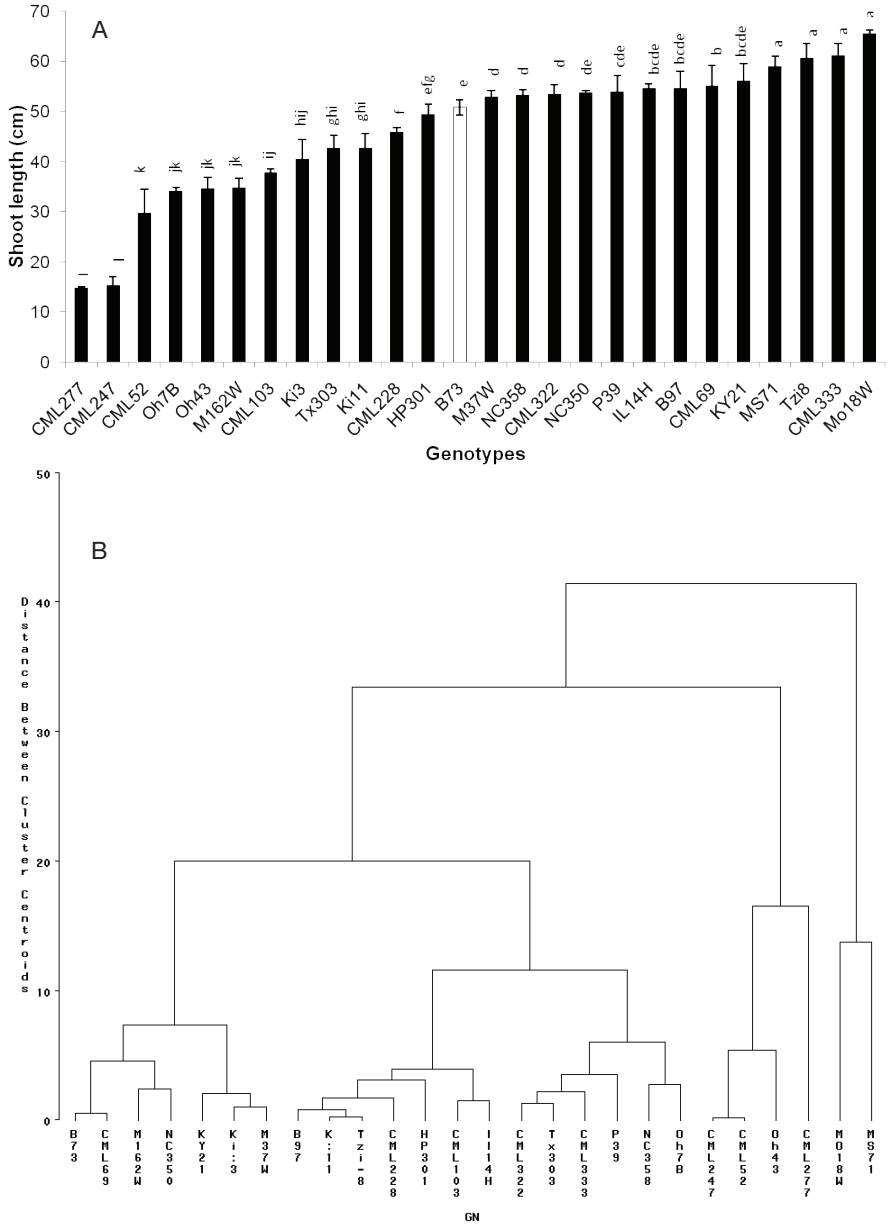

Figure 2 - A) Shoot length of 26 diverse inbred lines thirty days after sowing. Reference line B73 is represented by an open bar. Bars followed by different letters are significantly different according to t- test ($p < 0.001$ level). B) Clustering of shoot length of 26 diverse maize lines based on median distance.

tween the different traits measured in this study (Table 5). The root length was significantly correlated with shoot length, root, and shoot biomass (dry weight basis) at $p < 0.05$ level. Highest correlation was noticed between root and shoot dry weights ($r = 0.91$, $p < 0.001$). Similarly a tight correlation was exhibited between leaf area and traits like leaf number ($r = 0.85$), leaf width ($r = 0.84$) and shoot weight ($r = 0.91$). Surprisingly all the traits showed significant correlation with each other except for root length and leaf number between which no significant correlation was established.

Discussion

Constitutive differences in root traits like rooting depth play a major role in drought resistance of crops (Blum, 2002; Kamoshita et al, 2008). Genetic variability studies in maize for root architecture are limited due to the highly heterogeneous root architecture within and among different cultivars as a response to a complex soil matrix (Bohn et al, 2006). Studying roots extensively under field conditions is still limited due to the expenditure of time involved in destructive techniques like the core method and the likelihood of under-estimation of root depth and density with alter-

Table 3 - Root and shoot biomass traits of the diverse lines at 30 days after sowing: (Values are presented as mean ± SE of the mean (n=5)). Means followed by same letters are not significantly different at p <0.05 level based on Tukey's studentised range (HSD) test.

Genotype	Root		Shoot		Root/Shoot ratio
	Fresh weight (g)	Dry weight	Fresh weight	Dry weight	(DW basis)
B73	7.75 ± 0.76 abcd	2.94 ± 0.04 abc	8.26 ± 0.53 cdefg	3.03 ± 0.05 def	0.97 bcdef
B97	4.71 ± 0.47 defg	2.70 ± 0.02 efg	7.90 ± 0.58 defgh	2.98 ± 0.05 defg	0.91 ghijk
CML103	4.93 ± 0.05 defg	0.19 ± 0.01 g	6.44 ± 0.18 ghij	2.19 ± 0.02 ij	0.99 bcde
CML228	4.37 ± 0.09 fg	2.22 ± 0.01 h	6.74 ± 0.16 fghiij	2.35 ± 0.02 hi	0.94 egfhi
CML247	4.10 ± 0.03 fg	2.57 ± 0.01 g	5.27 ± 0.06 j	2.37± 0.00 h	1.08 a
CML277	5.07 ± 0.05 defg	2.16 ± 0.03 h	6.25 ± 0.00 hij	2.10 ± 0.05 j	1.03 abc
CML322	9.55 ± 0.90 a	2.94 ± 0.07 abc	8.47 ± 0.52 bcdef	3.12 ± 0.04 bcd	0.94 efghij
CML333	7.02 ± 0.57 abcde	2.80 ± 0.03 bcdef	11.24 ± 1.10 a	3.31 ± 0.11 a	0.85 lk
CML52	4.30 ± 0.11 fg	2.26 ± 0.01 h	5.65 ± 0.18 ij	2.18 ± 0.04 ij	1.03 ab
CML69	6.28 ± 0.84 bcde	2.84 ± 0.04 abcdef	8.98 ± 1.35 bcd	3.21 ± 0.13 abc	0.88 ijkl
HP301	3.94 ± 0.19 g	2.73 ± 0.04 defg	5.80 ± 0.38 ij	2.87 ± 0.05 fg	0.95 defgh
IL14H	4.68 ± 0.14 defg	2.83 ± 0.02 abcdef	7.58 ± 0.15 defghi	2.97 ± 0.01 defg	0.95 defgh
Ki11	4.75 ± 0.47 defg	2.88 ± 0.04 abcde	6.07 ± 0.76 hij	2.86 ± 0.07 ij	1.01 bcd
KY21	7.79 ± 0.65 abcd	2.86 ± 0.06 abcde	11.32 ± 1.26 a	3.25 ± 0.10 ab	0.88 jkl
Ki3	6.11 ± 0.70 bcde	2.88 ± 0.04 abcde	6.94 ± 0.66 efghi	2.93 ± 0.07 efg	0.98 bcdef
M162W	5.91 ± 0.57 bcde	2.77 ± 0.03 cdef	6.58 ± 0.98 fghij	2.84 ± 0.08 g	0.98 bcdef
M37W	8.89 ± 0.86 ab	2.97 ± 0.05 ab	7.89 ± 0.47 defg	3.07 ± 0.04 cde	0.97 cdefg
Mo18W	7.81 ± 0.84 abcd	2.66 ± 0.22 fg	10.05 ± 0.53 abc	3.14 ± 0.04 abcd	0.85 lk
MS71	8.65 ± 0.46 ab	3.00 ± 0.07 a	9.96 ± 0.29 abc	3.13 ± 0.02 bcd	0.96 defgh
NC350	6.48 ± 0.49 abcde	2.94 ± 0.02 abc	8.51 ± 0.24 bcdef	3.13 ± 0.02 bcd	0.94 efghij
NC358	7.13 ± 0.51 abcd	2.71 ± 0.16 efg	11.93 ± 1.51 a	3.22 ± 0.11 abc	0.84 l
Oh43	4.66 ± 0.10 defg	2.13 ± 0.02 h	6.02± 0.04 ij	2.18 ± 0.03 ij	0.98 bcdef
Oh7B	3.86 ± 0.06 g	2.14 ± 0.01 h	6.79 ± 0.21 fghij	2.17 ± 0.02 ij	0.98 bcdef
P39	5.36 ± 0.60 cde	2.84 ± 0.05 abcdef	8.92 ± 0.88 bcde	3.07 ± 0.09 cde	0.93 fghij
Tx303	4.63 ± 0.09 efg	2.18 ± 0.02 h	6.82 ± 0.16 fghij	2.22 ± 0.02 hij	0.98 bcdef
Tzi8	8.32 ± 0.92 abc	2.91 ± 0.05 abcd	10.34 ± 1.1 ab	3.24 ± 0.10 abc	0.90 hijkl
LSD $_{0.05}$	1.65	0.2	1.98	0.18	0.06

native methods like mini-rhizotron (Wiesler and Horst, 1994). Considering these points, the current study focused on using the maize inbred lines which are more homogeneous than diverse hybrids. In addition, this study utilized a growth medium closer to sand, but more porous than sand which prevents any differences arising from soil strength. The turface:sand medium was successfully used to estimate genotypic differences in root growth in crop species like cowpea and soybean (Petrie and Hall, 1992; Manavalan et al, 2010). The pot size and media did not seem to influence the root and shoot growth (Figure 3), however as seedling vigor or seed size might perplex with genetic potential for rooting ability, estimation of seedling vigor is suggested which was not performed in this study.

In maize, good seedling root morphology was associated with vigorous plant development at the early stages in both the growth chamber (Stamp, 1984; Richner et al, 1997) and the field (Richner et al, 1996, 1997). In the present study, significant differences were observed between the DLs for root length and root biomass at 30 days after sowing (at 4-5 leaved stage). Cluster analysis encompasses many diverse techniques for discovering structure within complex bodies of data (Sharma, 1996). The cluster analysis in this study resulted in separating the 26 DLs into four different groups. The genotypic clustering reflected the geographic adaptation/origin to some extent (e.g. group 1 & 2); but the classification would reveal distinct differences, if the plants were evaluated under water-deficit conditions due to the soil and climatic conditions in which they are being grown. More diversity was exhibited by CML lines for root length. This supports the genetic studies where tropical and subtropical maize diversity lines showed greater gene diversity and allele numbers (Liu et al, 2003).

Plants that have a long shoot system tend to have a deeper root system, while short plants tend to have shorter roots (Guerrero-Campo and Fitter, 2001). A significant correlation between shoot length and root length (r = 0.60) achieved in this study is concurrent with these findings. Similar results were reported in wheat (Key, 1973) and soybean (Taylor et al, 1978). No relation between seed weight and root length, shoot and root dry weight (r = 0.21, -0.09, -0.17, respectively) was observed. This indicates that the root length and biomass differences were due to inherent genetic variation and not contributed by seed size. A negative non-significant correlation between seed

Table 4A - Type III hypothesis tests for covariate analysis of phenotypic traits as a function of shoot length (GN-genotype; SL-shoot length).

Trait	Source	df	Type III sum of squares	Mean square	F value	Probability .F	CV (%)
Root length	GN	25	5121.59083	1004.86363	10.69	***	15.5
	SL	1	228.04534	228.04534	2.43	NS	
Leaf Number	GN	25	51.78306654	2.07132266	11.24	***	8.4
	SL	1	1.04177370	1.04177	5.65	*	
Leaf area	GN	25	6655378.240	266215.130	6.77	***	21.9
	SL	1	969731.754	969731.754	24.66	***	
Leaf width	GN	25	14.90904935	0.59636197	6.18	***	14.65
	SL	1	0.25656350	0.25656350	2.66	NS	
Root fresh weight	GN	25	219.2034601	8.7681384	6.75	***	18.64
	SL	1	18.1831700	18.1831700	14.00	**	
Shoot fresh weight	GN	25	152.3117257	6.0924690	4.03	***	15.39
	SL	1	61.9467144	61.9467144	41.01	***	
Root dry weight	GN	25	6.59510818	0.26380433	25.03	***	3.43
	SL	1	0.06916002	0.06916002	6.56	NS	
Shoot dry weight	GN	25	6.80015058	0.27200602	25.00	***	3.68
	SL	1	0.58248445	0.58248445	53.53	***	

*** Significant at the 0.001 level; ** Significant at the 0.01 level; * Significant at the 0.05 level; NS nonsignificant at the 0.05 level.

weight and root and shoot dry weight was found in maize seedlings (Hund et al, 2007). This is similar to the results of Leishman and Westboy (1994) who found no relationship between seed size, seedling emergence, and establishment in non-drying soil conditions in semiarid species. The covariance analysis of root traits (root length and root dry weight) with shoot length as a covariate indicated no significant difference for shoot length, which confirms that the phenotypic differences for root length is not affected by shoot growth.

Root-shoot fresh weight measurements were used as the final measure of growth in a given experiment. Even though root and shoot fresh weight was measured in this study, freshly cut plants have a high composition of water and the level of water in a tissue is dependent on the amount of water in its environment (which is very difficult to control). Hence using dry weight as a measure of plant growth tends to be more reliable. Significant variability was displayed by the DLs for root and shoot biomass in terms of dry weight. Similar findings for root biomass traits were reported in maize (Hund et al, 2004).

The study of phenotypic association between traits is an important aspect in breeding programs, as genetic change in a given trait may positively or negatively affect other traits (Neto et al, 2001). Significant correlation between maize root length density at 60-90 cm soil layer and shoot traits like plant height and yield was reported (Wiesler and Horst, 1994). A highly

Table 4B - Test of between subjects effects for dependent variable RL with SL as covariate.

Source	Type III sum of squares	df	Mean Square	F	Sig.	Partial Eta Squared (R^2)
Corrected Model	40242.438[a]	51	789.028	10.054	0.000	0.890
Intercept	341.416	1	341.416	4.545	0.037	0.064
GN	3669.493	25	146.780	1.954	0.016	0.425
SL	1.289	1	1.289	.017	0.896	0.000
GN*SL	3592.315	25	143.693	1.913	0.019	0.420
Total	506198.250	118				
Corrected Total	117					

[a] R squared=0.890

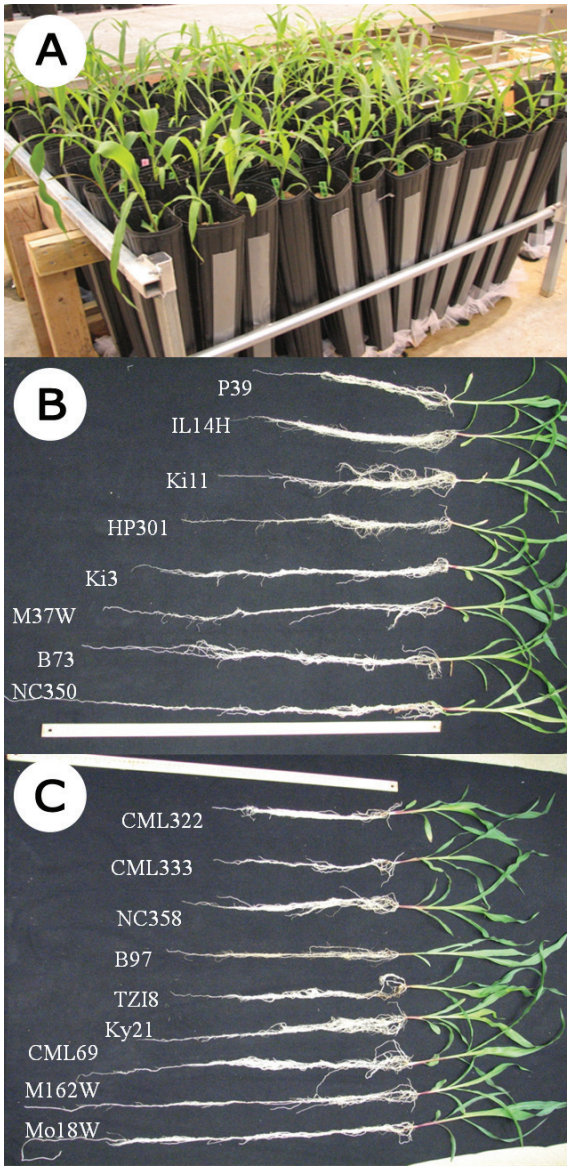

Figure 3 - A) screening system; B) and C) shoot and root morphology of representative diverse inbred lines at 30 days after sowing.

significant phenotypic correlation between root traits and shoot traits was observed in this study.

Similar levels of phenotypic relationships between root and shoot traits were reported in maize by Richner et al (1997) who suggested that seedling root traits with other secondary traits could be used as indirect selection for shoot performance in maize. A significant correlation noticed between root length and leaf area (r = 0.46) in this study was corroborated with the results reported by Hund et al (2007) in maize.

The maize diverse inbred lines have been characterized and reported to show genetic diversity for several kernel quality traits like seed carotenoid composition (Harjes et al, 2008), kernel quality (Flint-Garcia et al, 2009a), amino acid metabolism (Flint-Garcia et al, 2009b), and central carbon metabolism (Zhang et al, 2010). The diversity of these lines was utilized to capture important genetic components via the Nested Association Mapping (NAM) population for traits like flowering time (Buckler et al, 2009) and aluminum tolerance (Krill et al, 2010). In this study considerable genetic variation for root traits was exhibited by the diverse lines (MO18W, MS71, M162W, NC350 and B73 on the higher side and CML277, CML247, and CML52 on the lower side). The crosses between B73 and CML247, CML333, and NC350 were utilized to detect candidate genes for Aluminum tolerance in maize (Krill et al, 2010).

Table 5 - Pearson's correlation matrix showing the relationship among the six morphological traits (n=118) (SL: shoot length, SDW: shoot dry weight, LN: leaf number, LA: leaf area, LW: width of the first fully expanded leaf, RL: root length and RDW: root dry weight) for the 26 diverse inbred lines.

	SL	SDW	LN	LA	LW	RL
SDW	0.80***					
LN	0.70***	0.80***				
LA	0.81***	0.91***	0.86***			
LW	0.70***	0.84***	0.72**	0.92***		
RL	0.52***	0.49**	0.38	0.46*	0.51*	
RDW	0.60***	0.91***	0.65**	0.70***	0.70***	0.64**

***Significant at P <0.001, **P <0.01 and *P <0.05 level

The study of the root system at 30 days was sufficient to estimate the genotypic differences in the present study. However, maize cultivars differ in root length in all soil layers in the vegetative stage, and continue to increase until silking stage at deep soil layers (Wiesler and Horst, 1994). Maize plants have a basic pattern of root distribution which is influenced by large variations in time and space (Liedgens and Richner, 2001). Hence the consistency of the differences in root length exhibited by the DLs at later stages of plant growth and deeper soil layers must be carefully verified in future studies. Additionally, the use of screening methods like gel filled chambers (Bengough et al, 2004) coupled with imaging techniques (Manschadi et al, 2008; Iyer-Pascuzzi et al, 2010) are suggested to verify the rooting ability in different media to confirm the genetic variability in these diverse lines. This screening technique would be potentially useful to evaluate the NAM population for detection of novel alleles associated with root architectural traits.

Acknowledgements

We thank three undergraduate student assistants, Andrew Vanhooser, Austin Popham, and Steven Messmer for help in the study.

References

Banziger M, Araus JL, 2007. Recent Advances in Breeding Maize for Drought and Salinity Stress Tolerance. pp. 587-601. In: Advances in Molecular Breeding toward Drought and Salt Tolerant Crops. Jenks MA, Hasegawa PM, Jain MS (eds). Springer, Netherlands

Bengough AG, Gordon DC, Al-Menaie H, Ellis RP, Allan D, Keith R, Thomas WTB, Forster BP, 2004. Gel observation chamber for rapid screening of root traits in cereal seedlings. Plant Soil 262: 63–70

Blum A, 2002. Drought tolerance - is it a complex trait? pp. 17-24. In: Field Screening for Drought Tolerance in Crop Plants with Special Emphasis on Rice. NP Saxena, JC O'Toole (eds). International Crop Research Institute for Semi-arid Tropics, Rockefeller Foundation, New York, USA

Bohn M, Novais J, Fonseca R, Tuberosa R, Grift TE, 2006. Genetic evaluation of root complexity in maize. Acta Agronomica Hungarica 54: 291-303

Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz JC, Goodman MM, Harjes C, Guill K, Kroon DE, Larsson S, Lepak NK, Li H, Mitchelle SE, Pressoir G, Peiffer JA, Rosas MM, Rocheford TR, Romay MC, Romero S, Salvo S, Sanchez Villeda H, Da Silva HS, Sun Q, Tian F, Upadyayula N, Ware D, Yates H, Yu J, Zhang Z, Kresovich S, Mcmullen MD, 2009 The Genetic Architecture of Maize Flowering Time. Science 325: 714-718

Dwyer LM, Stewart DW, Balchin D,1988. Rooting characteristics of corn, soybeans and barley as a function of available water and soil physical characteristics. Can J Soil Sci 68:121–132

Fitter A, 2002. Characteristics and functions of root systems. pp. 15-32. In: The Hidden Half, Ed 3. Waisel Y, Eshel A, Kafkafi L (eds). Plant Roots, Marcel Dekker Inc, New York

Flint-Garcia SA, Thuillet A, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley JF, Kresovich S, Goodman MM, Buckler ES, 2005. Maize association population: A high resolution platform for QTL dissection. Plant J 44: 1054-1064

Flint-Garcia SA, Bodnar AL, Scott MP, 2009a. Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. Theor Appl Genet 119: 1129-1142

Flint-Garcia SA, Guill KE, Villeda HS, Schroeder SG, Mcmullen MD, 2009b. Maize amino acid pathways maintain high levels of genetic diversity. Maydica 54: 375-386

Guerrero-Campo J, Fitter AH, 2001. Relationships between root characteristics and seed size in two contrasting floras. Acta Oecologica 22: 77-85

Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, Stapleton AE, Vallabhaneni R, Williams M, Wurtzel ET, Yan JB, Buckler ES, 2008. Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science 319: 330-333

Hochholdinger F, Tuberosa R, 2009. Genetic and genomic dissection of maize root development and architecture. Current Opinion in Plant Biol 12: 172–177

Hund A, 2010. Genetic variation in the gravitropic response of maize roots to low temperatures. Plant Root 4: 22-30

Hund A, Richner W, Soldati A, Fracheboud Y, Stamp P, 2007. Root morphology and photosynthetic performance of maize inbred lines at low temperature. Europ J Agron 27: 52-61

Hund A, Fracheboud Y, Soldati A, Frascaroli E, Salvi S, Stamp P, 2004. QTL controlling root and shoot traits of maize seedlings under cold stress, Theor Appl Genet 109: 618–629

Iyer-Pascuzzi AS, Symonova O, Mileyko Y, Hao Y, Belcher H, Harer J, Weitz JS, Benfey PN, 2010. Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. Plant Physiol 152: 1148–1157

Kamoshita A, Babu RC, Boopathi NM, Fukai S, 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rain fed environments. Field Crops Res 109: 1-23

Key M, 1973. The wheat root. pp. 827-842. In: Proc. 4th International wheat Genetics Symposium, MO Agricultural Experimental Station, Columbia, MO, USA

Krill AM, Kirst M, Kochian LV, Buckler ES, Hoekenga OA, 2010. Association and Linkage Analysis of Aluminum Tolerance Genes in Maize. PLoS ONE 5(4): e9958

Leishman MR, Westboy M, 1994. The role of seed size in seedling establishment in dry soil conditions-experiment evidence from semiarid sps. J Ecol 82: 249-258

Liedgens M, Richner W, 2001. Minirhizotron observations of the spatial distribution of the maize root system. Agron J 93: 1097-1104

Liu K, Goodman M, Spencer M, Smith JS, Buckler ES, Doebley J, 2003. Genetic Structure and Diversity Among Maize Inbred Lines as Inferred From DNA Microsatellites. Genetics 165: 2117-2128

Manavalan LP, Guttikonda SK, Nguyen VT, Shannon JG, Nguyen HT, 2010. Evaluation of diverse soybean germplasm for root growth and architecture. Plant and soil 330: 503–514

Manschadi AM, Hammer GL, Christopher JT, De Voil P, 2008. Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant and soil 303: 115-129

Mcmullen MD, Kresovich S, Villeda HS, Bradbury P, Li H, Sun Q, Flint-Garcia S, Thornsberry J, Acharya

C, Bottoms C, Brown P, Browne C, Eller M, Guill K, Harjes C, Kroon D, Lepak N, Mitchell SE, Peterson B, Pressoir G, Romero S, Oropeza Rosas M, Salvo S, Yates H, Hanson M, Jones E, Smith S, Glaubitz JC, Goodman M, Ware D, Holland JB, Buckler ES, 2009. Genetic Properties of the Maize Nested Association Mapping Population. Science 325: 737-740

Neto F, Lopes A, Filho M, Branco De J, 2001. Genetic correlation between traits in the ESALQ-PB1 maize population divergently selected for tassel size and ear height. Sci agric 58: 119-123

Petrie CL, Hall AE, 1992. Water relations in cowpea and pearl millet under soil water deficits. II. Water use and root distribution. Aust J Plant Physiol 19: 591-600

Rafalski JA, 2010. Association genetics in crop improvement. Current Opinion in Plant Biol 13:174–180

Richner W, Soldati A, Stamp P, 1996. Shoot-to-root relations in field-grown maize seedlings. Agron J 88: 56-61

Richner W, Kiel C, Stamp P, 1997. Is seedling root morphology predictive of seasonal accumulation of shoot dry matter in maize? Crop Sci 37: 1237-1241

Salvi S, Tuberosa R, 2005. To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10: 297-304

Schroder JJ, Groenwold J, Zaharieva T, 1996. Soil mineral nitrogen availability to young maize plants as related to root length density distribution and fertilizer application method. Neth J Agric Sci 44: 209–225

Sharma S, 1996. Applied multivariate techniques. John Wiley and sons, New York.

Sharp RE, Davies WJ, 1985. Root growth and water uptake by maize plants in drying soil. J Exp Bot 170: 1441–1456

Stamp P, 1984. Chilling tolerance of young plants demonstrated on the example of maize (*Zea mays* L.). vol.7. In: Advances in Agronomy and Crop Science. Geisler G (ed). Verlag Paul Parey, Berlin.

Taylor HM, Burnett E, Booth GD, 1978. Taproot elongation rates of soybeans. J Agron and Crop Sci 146: 33-39

Tsonev S, Todorovska E, Avramova V, Kolev S, Abu-Mhadi N, Christov NK, 2009. Genomics assisted improvement of drought tolerance in maize: QTL approaches. Biotechnol & Biotechnol Eq 23: 1410-1413

Tuberosa R, Salvi S, Giuliani S, Sanguineti MC, Bellotti M, Conti S, Landi P, 2007. Genome-wide approaches to investigate and improve maize response to drought. Crop Sci 47: 120–141

Wiesler F, Horst WJ, 1994. Root growth of maize cultivars under field conditions as studied by the core and minirhizotron method and relationships to shoot growth. J Plant Nutr Soil Sci 157: 351-358

Yu J, Buckler ES, 2006. Genetic association mapping and genome organization of maize. Current Opinion in Plant Biol 17:155–160

Yu J, Holland JB, Mcmullen MD, Buckler ES, 2008. Genetic design and statistical power of nested association mapping in maize. Genetics 178: 539-551

Zaidi PH, Maniselvan P, Srivastava A, Yadav P, Singh RP, 2010. Genetic analysis of water-logging tolerance in tropical maize. Maydica 55: 17-26

Zhang N, Gur A, Gibon Y, Sulpice R, Flint-Garcia S, Mcmullen MD, Stitt M, Buckler ES, 2010. Genetic Analysis of Central Carbon Metabolism Unveils an Amino Acid Substitution That Alters Maize NAD-Dependent Isocitrate Dehydrogenase Activity. PLoS ONE 5(4): e9991

Zhu J, Alvarez S, Marsh EL, Lenoble ME, Cho IJ, Sivaguru M, Chen S, Nguyen HT, Wu Y, Schachtman DP, Sharp RE, 2007. Cell wall proteome in the maize primary root elongation zone. II. Region-specific changes in water soluble and lightly ionically bound proteins under water deficit. Plant Physiol 145: 1533–1548