

Reduced Lateral Root Branching Density Improves Drought Tolerance in Maize¹[OPEN]

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An emerging paradigm is that root traits that reduce the metabolic costs of soil exploration improve the acquisition of limiting soil resources. Here, we test the hypothesis that reduced lateral root branching density will improve drought tolerance in maize (*Zea mays*) by reducing the metabolic costs of soil exploration, permitting greater axial root elongation, greater rooting depth, and thereby greater water acquisition from drying soil. Maize recombinant inbred lines with contrasting lateral root number and length (few but long [FL] and many but short [MS]) were grown under water stress in greenhouse mesocosms, in field rainout shelters, and in a second field environment with natural drought. Under water stress in mesocosms, lines with the FL phenotype had substantially less lateral root respiration per unit of axial root length, deeper rooting, greater leaf relative water content, greater stomatal conductance, and 50% greater shoot biomass than lines with the MS phenotype. Under water stress in the two field sites, lines with the FL phenotype had deeper rooting, much lighter stem water isotopic signature, signifying deeper water capture, 51% to 67% greater shoot biomass at flowering, and 144% greater yield than lines with the MS phenotype. These results entirely support the hypothesis that reduced lateral root branching density improves drought tolerance. The FL lateral root phenotype merits consideration as a selection target to improve the drought tolerance of maize and possibly other cereal crops.

Suboptimal water availability is a primary limitation to crop productivity in both developed and developing countries (Lynch, 2007; Lobell et al., 2014). Climate change as well as decreased freshwater availability are likely to increase the frequency and severity of crop water stress (WS) in the future, causing significant yield loss (Tebaldi and Lobell, 2008; Brisson et al., 2010; IPCC, 2014). This will be a major obstacle to sustaining an increased human population, which is projected to reach 9.6 billion by 2050 (Lee, 2011). Therefore, the identification and understanding of traits improving crop drought tolerance have been the focus of the development of more drought-tolerant crops and cropping systems.

Root architecture regulates water and nutrient acquisition by positioning root-foraging activity in specific soil domains in time and space (Lynch, 1995, 2011; Gregory, 2006). Genotypic variation for root traits and their functional implications for soil resource acquisition and improved yields under nutrient and WS conditions have been reported in many crops. In the case of phosphorus (P), the most immobile macronutrient, whose availability is therefore greatest in the topsoil, the topsoil-foraging ideotype appears to be particularly important for genotypic adaptation to low-P soils (Lynch and Brown, 2001; Lynch, 2011; Richardson et al., 2011). For superior acquisition of water and nitrate, which are highly mobile in the soil, the Steep, Cheap, and Deep (SCD) ideotype has been proposed, consisting of architectural, anatomical, and physiological traits accelerating subsoil exploration (Lynch, 2013; Lynch and Wojciechowski, 2015). One element of the SCD ideotype is a low density of lateral roots per length of axial root and greater lateral root length of crown roots, traits that would reduce interroot competition, improve the metabolic efficiency of soil exploration, and accelerate the elongation of axial roots.

Lateral roots originate from a small number of differentiated cells situated in the subapical zone of the axial root. The development of lateral roots has been studied in detail in the model plant *Arabidopsis thaliana* (Nibau et al., 2008; Péret et al., 2009). Multiple genes, abscisic acid, and auxin are important in pre-branch site formation, lateral root initiation, and lateral root emergence (Swarup et al., 2008; Zhao et al., 2014).

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A.Z. designed and conducted the experiments, analyzed the results, and led the writing; H.S. analyzed the results and contributed to the writing; J.P.L. conceived and designed the study, supervised its execution, assisted with data analysis, and contributed to the writing.

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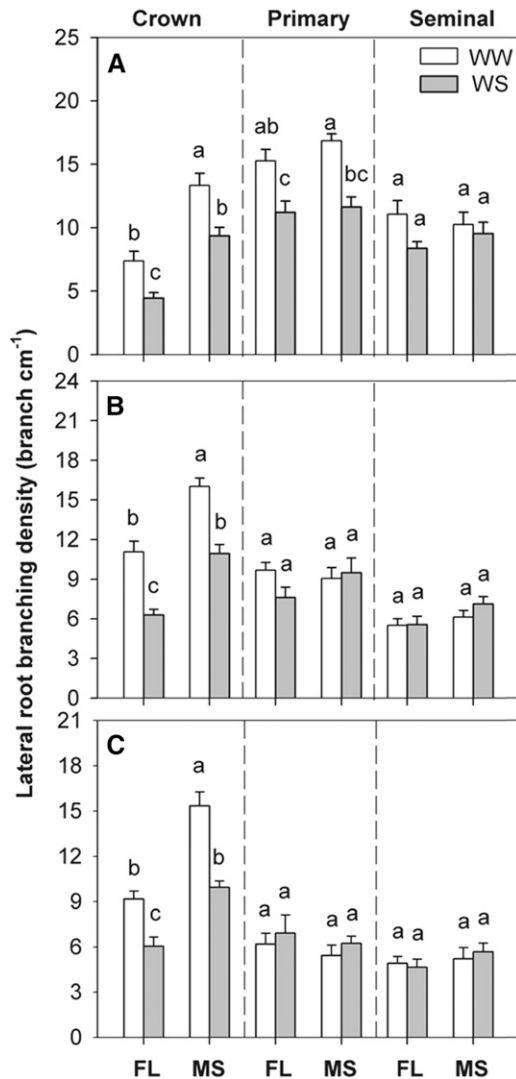


Figure 1. Lateral root branching density in GH (A) and in the field in AZ (B) and PA (C) under WW and WS conditions. Bars show means \pm SE of four replicates of the four genotypes in each phenotype class in WW or WS. Bars with the same letters are not significantly different within the same panel ($\alpha = 0.05$).

For example, in the prebranch site formation stage, the position of the lateral root primordium was demarked by auxin response DR5-driven luciferase, whose expression was dependent on AUXIN RESPONSE FACTOR6, AUXIN RESPONSE FACTOR7, AUXIN RESPONSE FACTOR8, and AUXIN RESPONSE FACTOR19 and on auxin repressor proteins INDOLE-3-ACETIC ACID8, INDOLE-3-ACETIC ACID19, and INDOLE-3-ACETIC ACID28 (De Rybel et al., 2010; Moreno-Risueno et al., 2010). A newly discovered adaptive mechanism, termed lateral root hydropatterning, was also involved in regulating prebranch sites (Bao et al., 2014). In addition, transcription factors such as LATERAL ORGAN BOUNDARIES-DOMAIN (LBD)16, LBD18, and LBD29, which belong to the LBD/ASYMMETRIC LEAVES-LIKE family, positively regulate lateral root formation (De Smet

et al., 2010). In cereals, a number of genes and growth regulators that regulate lateral root formation have been reported, including the rice (*Oryza sativa*) crown rootless1 mutants, which reduced lateral root number by 70% (Inukai et al., 2005), and in maize (*Zea mays*), lateral root initiation is inhibited when auxin transport is disrupted by the rootless with undetectable meristems1 mutation (Woll et al., 2005).

Lateral roots typically constitute the major portion of root systems, accounting for approximately 90% of the total root length (Pierret et al., 2006; Zobel et al., 2007). The formation of lateral roots increases the sink strength of the root system, promoting the development of greater root length and thereby greater soil resource acquisition (Varney and Canny, 1993; Postma et al., 2014). However, root construction and maintenance require metabolic investment, which can exceed 50% of daily photosynthesis (Lambers et al., 2002). Thus, the metabolic costs of the construction and

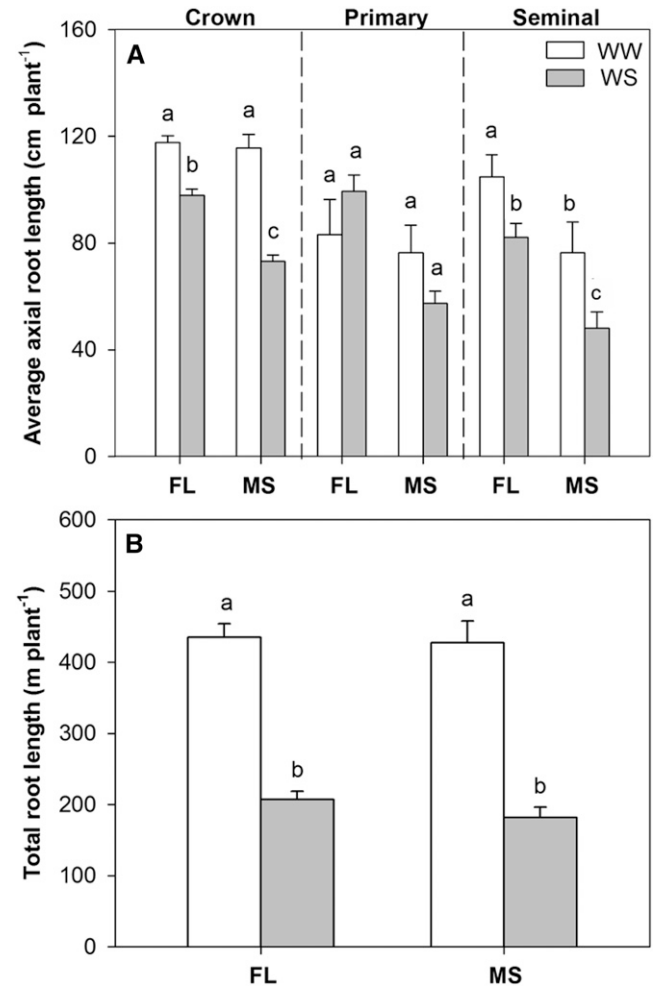


Figure 2. Average axial root length of crown, primary, and seminal roots (A) and total root length (B) in GH under WW and WS conditions. Bars show means \pm SE of four replicates of the four genotypes in each phenotype class in WW or WS. Bars with the same letters are not significantly different within the same panel ($\alpha = 0.05$).

maintenance for additional lateral roots, either calculated in units of carbon or in terms of other limiting resources, may reduce the growth of other roots, like axial roots (Borch et al., 1999, 2003; Lynch and Ho, 2005; Walk et al., 2006), potentially slowing axial root elongation into deep soil strata. This is especially important for the acquisition of water, whose availability is greater in deeper soil strata in most soils. A plant that is able to access water in deep soil domains at reduced metabolic cost will have superior productivity, because it will have more metabolic resources available for further resource acquisition, growth, and reproduction. Evidence in support of this hypothesis comes from empirical and modeling studies for maize and other cereal crops under water and edaphic stress (Lilley and Kirkegaard, 2011; Jaramillo et al., 2013; Uga et al., 2013; Chimungu et al., 2014a, 2014b; Lynch, 2014; Saengwilai et al., 2014a, 2014b). In addition, increased lateral root branching places roots closer together, which may increase competition for water among roots of the same plant, effectively reducing the uptake efficiency per unit of root length (Postma et al., 2014). The frequency and length of lateral branching determine the balance between the capture of mobile and immobile resources. Mobile resources are captured more efficiently by fewer but longer laterals capable of exploring larger volumes of soil with greater spatial dispersion among roots. Results from the functional-structural plant model *SimRoot* indicate that the optimal lateral root branching density for nitrogen (N) capture is less than that for P capture in maize (Postma et al., 2014). Previous research showed that reduced lateral root branching density improves N capture under N deficiency (Zhan and Lynch, 2015). Therefore, the few/long (FL) lateral root phenotype is

an element of the SCD ideotype for efficient water capture, because sparse lateral branching should conserve internal resources, reduce competition for water among neighboring lateral roots, and explore a greater volume of soil than the many/short (MS) lateral root phenotype.

The objectives of this research were to test the hypotheses that (1) reduced lateral root branching density decreases the respiration of maize roots and (2) maize genotypes with FL lateral roots have greater rooting depth under WS conditions, resulting in greater water acquisition and improving both plant growth and yield. To test these hypotheses, we compared the performance of maize recombinant inbred lines (RILs) sharing a common genetic background but having contrasting lateral root branching phenotypes under WS in greenhouse mesocosms (GH) and two field environments.

RESULTS

Lateral Root Branching and Root Length

Most genotypes selected for this study displayed stable lateral root branching density phenotypes, except MO327. In two replications of the Pennsylvania (PA) field site in WS conditions, MO327 displayed the MS lateral root phenotype rather than the FL lateral root phenotype. For the purposes of this study, MO327 was classified as the FL phenotype in all figures and statistics, which did not substantially affect statistical analyses (Supplemental Table S1).

In GH, WS significantly decreased lateral root branching density in crown and primary roots (Fig. 1). Compared with FL lines, MS lines had significantly greater lateral root branching density in crown roots, but no significant difference was found in primary and

Table 1. Root respiration of eight maize genotypes under two water treatments

Specific root respiration per unit of root length ($\eta\text{mol CO}_2 \text{ cm}^{-1} \text{ root length s}^{-1}$), root respiration of axial roots per unit of axial root length (axial root respiration; $\eta\text{mol CO}_2 \text{ cm}^{-1} \text{ axial root length s}^{-1}$), and root respiration of lateral roots per unit of axial root length (lateral root respiration; $\eta\text{mol CO}_2 \text{ cm}^{-1} \text{ axial root length s}^{-1}$) of eight maize RILs with contrasting lateral root branching density (FL or MS) under WW and WS conditions in GH are shown. Data are means ($n = 4$). The same letters within each column are not significantly different at $\alpha = 0.05$ (Tukey's honestly significant difference method). ANOVA data (at bottom) are shown for the above parameters as influenced by soil moisture treatment, genotype, and phenotype with associated F values and probabilities (NS, not significant; **, $P \leq 0.01$; and ***, $P \leq 0.001$).

Classification Based on Lateral Root Branching Density	RILs	Specific Root Respiration		Axial Root Respiration		Lateral Root Respiration	
		WS	WW	WS	WW	WS	WW
FL	67	164.44 b,c	292.63 a	10.24 a,b	17.82 a	3.50 b	14.11 a
	79	157.81 c	296.45 a	9.27 b	17.72 a	4.03 b	12.13 a
	86	149.62 c	253.62 a	12.76 a,b	17.34 a	5.27 b	14.11 a
	327	159.31 c	241.37 a	11.21 a,b	18.70 a	5.31 b	13.03 a
MS	134	233.19 a,b	259.49 a	9.79 a,b	19.71 a	11.35 a	13.59 a
	295	237.43 a	243.42 a	13.53 a,b	21.55 a	12.10 a	15.56 a
	321	236.21 a	279.96 a	15.37 a	18.15 a	12.24 a	11.26 a
	362	221.95 a,b	268.77 a	10.50 a,b	14.93 a	7.92 ab	14.45 a
ANOVA							
Treatment (T)			71.76***		57.61***		89.69***
Genotype (G)			3.42**		1.67 ^{NS}		5.32***
Phenotype (P)			9.45**		1.35 ^{NS}		27.37***
G × T			5.46***		0.97 ^{NS}		5.02***
P × T			21.24***		0.16 ^{NS}		21.64***

seminal roots. In the two field sites, lateral root branching density of crown roots in MS lines was significantly greater than that of FL lines in both WS and well-watered (WW) conditions (Fig. 1, B and C). WS significantly decreased lateral root branching density in crown roots, although no difference was found in primary and seminal roots.

Under WS in GH, the average axial root lengths of crown, primary, and seminal roots of the FL lines were greater, by 34%, 73%, and 71%, respectively, compared with the MS lines (Fig. 2). Total root length of FL and MS lines had equivalent values in either WS or WW, but WS significantly decreased total root length in both FL and MS lines (Fig. 2).

Lateral Root Branching Effects on Respiration and Rooting Depth

Water availability and genotype had significant effects on specific root respiration and root respiration of lateral roots per unit of axial root length (Table I). WS decreased specific root respiration in the mesocosms by 37%. Under WS conditions in mesocosms, specific root respiration of FL lines was 47% less than that of MS lines (Table I). Specific root respiration was positively correlated with lateral root branching density of crown roots under WS ($r^2 = 0.86$, $P = 0.0005$; Fig. 3). Root respiration of axial and lateral roots per unit of axial root length was significantly affected by water treatment (Table I). Root respiration of axial roots per unit of axial root length showed no significant difference among genotypes in either WS or WW, but root respiration of axial roots per unit of axial root length in WS was 37% less than that in WW. Root respiration of lateral roots per unit of axial root length in WS was 43% less than in WW (Table I). Under WS, root respiration of lateral roots per unit of axial root length in FL lines had 141% less respiration than in MS lines. Root respiration of lateral roots per unit of axial root length was positively correlated with the lateral root branching density of crown roots under WS ($r^2 = 0.95$, $P < 0.0001$; Fig. 3).

WS decreased root length density in the GH and in the field in Arizona (AZ) and PA, and the FL lines had greater root length density in deep soil layers than MS lines (Fig. 4). Under WS in AZ and PA, FL lines had significantly greater depth above which 95% of total root length is located in the soil profile (D_{95}) than MS lines (Supplemental Table S2). FL lines under WS had D_{95} values of 118, 54, and 55 cm in GH, AZ, and PA, respectively, compared with 88, 43, and 45 cm in MS lines. Lateral root branching density of crown roots was negatively correlated with D_{95} under WS in all three environments (Fig. 5).

Lateral Root Branching Effects on Leaf Relative Water Content and Isotopic Signature

Water treatment and genotype had significant impacts on leaf relative water content (LRWC) in PA and

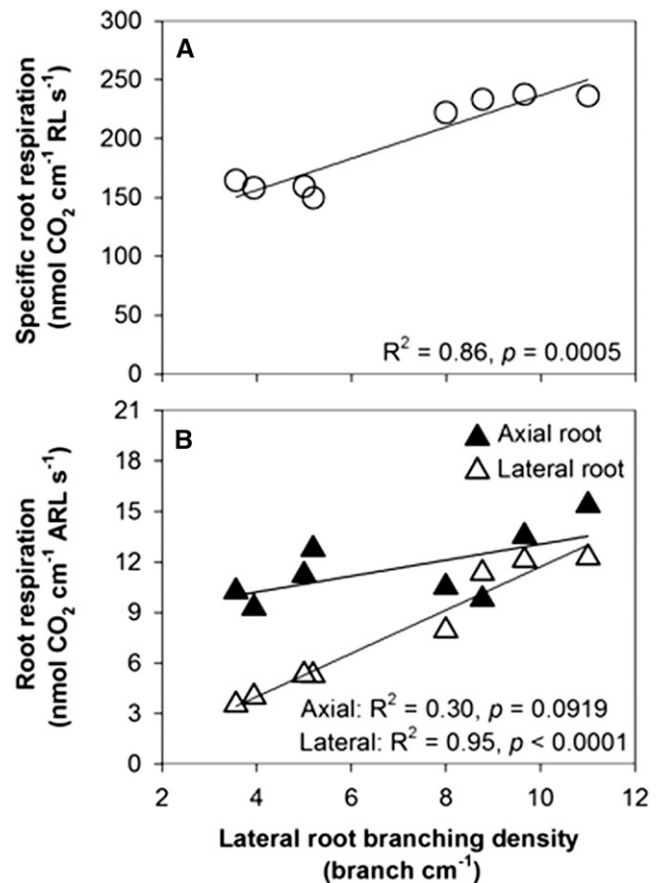


Figure 3. Correlation between lateral root branching density of crown roots and specific root respiration per unit of root length (RL; A) and root respiration per unit of axial root length (ARL; B) under water stress in GH. Each point is the mean of four replicates of each genotype.

mesocosms (Supplemental Table S2). Under non-stressed conditions, LRWC in the GH and AZ was not significantly different between FL and MS lines. Under WS, the LRWC of FL lines in GH and PA was significantly greater, by 8% and 13%, than that of MS lines. Under WS, LRWC was positively correlated with rooting depth in GH ($r^2 = 0.80$, $P = 0.0017$) and PA ($r^2 = 0.92$, $P = 0.0001$; Fig. 6).

Under WS conditions, analysis of soil water isotopic signature ($\delta^{18}\text{O}$) in both AZ and PA showed progressively lighter $\delta^{18}\text{O}$ of water with increasing depth (Fig. 7). In AZ, the majority of change in this signature was found in the top three soil layers (0–10, 10–20, and 20–30 cm; approximately 1.97‰), while in PA, this change was mainly found in the top two soil layers (0–10 and 10–20 cm; approximately 4.19‰). No significant difference was found in the deepest three soil layers, which were aggregated as deep water for subsequent analyses. The values of stem water $\delta^{18}\text{O}$ of the eight genotypes varied by 3.25‰ in AZ and 3.45‰ in PA (Table II). The FL lines in AZ and PA had 46% and 44% lighter stem water signatures, respectively, than the MS lines. Soil water $\delta^{18}\text{O}$ values were used in an

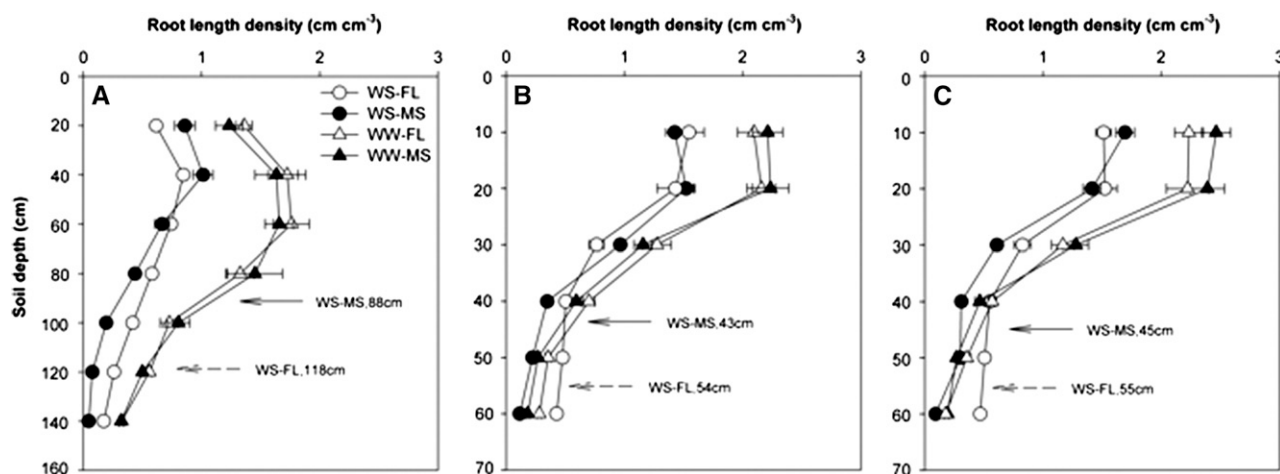


Figure 4. Root length density by soil depth of maize RILs in GH (A) and in the field in AZ (B) and PA (C) under WS (circles) and WW (triangles) conditions. The data shown are means \pm se of four replicates of the four genotypes in each phenotype class in WS or WW. The average value of D_{95} for four FL (dashed arrows) and four MS (solid arrows) genotypes under WS are shown in each panel.

isotopic mixing model to determine water sources contributing to the $\delta^{18}\text{O}$ signature for stem water, assuming that any water acquired below 30 cm depth was deep water. Under WS in AZ and PA, the FL lines mainly absorbed deep water, averaging 66% and 66% of stem water, respectively, while the MS lines had greater reliance on the two most shallow soil layers (Table II). Lateral root branching density of crown roots was negatively correlated with the $\delta^{18}\text{O}$ signature for stem water in AZ ($r^2 = 0.83$, $P = 0.0011$) and PA ($r^2 = 0.54$, $P = 0.0224$; Fig. 8).

Lateral Root Branching Effects on Plant Growth and Yield

In all three environments, WS significantly decreased CO_2 exchange rate and stomatal conductance (Fig. 9). Under WS, FL lines had significantly greater leaf CO_2 assimilation than MS lines, by 58% in GH, 42% in AZ, and 79% in PA. Stomatal conductance in GH, AZ, and PA were 84%, 73%, and 65% greater in FL lines than in MS lines, respectively, under WS conditions (Fig. 9).

Relative shoot dry weight in GH, AZ, and PA were significantly influenced by water treatment, and that in the two field sites was influenced by genotype (Supplemental Table S2). Under WS, the FL lines had 50%, 51%, and 67% greater relative shoot dry weight at 42 d after planting in the GH and at anthesis in AZ and PA, respectively, than MS lines (Fig. 10). Relative shoot dry weight was negatively correlated with lateral root branching density of crown roots (GH, $r^2 = 0.86$, $P = 0.0006$; AZ, $r^2 = 0.51$, $P = 0.0279$; PA, $r^2 = 0.45$, $P = 0.0402$; Fig. 11A). In PA, the lateral root branching density of crown roots was negatively correlated with yield ($r^2 = 0.50$, $P = 0.0307$; Fig. 11B). Under WS, compared with MS, FL lines improved yield by 144% (Fig. 11B).

DISCUSSION

We hypothesized that reduced lateral root branching density would decrease the metabolic cost of soil exploration, thereby improving water acquisition, plant growth, and yield under WS. Our results from

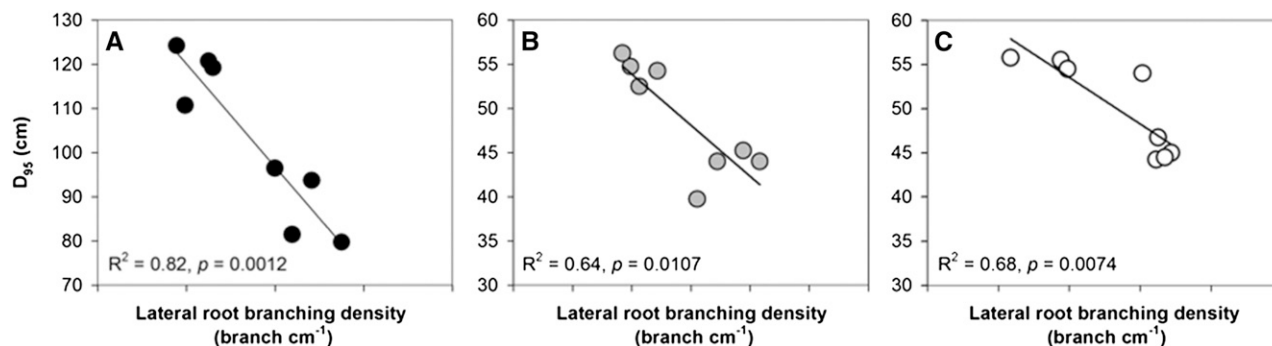


Figure 5. Correlation between lateral root branching density of crown roots and D_{95} in GH (A) and in the field in AZ (B) and PA (C) under WS. Each point is the mean of four replicates of each genotype.

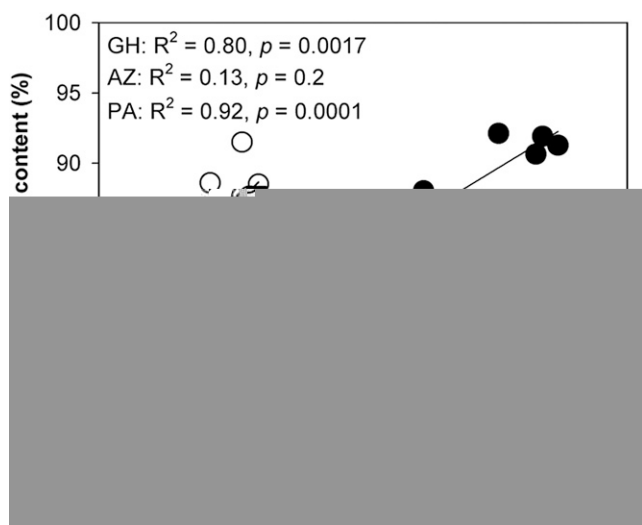


Figure 6. Correlation between D_{95} and LRWC in GH and in the field in AZ and PA under WS conditions. Each point is the mean of four replicates of each genotype.

GH and two field environments entirely support the hypothesis that, under WS, root phenotypes with FL lateral roots have less specific root respiration, greater rooting depth, greater acquisition of deep soil water, improved plant water status, leaf photosynthesis, stomatal conductance, and hence greater plant growth and yield. These results support the inclusion of this lateral root phenotype in the SCD ideotype for optimal acquisition of water and N (Lynch, 2013).

In order to impose terminal drought by progressive reduction of soil water content, we used GH, reduced irrigation in AZ, and automated rainout shelters in PA. The combination of results from three distinct environments is noteworthy. Mesocosms are simplified, controlled environments, yet they permit detailed analysis of root distribution by depth and intact root respiration, as entire root systems can be excavated. The field environments include variable environmental factors such as temperature, rainfall, soil biota, and soil physical properties that may affect results, and the two field environments had contrasting soil physical properties. The fact that results from these contrasting WS environments are in agreement with each other suggests that potentially confounding factors of any given environment are not driving the results. In addition, we used RILs that share a common genetic background (i.e. all lines descend from the same two parents) without artificially induced mutations or transformation events. Each RIL is a distinct genotype, and comparison of several RILs allows the analysis of a phenotype in distinct genomes, thereby minimizing the risk of confounding effects from pleiotropy, epistasis, or other genetic interactions (Zhu and Lynch, 2004). RILs are particularly valuable in the analysis of phenotypic traits governed by multiple genes, as is the case for lateral rooting in maize (Zhu et al., 2005b; Burton et al., 2014).

We have proposed that reduced lateral root branching density may be a useful adaptation to drought by reducing the metabolic costs of soil exploration (Lynch, 2013). The metabolic costs of soil exploration by root systems are substantial and can exceed 50% of daily photosynthesis (Lambers et al., 2002). The fewer roots that are initiated, the fewer carbon and other resources that need to be invested in root growth and maintenance, which could save photosynthate and improve the growth of shoots and other roots and may enhance reproduction (Lynch, 2007). Root respiration associated with growth, maintenance, and ion uptake is a major component of root metabolic costs (Lambers et al., 2002; Lynch and Ho, 2005). In this study, decreasing lateral root branching of crown roots from 11 to 3 branches cm^{-1} was associated with a 58% reduction of specific root respiration and a 71% reduction of lateral root respiration per unit of axial root length (Fig. 3). Empirical and modeling results indicate that the optimal density of lateral branching of maize roots decreases at low N availability (Postma et al., 2014; Zhan and Lynch, 2015). In this study, results from mesocosms and one field site show that reduced lateral root branching density increases rooting depth and improves plant water status and stomatal conductance (the lack of effect of lateral root branching density on LRWC and stomatal conductance in AZ was due to rainfall at the time of

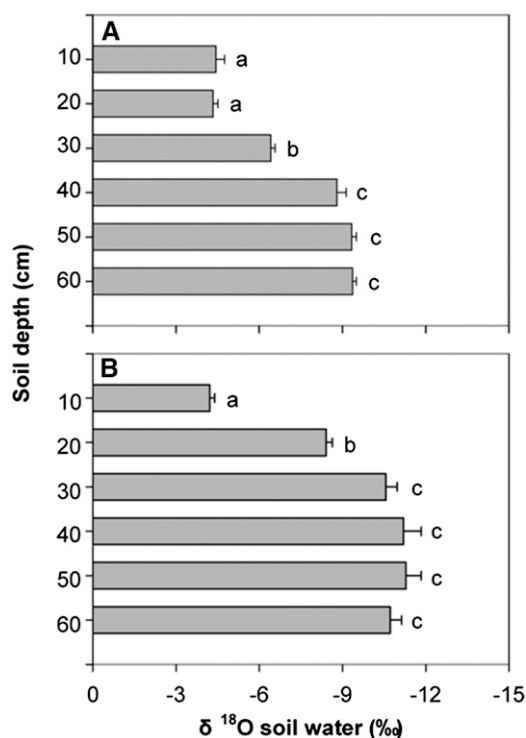


Figure 7. Mean $\delta^{18}\text{O}$ of soil water along the soil profile in the field in AZ (A) and PA (B) under WS conditions. Values are means \pm SE of four observation points. Bars with the same letters are not significantly different within the same panel ($\alpha = 0.05$).

Table II. Stem water $\delta^{18}\text{O}$ of eight maize genotypes and proportional water use by depth

Data are means \pm SE of $\delta^{18}\text{O}$ of stem water ($n = 4$) measured for eight maize genotypes contrasting in lateral root branching density (FL or MS) and proportional water use by depth from different soil layers (deep is the aggregate of three deep soil layers) under WS conditions at anthesis in AZ and PA.

Classification Based on Lateral Root Branching Density	RILs	$\delta^{18}\text{O}$ of Stem Water		Proportional Water Use by Depth					
		AZ	PA	AZ			PA		
				10 cm	20 cm	Deep	10 cm	20 cm	Deep
				%					
FL	67	-7.66 ± 0.17	-9.69 ± 0.11	10.20	10.20	61.15	9.65	25.58	64.78
	79	-7.89 ± 0.21	-9.94 ± 0.51	9.28	8.90	65.58	8.18	25.83	66.00
	86	-7.62 ± 0.13	-9.76 ± 0.35	10.98	10.70	59.28	9.18	25.60	65.28
	327	-8.46 ± 0.36	-9.91 ± 0.40	5.60	5.15	80.10	8.23	24.43	67.33
MS	134	-5.39 ± 0.23	-6.49 ± 0.26	26.85	41.60	11.20	56.38	27.03	16.65
	295	-5.21 ± 0.22	-6.98 ± 0.027	36.45	35.80	9.15	41.68	41.43	23.90
	321	-5.59 ± 0.21	-7.01 ± 0.37	29.88	28.80	14.70	47.55	32.60	19.85
	362	-5.44 ± 0.25	-6.87 ± 0.11	30.65	33.03	12.85	48.53	32.15	19.33

sampling) under drought conditions. Reduced lateral branching directly reduces the respiratory costs associated with sustaining more lateral roots, thereby permitting the axial root to elongate faster. An indirect benefit of reduced lateral branching is that for mobile resources like water and nitrate, greater spatial dispersion of lateral roots increases the soil volume explored per unit of root cost and reduces resource competition among roots of the same plant, which improves the metabolic efficiency of soil exploration (Postma et al., 2014). This has practical implications, since in many rainfed or drought environments, the topsoil dries before the subsoil, and, as drought progresses, roots must exploit increasingly deeper soil strata to capture water. Therefore, genotypes with deep root systems would have the capability to capture water from deep soil strata and resist WS (Lopes and Reynolds, 2010; Wasson et al., 2012; Lynch and Wojciechowski, 2015).

Reduced lateral root branching density is important for drought tolerance because this phenotype determines the balance between the capture of mobile and immobile resources (Lynch, 2013). Greater lateral root branching increases the rate at which a soil domain is depleted of resources, especially for immobile resources like P. For example, results from a recent modeling study showed that a greater density of lateral branches in the topsoil can improve P uptake from low-P soil in wheat (*Triticum aestivum*) by 142% (Heppell et al., 2015). However, for highly mobile resources, like N and water, depletion zones are larger and the greater lateral root branching creates overlapping resource depletion zones around roots of the same plant, thereby decreasing resource capture efficiency (Ge et al., 2000). Therefore, lateral root phenotypes to optimize mobile resources should be long and dispersed along the axial roots. Results from the structural-functional simulation model *SimRoot* have shown that the optimal density of lateral branching of maize roots at low N availability is less than that at low P availability (Postma et al., 2014), a result later confirmed at low N in field and mesocosm studies

(Zhan and Lynch, 2015). Here, we show that reduced lateral root branching density improves plant water capture under WS (Table II; Fig. 6).

An additional benefit to reducing root cost is that extra resources from reduced root metabolic demand can contribute to growth and yield (Lynch, 2014), which are competing sinks for current photosynthate. In our mesocosm study, decreasing lateral branching of crown roots from 11 to 3 branches cm^{-1} was associated with an 11% increase of relative shoot biomass (Fig. 11A). In the field experiments, decreasing lateral branching of crown roots from 11 to 4 branches cm^{-1} in AZ and from 12 to 5 branches cm^{-1} in PA was associated with 40% and 37% increased relative shoot biomass, respectively (Fig. 11A). Simulation results indicate that, without root maintenance respiration, maize plants had up to 72% greater growth under limiting nutrient supply (Postma and Lynch, 2011a,

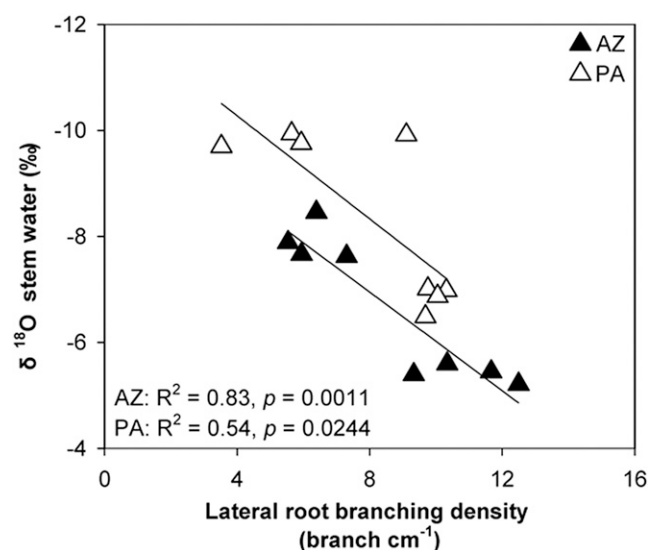
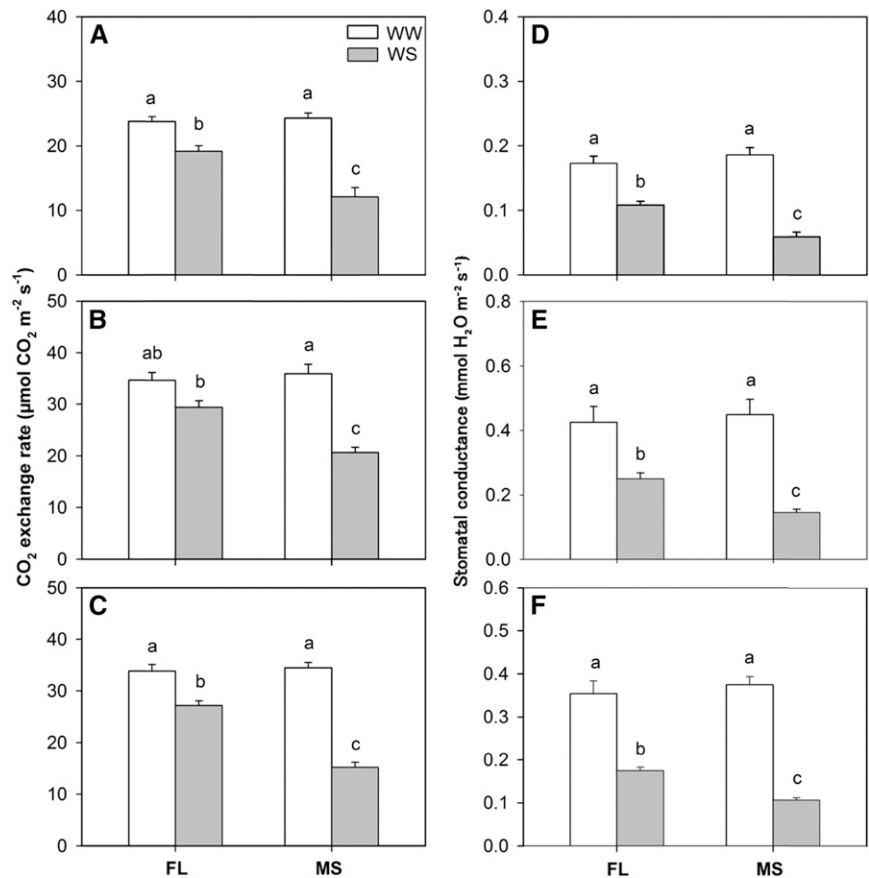


Figure 8. Correlation between lateral root branching density and stem water $\delta^{18}\text{O}$ in the field in AZ and PA under WS conditions. Each point is the mean of four replicates of each genotype.

Figure 9. CO₂ exchange rate (A–C) and stomatal conductance (D–F) of FL and MS phenotypes in GH (A and D) and in the field in AZ (B and E) and PA (C and F) under WW and WS conditions. Bars show means \pm SE of four replicates of the four genotypes in each phenotype class in WW or WS. Bars with the same letters are not significantly different within the same panel ($\alpha = 0.05$).



2011b). Therefore, reduced root carbon demand in FL genotypes may be beneficial by increasing carbohydrate availability (Fig. 11A). These results support the hypothesis that genotypes with less costly root tissue could develop the extensive, deep root systems required to fully utilize soil water resources in drying soil without as much yield penalty.

Hydrogen and oxygen stable isotope analysis provides an effective approach for studying root water uptake. Normally, natural discrimination by evaporation against heavy isotopes increases the concentration of heavy isotopes of oxygen in water at the soil surface (Durand et al., 2007). Under dry conditions, this results in a relative enrichment in heavy isotopes of elements of water (deuterium and ¹⁸O) in the topsoil, while deeper soil strata maintain the average isotopic composition of regional precipitation (Durand et al., 2007; Fig. 7). No hydrogen and oxygen isotope fractionation occurs during soil water uptake by root systems (Ehleringer and Dawson, 1992), so the water absorbed by plant roots can be considered as the mixture of water acquired from different soil depths. In this study, stem water $\delta^{18}\text{O}$ showed that the FL phenotype had lighter $\delta^{18}\text{O}$ and greater dependency on deep soil water than the MS phenotype (Table II). The difference in the depths of root water acquisition between the FL and MS genotypes could be attributed to their rooting depth (Figs. 4 and 5; Supplemental Table S2).

Studies have shown that lateral root formation for embryonic (seminal and primary roots) and postembryonic (nodal roots, including crown and brace roots) roots is controlled by multiple pathways or different sensitivities to signals in lateral root formation (Hochholdinger and Feix, 1998; Hochholdinger et al., 2001).

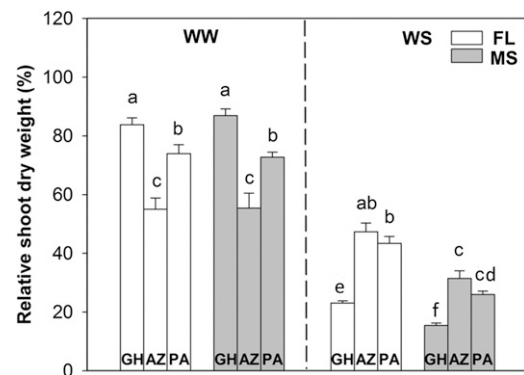


Figure 10. Relative shoot dry weight (percentage of greatest shoot dry weight within each location) of FL and MS phenotypes in GH and in the field in AZ and PA under WS and WW conditions. Bars show means \pm SE of four replicates of the four genotypes in each phenotype class in WW or WS. Bars with the same letters are not significantly different within the same panel ($\alpha = 0.05$).

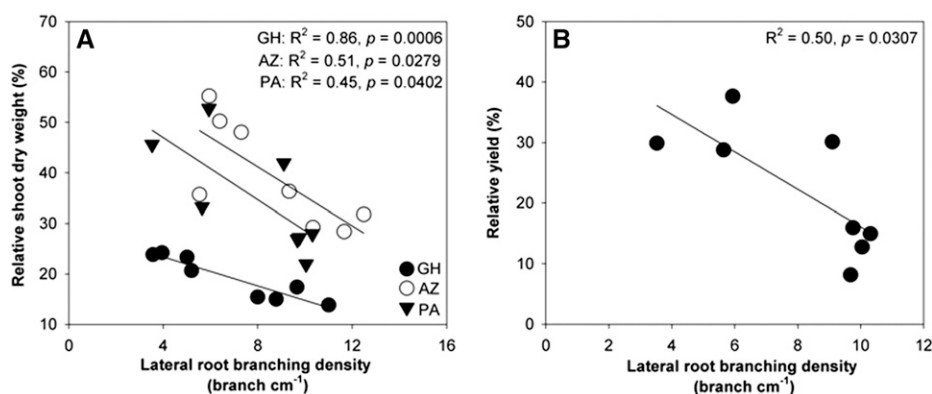


Figure 11. Correlation between lateral root branching density of crown roots and relative shoot dry weight (percentage relative to greatest shoot dry weight within each location) in GH and in the field in AZ and PA (A) and relative yield (percentage relative to greatest yield) in PA under WS conditions (B). Each point is the mean of four replicates of each genotype.

In maize, the *lateral rootless1* mutant is deficient in the initiation of lateral roots in the primary roots, seminal roots, and crown roots emerging from the coleoptilar node; however, crown roots from subsequent nodes have normal lateral root formation (Hochholdinger and Feix, 1998). In addition, *short lateral root1* (*slr1*) and *slr2* maize mutants display reduced elongation of lateral roots from roots of the embryonic root system and normal lateral root formation from roots in the post-embryonic root system (Hochholdinger et al., 2001). In this study, primary and seminal roots did not show the same lateral root branching phenotypes as the crown roots, and phenotypes for branching densities were intermediate rather than in distinct groups of MS or FL (Fig. 1). These results are evidence that lateral branching density for the embryonic and postembryonic root system is under distinct genetic control. However, during vegetative growth of the plant, the crown roots capture the majority of the soil resources (Lynch, 2013). The SCD ideotype proposes an increased lateral branching density of seminal roots to optimize P and ammonium capture during seedling establishment and a decreased lateral root branching density of crown roots to improve the capture of nitrate and water during vegetative growth (Lynch, 2013). Results from this study support the SCD ideotype. The FL lateral branching phenotype on crown roots improved plant water status, plant growth, and yield in WS conditions. The genotypes selected for this study did not have clear MS or FL lateral branching phenotypes for primary and seminal roots, which had intermediate branching densities. To further examine the SCD ideotype for primary and seminal roots, additional studies should be conducted using genotypes contrasting for FL and MS of primary and seminal roots.

Lateral branching is a heritable trait (Zhu et al., 2005b) and genetically controlled (Doebley et al., 1995; Takeda et al., 2003). Genotypes selected for this study generally displayed stable lateral root branching density phenotypes regardless of treatment or environment. One exception to this was the genotype MO327, in two replications of the PA field site in WS conditions, which displayed the MS phenotype rather than the FL phenotype. For the purposes of this study, all

figures and statistics include MO327 classified as the FL phenotype, which minimally impacts statistics (Supplemental Table S1). Although genotypes remained stable in terms of phenotypic classes for lateral root branching density throughout the experiment, shifts of lateral branching density were observed in the data. For example, in WS conditions in PA, the average lateral root branching density was 5 branches cm^{-1} for FL and 9.8 branches cm^{-1} for MS, whereas in AZ, the average lateral root branching density was 6.3 branches cm^{-1} for FL and 11 branches cm^{-1} for MS. This plasticity response may reflect varying soil and environmental conditions at the field sites, which is to be expected, as previous studies have shown that genetic variation exists for plasticity in root traits (Zhu et al., 2010).

Root depth is one of the most important traits for plant resistance to WS (Wasson et al., 2012; Lynch and Wojciechowski, 2015). Modeling studies indicate that selection for deeper, more effective roots could significantly improve the capture of water and N in wheat (Manschadi et al., 2006; Asseng and Turner, 2007; Lilley and Kirkegaard, 2011). In rice, maximum root length, root depth, and basal thickness are correlated with yield under WS (Champoux et al., 1995; Li et al., 2005). When introduced into a shallow-rooting rice cultivar, *Deeper rooting1* improved yield under drought conditions by increasing rooting depth (Uga et al., 2013). Root depth also has been positively correlated with yield in soybean (*Glycine max*; Cortes and Sinclair, 1986). Our results in GH and two field experiments clearly show that the FL phenotype increases rooting depth (Figs. 4 and 5), improves water capture from deep soil (Table II; Figs. 6 and 8), and improves plant water status, growth, and yield (Figs. 6, 10, and 11). Although this study focuses on maize, we suggest that the phenotype of FL lateral roots would improve water capture in other species, like sorghum (*Sorghum bicolor*), which has a root system architecture similar to that of maize (Lynch, 2013). Other Poaceae species have the same basic root structure as maize and may also benefit from this phenotype, like wheat, rice, and barley (*Hordeum vulgare*), although greater density of nodal roots in tillering species may change the relationship of lateral root

branching density and resource capture. Our results are entirely supportive of the inclusion of reduced lateral root branching as a component of the SCD ideotype (Lynch, 2013) for improved capture of N (Zhan and Lynch, 2015) and water (this article) when those resources limit growth. The SCD ideotype applies to both water and N capture, since both of these soil resources are often localized in deep soil strata under limiting conditions.

Plant breeders rarely select for root traits because they are challenging to phenotype, many traditional metrics of root phenotypes are actually phenic aggregates with low heritability, and root phenotypes often display plasticity in response to soil conditions (Tuberosa et al., 2002; Malamy, 2005; York et al., 2013; Lynch, 2014). As shown here and in previous literature, genotypic differences in lateral root number and length exist in maize (Zhu et al., 2005b; Trachsel et al., 2011; Lynch, 2013; Burton et al., 2014). Previous studies indicate that lateral branching is a heritable trait (Zhu et al., 2005b), and genes affecting lateral branching have been identified in several species, including maize (Doebley et al., 1995) and rice (Takeda et al., 2003), making lateral branching and length feasible targets for plant breeding. Our results from three distinct environments, GH and two field sites, are entirely consistent with the hypothesis that the FL lateral root phenotype increases rooting depth by reducing root metabolic costs, resulting in greater water acquisition from deep soil strata and improved plant growth and yield under WS. We suggest that lateral root number and length deserve consideration as a root phenotype to improve drought tolerance in crop breeding programs.

MATERIALS AND METHODS

GH Experiment

Plant Materials

Eight RILs of maize (*Zea mays*), genotypes MO067, MO079, MO086, MO134, MO295, MO321, MO327, and MO362, from the intermated B73 × MO17 population were obtained from Dr. Shawn Kaeppeler (University of Wisconsin, Madison; Genetics Cooperation Stock Center). Our previous screening for lateral root branching and length in this population indicated that RILs MO067, MO079, MO086, and MO327 had the FL phenotype and RILs MO134, MO295, MO321, and MO362 had the MS phenotype (Trachsel et al., 2011, 2013). Thus, in this study, we consider RILs MO067, MO079, MO086, and MO327 to have the FL phenotype and RILs MO134, MO295, MO321, and MO362 to have the MS phenotype.

Experimental Design

The greenhouse experiment was a randomized complete block design. The factors were two water treatments (WW and WS) and eight RILs (MO067, MO079, MO086, MO134, MO295, MO321, MO327, and MO362), with four replicates.

Growth Conditions

Plants were grown from March 19 to April 30, 2014, in a greenhouse located on the campus of Pennsylvania State University in University Park (40° 48' N, 77° 51' W) under constant conditions (14 h of day at 28°C/10 h of night at 24°C, 40%–70% relative humidity). Seeds of eight genotypes were surface sterilized in 0.05% (v/v) NaOCl for 30 min and imbibed for 24 h in aerated 1 mM

CaSO₄, then were placed in darkness at 28°C ± 1°C in a germination chamber for 2 d. Three seedlings of similar size were transplanted to mesocosms consisting of polyvinylchloride (PVC) cylinders 15.7 cm in diameter and 155 cm in height, with plastic liners made of 4-mil (0.116-mm) transparent high-density polyethylene film, which was used to facilitate root sampling, then thinned to one seedling per mesocosm 5 d later after planting. The growth medium consisted of 50% (v/v) medium size (0.5–0.3 mm) commercial-grade sand (Quikrete), 35% (v/v) horticultural size 3 vermiculite, 5% (v/v) perlite (Whittemore), and 10% (v/v) topsoil. The topsoil was collected from the Russell E. Larson Agricultural Research Center in Rock Springs, Pennsylvania (fine, mixed, semiactive, mesic Typic Hapludalf, pH 6.7, silt loam). To ensure a consistent bulk density, a uniform volume (29 L) of the soil mixture was used in each mesocosm. Mineral nutrients were provided by mixing the medium with 70 g per column of Osmocote Plus fertilizer consisting of 15% (w/w) N, 9% (w/w) P, 12% (w/v) potassium, 2.3% (w/w) sulfur, 0.02% (w/w) boron, 0.05% (w/w) copper, 0.68% (w/w) iron, 0.06% (w/w) manganese, 0.02% (w/w) molybdenum, and 0.05% (w/w) zinc (Scotts-Sierra Horticultural Products). Two days before planting, each cylinder was irrigated with 4.5 L of deionized water. In the first 4 d, plants received 100 mL of deionized water every day. Then, 200 mL of deionized water was irrigated for the WW treatment every 2 d, and the WS treatment received no further irrigation. Additional light was provided with 400-W metal-halide bulbs (Energy Technics) for 14 h per day to a maximum illumination of 1,200 μmol photons m⁻² s⁻¹. Average daytime temperature in the greenhouse was approximately 28°C.

Root Respiration and Root Harvest

Three days before harvesting, the head-space approach of sampling air flow over the soil surface was used in this study to measure intact root respiration. In short, a PVC plate was placed on top of the pot to seal off the root system from the shoot. An air pump provided a stable flow of air through the head-space compartment of the pot. The air flow rate was 1,200 μmol s⁻¹. The measurements were conducted in early morning with the Li-6400 portable infrared gas analyzer (Li-Cor Biosciences). Intact root respiration was measured for a short time (approximately 5 min; Fan et al., 2003; Zhu et al., 2005a). In this study, we assumed that the amount of natural soil and the respiration of microbes were the same in all cylinders (Bouma et al., 1997a, 1997b) and used the intact root system plus medium respiration as a proxy for total root respiration. The intact root system respiration values were divided by the total root length obtained by WinRhizo scanning (described below) to obtain the specific root respiration per unit of root length (μmol CO₂ m⁻¹ root length s⁻¹).

At harvest (April 30, 2014), the plastic sleeve was removed from the supporting PVC cylinder and cut open, and roots were separated from the soil by vigorous rinsing at low pressure with water. Root respiration of axial and lateral roots was measured. Three representative 10-cm root segments from the third crown root were excised 20 cm from the base. Lateral roots of axial roots were removed with a Teflon blade (Electron Microscopy Sciences). Excised axial and lateral root samples were patted dry and placed in a 40-mL custom chamber connected to the Li-6400 infrared gas analyzer (Li-Cor Biosciences) separately. The temperature of the chamber was maintained at 26°C ± 1°C using a water bath while respiration was measured. Carbon dioxide evolution from the root segments was recorded every 5 s for 180 s. Axial root length of crown and seminal roots was collected from three representative root samples representing the average growth of each root class. Root number in each whorl of crown roots and seminal roots was counted manually. Average axial root length of crown roots was calculated using a weighted average from all roots. Roots from each 20-cm soil layer were collected, and lateral root number from three representative roots was obtained by scanning with image-analysis software (WinRhizo Pro; Régent Instruments) as described below. The total root length of each plant was the sum of the root length in each layer.

Plant Measurements and Shoot Dry Weight

One day before harvesting, LRWC was measured. To measure LRWC, four fresh leaf discs (1 inch in diameter) were collected from the third fully expanded leaf and weighed immediately to determine fresh weight (FW). After this, the discs were immediately hydrated to full turgidity by soaking them in distilled water for 8 h. After 8 h, the discs were patted dry and weighed again to determine turgid weight (TW). The discs were then dried at 70°C for 72 h, and dry weight (DW) was determined. LRWC was calculated according to the following equation: LRWC (%) = 100 × (FW – DW)/(TW – DW).

The CO₂ exchange rate and stomatal conductance of the third fully expanded leaf were measured with the Li-6400 portable photosynthesis system (Li-Cor Biosciences) using a red-blue light at a photosynthetically active radiation intensity of 1,200 μmol photons m⁻² s⁻¹, CO₂ concentration of 400 μL L⁻¹, and leaf temperature of 25°C. The measurements were conducted between 9 and 11 AM. At harvest, shoots were collected and dried at 70°C until constant weight for biomass determination.

Field Studies

Field Conditions, Experimental Design, and Plant Materials

Field experiments were carried out from April to July 2014 at the Apache Root Biology Center, Willcox, Arizona (32° 2' 0'' N, 109° 41' 30'' W), and from May to August 2014 at the Russell Larson Research and Education Center of Pennsylvania State University in Rock Springs, PA (40° 42' 37'' N, 77° 57' 07'' W). The soils at the experimental sites were a Grabe loam (coarse-loamy, mixed, thermic Typic Torrifluent) in AZ and a Hagerstown silt loam (fine, mixed, mesic Typic Hapludalf) in PA.

The two experiments were arranged in a split-plot design replicated four times with two water treatments (WS and WW). The main plots were composed of two moisture regimes, and the subplots were eight genotypes (RILs MO067, MO079, MO086, MO134, MO295, MO321, MO327, and MO362) in each experiment. In AZ, the experiments were planted in five 6-m row plots with 25 cm distance between plants and 75 cm wide between rows. The WS treatment was initiated starting 40 d after planting by withholding water application in AZ. In PA, each subplot consisted of three rows, each row was 3 m long, 25 cm between plants, and 75 cm between rows. The drought treatment was initiated 30 d after planting using an automated rainout shelter in PA. The shelters (10 × 30 m) were covered with a clear greenhouse plastic film (0.184 mm) and were automatically triggered by rainfall to cover the plots, excluding natural precipitation. Adjacent nonsheltered control plots were rainfed to maintain the soil moisture close to field capacity throughout the growing season; drip irrigation was applied when necessary. Soil water content in AZ and PA was monitored using soil moisture probes (PR2; Dynamax) and the TRIME FM system (IMKO Mocomoduletechnik), respectively, both in WS and WW treatments. At each location, the recommended fertilizer rate was applied before planting. Pest control and irrigation were carried out as needed.

Plant Measurements

Two days before harvest, CO₂ exchange rate and stomatal conductance of the ear leaf were measured with the Li-6400 portable photosynthesis system (Li-Cor Biosciences) using a red-blue light at radiation intensity of 1,800 μmol photons m⁻² s⁻¹, constant CO₂ concentration of 400 μL L⁻¹, and leaf temperature of 28°C. The measurements were conducted between 9 and 11 AM. In both field experiments, LRWC was measured as described above, except that nine fresh leaf discs were collected from the ear leaf for three representative plants per plot (three fresh leaf discs per plant). At physiological maturity, grain yield was collected in PA. Yield was not collected in AZ because of uneven anthesis of these RILs in response to the temperature and photoperiod regime at this location.

Shoots and roots were evaluated 10 weeks after planting at AZ and 12 weeks after planting at PA (anthesis stage). Three representative, adjacent plants were randomly selected in the same row for shoot dry weight per replicate and dried at 70°C for 72 h before being weighed. Roots were excavated by removing a soil cylinder approximately 40 cm in diameter and 25 cm in depth with the plant base as the horizontal center of the soil cylinder. The excavated root crowns were cleaned by vigorous rinsing at low pressure with water. The clean roots were subsequently used to measure lateral root number. All nodal roots emerging belowground were classified as crown roots. Three 5-cm root segments were taken 5 cm from the base of each whorl of crown, primary, and seminal roots, and lateral root number of the corresponding roots was based on the counts.

Root Harvest and Rooting Depth

Roots were excavated by removing a soil cylinder of approximately 40 cm diameter and 25 cm depth with the plant base as the horizontal center of the soil cylinder. The excavated root crowns were cleaned by vigorous rinsing at low pressure with water. The clean roots were subsequently used to measure lateral root number. All nodal roots emerging belowground were classified as crown roots. Three representative 5-cm root segments were taken 5 cm from the base

of each whorl of crown, primary, and seminal roots, and lateral root number of the corresponding roots was based on the counts.

In both field sites, soil cores were collected at flowering to determine root distribution in the soil profile. A soil coring tube (Giddings Machine) 5.1 cm in diameter and 60 cm long was used for sampling. The core was taken within a planting row midway between two plants. The soil core was subdivided into 10-cm segments, and roots were extracted from each segment. Extracted root samples were scanned using a flatbed scanner (Perfection V700 Photo; Epson America) at a resolution of 23.6 pixels mm⁻¹ (600 dots per inch) and analyzed using the image-processing software WinRhizo Pro (Regent Instruments). Percentages of root length at each depth were calculated in each soil core. D₉₅ was calculated by linear interpolation between the cumulative root lengths (Trachsel et al., 2013).

Shoots and roots were evaluated 10 weeks after planting at AZ and 12 weeks after planting at PA (anthesis stage). Three representative, adjacent plants were randomly selected in the same row for shoot dry weight per replicate and dried at 70°C for 72 h before being weighed. Roots were excavated by removing a soil cylinder approximately 40 cm in diameter and 25 cm in depth with the plant base as the horizontal center of the soil cylinder. The excavated root crowns were cleaned by vigorous rinsing at low pressure with water. The clean roots were subsequently used to measure lateral root number. All nodal roots emerging belowground were classified as crown roots. Three 5-cm root segments were taken 5 cm from the base of each whorl of crown, primary, and seminal roots, and lateral root number of the corresponding roots was based on the counts.

Soil and Plant Sampling for δ¹⁸O Analysis

In both AZ and PA, soil samples for δ¹⁸O analysis were collected adjacent to plants in the WS treatment at flowering stage using a 5-cm-diameter soil core. Soil cores were taken to the maximum achievable depth of 60 cm. The cores were separated into 10-cm increments: 10, 20, 30, 40, 50, and 60 cm. In each replicate, three cores were collected from different positions and mixed as one sample for each depth. The maize stems were collected at the same time, approximately 8 to 10 cm of the stem was collected just above ground level, and the epidermis was immediately removed. Soil and maize stem samples were put into snap vials, sealed with Parafilm to prevent evaporation, and refrigerated immediately. Cryogenic vacuum distillation (Ehleringer and Osmond, 1989) was used to extract soil water and crop stem water. In cryogenic vacuum distillation, two glass tubes were attached to a vacuum pump. The sample was placed in one tube and frozen by submerging the tube in liquid N, and then both tubes were evacuated by vacuum pump to create a closed U-shaped configuration. After that, the tube containing the sample was heated to 100°C, while the collection tube was still immersed in liquid N to collect evaporated water. Samples were weighed and oven dried after extraction to ensure that the extraction time was sufficient to vaporize all the water in the samples. The water samples were analyzed using the L2130-i δD/δ¹⁸O Ultra High Precision Isotopic Water Analyzer (Picarro). Results were expressed as parts per 1,000 deviations from the Vienna Standard Mean Ocean Water. To determine the percentage contribution of soil water from different depths to the signature of water within the plant tissue, an isotopic mixing model was used (Phillips et al., 2005). IsoSource version 1.3.1 (Phillips and Gregg, 2003) was used to evaluate the relative contribution of each soil layer to the tissue water signature. The fractional increment was set at 1%, and tolerance was set at 0.1.

Statistical Analysis

The experimental data were statistically analyzed by ANOVA, and Tukey's honestly significant difference method ($\alpha = 0.05$) was used for multiple comparisons with SAS 8.0 software (SAS Institute). Linear regression analysis and Pearson correlation coefficients were calculated using SigmaPlot 10.0 software (Systat Software).

Supplemental Data

The following supplemental materials are available.

Supplemental Table S1. Analysis of the effect of plasticity of genotype MO327 on results in WS conditions at the PA field site.

Supplemental Table S2. Summary of ANOVA results.

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