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Reduced root cortical cell file number improves drought tolerance in maize

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Summary: Reduced root cortical cell file number substantially reduces root respiration, permitting greater root growth and exploration of deep soil domains, thereby improving water acquisition, plant growth, and yield under drought.

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Abstract

We tested the hypothesis that reduced root cortical cell file number (CCFN) would improve drought tolerance in maize (*Zea mays* L.) by reducing the metabolic costs of soil exploration. Maize genotypes with contrasting CCFN were grown under well-watered and water-stressed conditions in greenhouse mesocosms and in the field in the USA and Malawi. CCFN ranged from 6 to 19 among maize genotypes. In mesocosms reduced CCFN was correlated with 57% reduction of root respiration per unit root length. Under water stress in the mesocosms, genotypes with reduced CCFN had between 15% and 60% deeper rooting (D_{95}), 78% greater stomatal conductance, 36% greater leaf CO_2 assimilation, and between 52% to 139% greater shoot biomass than genotypes with many cell files. Under water stress in the field, genotypes with reduced CCFN had between 33% and 40 %deeper rooting (D_{95}), 28% lighter stem water $\delta^{18}O$ signature signifying deeper water capture, between 10% and 35% greater leaf relative water content, between 35% and 70% greater shoot biomass at flowering, and between 33% and 114% greater yield than genotypes with many cell files. These results support the hypothesis that reduced CCFN improves drought tolerance by reducing the metabolic costs of soil exploration, enabling deeper soil exploration, greater water acquisition, and improved growth and yield under water stress. The large genetic variation for CCFN in maize germplasm suggest that CCFN merits attention as a breeding target to improve the drought tolerance of maize and possibly other cereal crops.

Key words: Cortical cell file number, root costs, *Zea mays* L., (Maize), drought

Introduction

Drought is a primary constraint to global crop production (Schmidhuber and Tubiello, 2007) and global climate change is likely to increase the risk of drought, especially in rain-fed agriculture (Battisti and Naylor, 2009; Burke et al., 2009; Mishra and Cherkauer, 2010; Lobell et al., 2011). The development of crops with greater drought tolerance is therefore an important global objective. Yield under drought is often not an efficient selection criterion in drought breeding programs, since yield is affected by many elements of the phenotype and the environment, interacting in complex and often unknown ways. Trait-based selection or ideotype breeding is generally a more efficient selection strategy, permitting the identification of useful sources of variation among lines that have poor agronomic adaptation, elucidation of genotype by environment interactions, and informed trait stacking (Araus, 2002; Manschadi et al., 2006; Lynch, 2007b; Araus et al., 2008; Lynch, 2011, York et al., 2013).

In most agroecosystems the topsoil dries before the subsoil as drought progresses. In such environments plants with deeper roots are able to acquire water available in deeper soil domains that may not be available to plants with shallower roots (Ludlow and Muchow, 1990; Ho et al., 2005; Hammer et al., 2009). An ideotype has been proposed to guide the breeding of crops with deeper roots and therefore greater water acquisition from drying soil called 'steep, cheap and deep', integrating architectural, anatomical, and physiological phenes (Lynch, 2013). The term 'cheap' denotes phenes that reduce the metabolic cost of soil exploration, which is an important limitation to the acquisition of scarce soil resources, including water in dry soil (Fan et al., 2003; Lynch, 2007b; Zhu et al., 2010; Postma and Lynch, 2010; Postma and Lynch, 2011; Jaramillo et al., 2013). Plant resource allocation to root growth typically increases under drought to enhance water acquisition, and therefore the metabolic cost of root growth becomes a significant component of plant fitness and adaptation under drought (Lynch, 2007b; Lynch, 2013). Therefore, 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 modelling studies for maize under water and edaphic stress (Lynch, 2007a; Zhu et al., 2010; Postma and Lynch, 2010; Postma and Lynch, 2011; Jaramillo et al., 2013).

Root cortical aerenchyma (RCA) is the enlarged air space in the root cortex that forms either through cell death or cell separation (Evans, 2004). RCA is associated with a disproportionate reduction of root respiration in maize by converting living cortical tissue to air volume (Fan et al., 2003; Zhu et al., 2010). Reduction of root metabolic costs permits more internal resources to be allocated to greater root growth and consequently greater soil resource acquisition. RCA formation is also associated with reduction of phosphorus content in root tissue on a volume basis, since air spaces do not contain phosphorus (P) (Fan et al., 2003), and with improved growth in low-P soil (Lynch, 2011). RCA also reduces the N content of root tissue, and is beneficial for N capture and maize growth on low N soils (Saengwilai 2013). Modelling studies suggest that RCA improves crop adaptation to suboptimal nutrient availability by reducing the metabolic costs of soil exploration (Postma and Lynch, 2010; Postma and Lynch, 2011). Under drought Zhu et al. (2010) found that maize genotypes with more RCA had 5 times greater biomass and 8 times greater yield than genotypes with less RCA. Living Cortical Area (LCA) is total transverse root cortical area minus RCA area. Jaramillo et al. (2013) found that root respiration is positively correlated with LCA, and a 3.5-fold reduction in LCA is associated with

a 2.5-fold improvement in plant growth under drought. These results indicate that the metabolic demand of living cortical tissue is a primary determinant of root growth, soil exploration and resource acquisition in soil environments with suboptimal resource availability.

The current study builds on earlier studies indicating that substantial reduction of root metabolic cost is associated with variation in LCA. The cortex of the maize root is composed of several concentric layers of parenchyma cells, the number of which we refer to as 'cortical cell file number' (CCFN). Recently Burton et al., (2013) reported that there is 3-fold variation for CCFN in *Zea* species. In that study the variation was wider in landraces (6-16 cell files) than in teosinte (7-13 cell files). It has been proposed that reduced CCFN would decrease the metabolic costs of root growth and maintenance, both in terms of the carbon cost of root respiration as well as the nutrient content of living tissue, by reducing the proportion of root volume occupied by living cortical tissue, which has greater metabolic demands than the stele (Lynch, 2013). However, the physiological utility of CCFN has not been explored.

The objective of this study was to test the hypothesis that reduced CCFN would reduce root respiration, permitting greater rooting depth, thereby enhancing water acquisition and improving both plant growth and yield under water stress.

Results

We observed substantial phenotypic variation for CCFN within maize recombinant inbred lines (RILs) (Fig 1). In mesocosms (GH1), CCFN ranged from 8 to 17 in the IBM population (Fig 2A). In the field in Malawi (MW2011), CCFN ranged from 6 to 19 among lines from the maize breeding program at LUANAR (Lilongwe University of Agriculture and Natural Resources; Fig 2B). The stability of CCFN across environments was estimated as the correlation coefficient between CCFN measured on the same genotypes in mesocosms (30 day old plants, GH2) and in the field (70 day old plants, PA2011) and across environments in the field, Bunda (BU2012) and Chitala (CH2012). Strong positive correlations were found between CCFN in mesocosms (GH2) and in the field (PA2011) ($r = 0.85$, $P < 0.05$), and between CCFN measured in two environments in Malawi (BU2012 and CH2012) ($r = 0.68$, $P < 0.05$).

To understand the effects of CCFN on root respiratory costs, CO_2 production from excised root segments was measured in diverse sets of maize lines in mesocosms (GH 1,2,3). Reduced CCFN was correlated with reductions of specific root respiration by 57% (GH1-IBM), 46% (GH1-NyH), 52% (GH2), and 69% (GH3) (Fig. 3). However, there was no significant difference in respiration rates between well-watered and water-stressed roots in GH2 and GH3 (Table 1). In GH1, CCFN was correlated with specific root length (SRL) both in IBM lines ($r = -0.55$, $p < 0.05$) and NyH lines ($r = -0.48$, $p < 0.05$). CCFN was a better predictor of root respiration than SRL (Table 2). In well-watered mesocosms, CCFN had no relationship with rooting depth, stomatal conductance, photosynthesis rate or plant biomass. Under water stress, genotypes with reduced CCFN had 15% (GH1) and 60% (GH2) deeper rooting, 78% greater stomatal conductance (GH3), 36% greater leaf photosynthetic rate (GH3), and 52% (GH2) and 139% (GH3) greater biomass than genotypes with many cell files (Table 1, Figs. 4, 5, 6). Reduced CCFN genotypes proliferated more roots in soil domains below 60 cm compared to many CCFN genotypes under water stressed conditions (Supplemental figure S1A).

In the field at Rock Springs, PA, under water stress genotypes with reduced CCFN had 33% (PA2011) and 40% (PA2012) deeper rooting depth (D_{95}), and 10% (PA2011) and 35% (PA2012) greater leaf relative water content than genotypes with many cell files (Table 3, Fig. 7, 9 A&B). D_{95} is the depth above which 95% of total root length is located in the soil profile. In addition, genotypes with deeper D_{95} had greater leaf water status than genotypes with shallow D_{95}, while there was no relationship in well watered conditions (PA2011) (r=0. 51, p<0.000). In addition reduced CCFN genotypes proliferated more roots in soil domains below 30 cm compared to many CCFN genotypes under water stressed conditions (Supplemental figure S1B).

Analysis of soil water $\delta^{18}O$ showed progressively lighter isotopic signature of water with increasing depth in water stress conditions (Fig 8). However, the majority of change in this signature was in the top two layers: 0-10 and 10-20 cm depth (approximately 2.09‰). The values of soil water signature below 30 cm depth showed no significant difference with depth (Fig 8) and were aggregated as 'deep water' for further analysis. The average values of xylem water $\delta^{18}O$ for genotypes varied by 3.19‰ (Table 4). Genotypes with reduced CCFN had a collective xylem water signature that was 28% lighter than that of genotypes with many cell files (Table 4). Soil water $\delta^{18}O$ values were used in an isotopic mixing model to determine water sources contributing to the $\delta^{18}O$ signature for xylem water, assuming that any water acquired below 30 cm depth was 'deep water'. Genotypes with reduced CCFN had greater average reliance on 'deep water' and were the least reliant on shallow water from top two soil layers than genotypes with many cell files (Table 4). The proportion of deep water acquired by genotypes with reduced CCFN ranged from 21 to 81% while for two genotypes with many cell files this value was zero. The only exception was genotype 181 which was classified as having many cell files but had relatively greater dependency on deep water of 32%.

Water stress reduced shoot biomass by 30% (PA2011) and 33% (PA2012), and reduced yield from 26% to 68% (PA2011) and from 33% to 75% (PA2012) compared with well-watered plants (Table 3, Fig 9 C, D, E, F). Genotypes with reduced CCFN had 35% (PA2011) and 45% (PA2012) greater shoot biomass, and 38% (PA2011) and 114% (PA2012) greater yield than lines with many cell files under water stress (Table 3, Fig 9 C, D, E, F).

In the field across two maize growing environments in Malawi, water stress reduced leaf relative water content by 22% (BU2012) and 25% (CH2012), shoot biomass by 43% (BU2012) and 54% (CH2012), and grain yield by 59% (BU2012) and 53% (CH2012) (Fig. 9). Under water stress genotypes with reduced CCFN had 20% (BU2012) and 19% (CH2012) greater leaf relative water content, 70% (BU2012) and 57% (CH2012) greater shoot biomass, and 93% (BU2012) and 33% (CH2012) greater yield than genotypes with many cell files under water stress (Table 5, Fig. 10)

DISCUSSION

We hypothesized that reduced CCFN would reduce root respiration per unit root length, permitting greater root growth and exploration at depth, thereby enhancing water acquisition, improving plant growth and yield under drought. Our results extend from observations of young plants in greenhouse mesocosms to mature plants in the field in the USA and two environments in Malawi. Our results entirely support our hypotheses: CCFN varied substantially among maize genotypes, and genotypes with reduced CCFN had lower specific root respiration, and under

water stress genotypes with reduced CCFN had greater rooting depth, greater acquisition of deep soil water, better plant water status, greater leaf photosynthesis, better growth, and better yield.

The utility of CCFN was evaluated using diverse sets of genotypes contrasting in CCFN in greenhouse mesocosms, in the field using moveable rainout shelters, and with differential irrigation in Malawi. The greenhouse mesocosms and movable rainout shelters in the field allowed us to simulate terminal drought by the progressive reduction of soil water content (Fig. 10). The mesocosms also permit a detailed analysis of root distribution by depth and root respiration, since entire root systems can be recovered. The field environments in Malawi were natural drought environments in which rainfall varied but was insufficient to meet plant water requirements. The combination of results from the field and mesocosms lends credence to our conclusions, as the field includes variable environmental factors such as soil temperature, biota, and soil physical properties, while mesocosms permit greater environmental control and more detailed measurement of root properties. RILs sharing the same genetic lineage were employed to minimize the effects of genetic interaction, epistasis, and pleiotropy, which may confound the interpretation of results from comparison of unrelated lines (Zhu and Lynch, 2004). CCFN is a quantitative trait associated with multiple genetic loci in maize (Saengwilai, 2013). For evaluation of the utility of quantitative traits such as CCFN, RILs are useful since they permit the comparison of lines differing in CCFN expression among a set of genotypes sharing common parents.

Maize has substantial genetic variation for root architectural and anatomical phenes (Hochholdinger, 2009; Bayuelo-Jiménez et al., 2011; Trachsel et al., 2011; Burton et al., 2013; Lynch, 2013). Genotypic variability observed here was consistent with previous studies which found that CCFN varies in the range from 7-16 for CCFN in *Zea* species (including maize landraces and teosinte) (Burton et al., 2013). Cortical cell files are formed by several successive asymmetric periclinal divisions in the root apical meristem (Baum et al., 2002; Chapman et al., 2003; Lux et al., 2004). It has been documented that the number of such periclinal divisions varies among species, genotypes and root types (Lux et al., 2004; Coudert et al., 2010), which might generate differences in CCFN as observed here. Maize, like other monocots, has no secondary growth in its roots (Esau, 1965). Hence, CCFN variation along the longitudinal axis of a root represent the radial patterning in the root apical meristem. Therefore CCFN variation observed in this study was largely due to genotypic differences. However, the genetic and physiological mechanism of this variation in maize is not yet known and deserves further exploration.

CCFN can be easily observed with a microscope and is therefore amenable to direct phenotypic selection in crop improvement programs. In this study we showed that CCFN measured on young plants from greenhouse mesocosms 30 days after planting were accurate reflections of CCFN measured on mature plants in the field 70 days after planting. We also observed correlation between CCFN measured in the field across two contrasting maize growing environments in Malawi. These results indicate that CCFN was stable across environments in this study.

We have proposed that reduced CCFN may be a useful adaptation to drought by reducing the metabolic costs of soil exploration (Lynch 2013). Previous studies have associated reduction of root respiration with RCA formation (Fan et al., 2003; Zhu et al., 2010). Jaramillo et al. (2013)

found that reduced LCA substantially reduces root respiration in maize. In that study, it was concluded that LCA is a stronger predictor of root respiration than either RCA or root diameter, since it takes into account the differing cortical areas among root classes. Two key determinants of LCA are cell file number and cell size, and altering either one may affect the size of LCA, consequently affecting root metabolic costs. We have recently shown that large cortical cell size in maize is associated with reduced root respiration, and greater root depth, water acquisition, plant growth, and yield under drought (Chimungu et al 2014). As shown in this study, decreasing CCFN from 16 to 8 was associated with a 57% reduction of root respiration (Fig 3). This respiratory pattern may reflect the effect of decreasing the proportion of metabolically active cells in the cortex and increasing the proportion of nonrespiring tissues such as sclerenchyma and xylem vessels.

Root respiration associated with growth, maintenance, and ion uptake are major components of root metabolic costs (Lambers, 1979; van der Werf et al., 1988; Peng et al., 1993; Lambers et al., 2002; Lynch and Ho, 2005). In this study, root respiration was measured in the mature region of the root, therefore, total respiration in this region is primarily the respiration for tissue maintenance. Root construction cost is assumed to be a one-time cost that occurs when the root is formed (Yanai et al., 1995). In contrast, maintenance costs accumulate over time, and can quickly exceed initial construction costs, and therefore maintenance cost are important determinants of root metabolic cost (Eissenstat and Yanai, 1997; Lynch and Ho, 2005; Lynch and Brown, 2008). For example Postma and Lynch (2011) reported that maize plants without maintenance respiration had up to 72% greater growth under nutrient limiting conditions than plants with root maintenance respiration. The importance of maintenance costs is clearly shown by the case of root cortical aerenchyma (RCA), which reduces the maintenance respiration and nutrient content of mature root tissue by converting living cortical cells to air space. Differential RCA formation among maize genotypes is associated with reduced maintenance respiration of root tissue, which when plants are stressed by suboptimal availability of water, N, P, or K, results in greater root growth, greater acquisition of soil resources, greater plant growth, and greater yield (Fan et al. 2003; Zhu et al. 2010; Postma and Lynch 2010; Postma and Lynch 2011; Saengwilai et al., 2014a). Since RCA is formed in mature regions of the cortex it affects root maintenance costs rather than root construction costs. Although CCFN affects both construction and maintenance costs, we believe that by analogy with RCA, the effects of CCFN on maintenance costs are more important for plant adaptation to stress than effects on construction costs. A more detailed analysis of this issue would be possible using the structural-functional plant model *SimRoot* (Lynch et al., 1997). *SimRoot* is a structural-functional plant model that simulates the three-dimensional architecture and soil resource acquisition of a root system as it develops over time. It is difficult to quantify both construction and maintenance costs in greenhouse and field studies, because of the tightly coupled integration between the two costs. *SimRoot* may provide useful insights in this context by allowing the quantification and independent manipulation of maintenance and construction costs in plants contrasting for CCFN. *SimRoot* has provided such insights in the context of the effects of RCA on maintenance and construction costs in maize (Postma and Lynch 2010; Postma and Lynch 2011)

CCFN was a stronger predictor than SRL for root segment respiration, with a slightly greater coefficient of determination (Table 2). Generally, greater SRL permits more efficient soil exploration (Eissenstat, 1992). SRL is influenced by root diameter as well as root anatomy, or "tissue mass density" (Wahl and Ryser, 2000). However, specific root length varies widely with

environmental conditions and the direction of change in SRL is not always predictable based on resource supply (Eissenstat et al., 2005). In addition, SRL is a coarse metric that aggregates many distinct phenes to provide an overall estimate of mass per unit root length, without indicating how mass varies or the composition and hence energy content of the mass. SRL also does not indicate whether the root mass is living or dead tissue and therefore it is not well correlated with variation for maintenance respiration among root classes and ages. Therefore, CCFN should be a more direct predictor of root respiratory costs than SRL (Table 2) since it takes into account the differing cortical areas which generally have high metabolic rate. For example (Hall et al., 1971) working with maize showed that fresh isolated cortex had greater respiration than fresh steles. CCFN therefore is an important determinant of root metabolic cost. Lynch (2013) proposed that large cortical cells may also substantially reduce root respiration, since larger cells have a higher ratio of vacuolar to cytoplasmic volume and hence reduced respiration per unit of tissue volume. For this reason we propose that the benefit of reduced CCFN should be strongest in roots with small cortical cells.

The benefits of reduced metabolic cost of soil exploration were greater under water stress (Figs. 3, 4, 5, 6, 7, 9, & 10). The greater utility of reduced CCFN under drought is associated with the fact that the genotypes with less costly root tissue had deeper rooting, better access to water and therefore extra carbon gain through photosynthesis, which in turn will increase root growth further, creating a positive feedback for plant growth under water stress. We found that reduced CCFN was associated with increased rooting depth (D_{95}) in the field under water stress, but did not affect rooting depth in well-watered conditions (Fig, 7, Supplemental figure S1 A&B). In addition, our results show that genotypes with reduced CCFN and deeper D_{95} were able to maintain greater RWC in the field and stomatal conductance in mesocosms under water stress than genotypes with many cell files (Fig. 5, 9 A&B, 10 A&B). These results suggest that increased availability of carbon from reduced respiration allows the plant to grow more roots under drought. Root growth in deep soil domains under water stress resulted in increased water acquisition, greater plant water status, and greater photosynthesis, which benefits overall plant growth and yield.

Xylem water reflects the oxygen isotopic composition of water acquired by the plant from the soil as no isotopic fractionation occurs during water uptake and transport (Ehleringer and Dawson, 1992; Dawson and Pate, 1996; Ehleringer et al., 2000). In this study we used natural variation in the isotopic signature of soil water to provide insight into the potential between root depth and water acquisition (Fig 4, 7, & 8). The isotopic signature of soil water observed in this study is determined by evaporation from the soil, precipitation and irrigation. Because soil cores were collected 30 days after the last irrigation or rainfall, the surface soil water was isotopically enriched due to evaporation. For the subsoil water, the isotope signature could be attributed to the combination of the evaporation effect and the isotopic signatures of irrigation water and rainfall, resulting in a gradient of $\delta^{18}O$ with soil depth (Fig. 8). Xylem water $\delta^{18}O$ signatures showed that genotypes with reduced CCFN had lighter isotope signatures and greater dependency on deep soil water than genotypes with many cell files (Table 3). The difference in the depths of root water acquisition between genotypes with reduced CCFN and many cell files could be attributed to their rooting depth (Fig 4 & 7).

The additional benefit of reducing root costs in annual crops like maize is that extra resources from reduced root metabolic demand can contribute to crop yield, by enhancing plant

reproductive growth, since reproduction and roots are competing sinks for current photosynthate. In this study we found that irrespective of maize population, environment, soil type, and trial management, genotypes with reduced CCFN had both greater shoot biomass and grain yield than genotypes with many cell files under water stress (Fig. 6, 9 (C,D,E,F), & 10 (C,D,E,F)). 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.

The physiological utility of a phene may depend on interactions with other phenes in integrated phenotypes (York et al., 2013). These interactions among phenes could result in synergistic or antagonistic effects on resource acquisition. Understanding phene synergisms is essential in developing ideotypes for breeding crops with greater tolerance of edaphic stress. We also recognize that a phene can be beneficial for multiple stresses. Root phenes, such as reduced CCFN, that influence the metabolic cost of soil exploration may be important to plants in low-input systems by increasing rooting depth. Rooting depth is important for the acquisition of mobile nutrients including nitrate and sulfate, particularly in soils with high leaching potential. Evidence for this comes from modelling studies, where it has been shown that deeper roots could significantly improve acquisition of nitrogen (Dunbabin et al., 2004; Postma and Lynch, 2011; Dathe et al., 2013). Reduced CCFN may also affect root hydraulic conductivity, because a smaller radial path is associated with greater hydraulic conductivity and consequently greater water acquisition (Rieger and Litvin, 1999). We anticipate that root radial hydraulic conductivity would increase with reduced CCFN. Reduced CCFN may exhibit tradeoffs when soil hardness restricts root penetration, since the capacity to penetrate hard soil is associated with larger root diameter (Materechera et al., 1992; Bengough et al., 2006; Bengough et al., 2011). Further research is needed to understand these potential tradeoffs and synergisms before the deployment of this trait in crop improvement programs.

In this study we have demonstrated the utility of reduced CCFN for maize under water stress. We propose that the utility of reduced CCFN may be applicable to the acquisition of N in leaching environments, and should be applicable to other plant species, especially graminaceous species lacking secondary root growth, including rice (*Oryza sativa*), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa*), sorghum (*Sorghum bicolor*), millet (*Pennisetum glaucum*) etc.

These results support the hypotheses that phenes and phenes states that reduce the metabolic cost of soil exploration improve the acquisition of limiting soil resources (Lynch and Ho, 2005; Lynch, 2014; Lynch et al., 2014). Such phenes include production of an optimal number of root axes, biomass allocation to metabolically efficient root classes, and reduced tissue respiration (Miller et al., 2003; Jaramillo et al., 2013; Lynch, 2014; Saengwilai et al., 2014b). CCFN is an example of the third category, i.e. an anatomical phene that affects the metabolic costs of soil exploration by affecting tissue respiration. Another example in this category is root cortical aerenchyma (RCA), which destroys living cortical cells. Maize genotypes with abundant RCA have reduced root respiration, greater rooting depth, greater water acquisition under drought (Zhu et al., 2010), greater N acquisition under N limitation (Postma and Lynch, 2011; Saengwilai et al., 2014a), and greater P and K acquisition in soils with suboptimal availability of those resources (Postma and Lynch, 2011). Similarly, maize genotypes with greater cortical cell size (CCS) have less root respiration, greater rooting depth, and greater water acquisition under

drought (Chimungu et al., 2014). The deployment of root phenotypes with greater metabolic efficiency of soil exploration represents a novel, unexploited paradigm to develop crops with greater resource efficiency and resilience (Lynch, 2014).

Materials and methods

Plant materials

Maize genotypes from IBM, NyH populations and advanced lines from the Malawi maize breeding program were utilized in this study. The IBM lines are from the intermated population of B73xMo17 and were obtained from Shawn Kaeppler, University of Wisconsin, Madison, WI, USA (Genetics Cooperation Stock Center, Urbana, IL, USA) and designated as Mo (Supplemental table S1). The NyH lines are from the Ny821xH99 population (Shawn Kaeppler, University of Wisconsin, Madison, WI, USA). Based on previous experiments carried out under optimum growing conditions (Burton, 2010), a subset of 15 IBM lines and 11 NyH lines was selected to assess the phenotypic variation of CCFN and its impact on root respiration (GH1). A set of six IBM lines contrasting in CCFN was used in 2011 experiments (GH2 and PA2011) and another set of six IBM lines also contrasting in CCFN for 2012 experiments (GH3 and PA2012) to evaluate utility of CCFN under water limited conditions (Supplemental table S1). In Malawi, a set of 70 breeding lines was used to assess phenotypic variation of CCFN in Malawian germplasm (MW2011). These lines originated from the Malawi national maize breeding program and were selected to represent a broad range of gene pools. A subset of 33 lines contrasting in CCFN was used in two field experiments (BU2012 and CH2012) to evaluate the utility of CCFN under water limited conditions and across sites in Malawi (Supplemental table S1). In all experiments genotypes were classified as 'reduced' CCFN (≤ 10 cell files) and as 'many' CCFN (≥13 cell files) (Supplemenatal table S1).

Greenhouse experiments

Three experiments were conducted under the same conditions in two consecutive years (GH1,2,3) (Supplemental table S1). The experiments were conducted in a greenhouse at University Park, PA, USA (40°48'N, 77°51'W,) under constant conditions (14-h/10-h day/night: 23°C/20°C day/night: 40-70% relative humidity), with maximum illumination of 1200 μmol photons m^{-2} s^{-1} and additional light was provided when necessary with 400-W metal-halide bulbs (Energy Technics, York, PA, USA). Plants were grown in mesocosms (Supplemental figure S2) consisting of PVC cylinders 1.5 m in height by 0.15 m in diameter, with plastic liners made of 4-mil (0.116-mm) transparent hi-density polyethylene film, which was used to facilitate root sampling. The growth medium consisted of (by volume) 50% commercial grade sand (Quikrete Companies Inc. Harrisburg, PA, USA), 35% vermiculite (Whittemore Companies Inc., Lawrence, MA, USA), 5% Perlite (Whittemore Companies Inc., Harrisburg, PA, USA), and 10% topsoil (Hagerstown silt loam top soil (fine, mixed, mesic Typic Hapludalf)). Mineral nutrients were provided by mixing the media with 70g per column of OSMOCOTE PLUS fertilizer consisting of (in %); N (15), P (9), K (12), S (2.3), B (0.02) Cu (0.05), Fe (0.68), Mn (0.06), Mo (0.02), and Zn (0.05) (Scotts-Sierra Horticultural Products Company, Marysville, Ohio, USA) for each column. The seeds were germinated by placing them in darkness at 28 ± 1 °C in a germination chamber for two days prior to transplanting two seedlings per mesocosm, thinned to one uniform seedling per mesocosm 5 days later after planting.

At harvest, the shoot was removed, and the plastic liner was pulled out of the PVC column and placed on a washing bench. The plastic liner was cut open and the roots were washed carefully by rinsing the media away with water. This allowed us to recover the entire plant root system. Samples for root respiration measurement were collected 10-20 cm from the base of three representative second whorl crown roots per plant. Root respiration (CO_2 production) was measured using an infrared gas analysis system (LI-COR 6400 Biosciences, Lincoln, NE, USA) equipped with a custom 56 ml closed chamber of plastic tubing (1.5 cm diameter) having connection points sealed with silicon grease. The change in CO_2 concentration in the chamber was monitored for 3 minutes. During the time of measurement the chamber was placed in a temperature controlled water bath at 27± 1 °C to maintain constant temperature. Following respiration measurements, root segments were preserved in 75% ethanol for anatomical analysis as described below.

Root length distribution was measured by cutting the root system into 7 segments of 20 cm depth increments. Roots from each increment were spread in a 5 mm layer of water in transparent plexiglass trays and imaged with a flatbed scanner equipped with top lighting (Epson Perfection V700 Photo, Epson America, Inc. USA) at a resolution of 23.6 pixel mm^{-1} (600 dpi). Total root length for each segment was quantified using WinRhizo Pro (Regent Instruments, Québec, Canada). Following scanning the roots were dried at 70^{o}C for 72 hours and weighed. To summarize the vertical distribution of the root length density we used the D_{95} (Schenk and Jackson, 2002), i.e. the depth above which 95 % of the root length was located in the column or soil profile. Specific root length was calculated by dividing root length with corresponding weight.

Root segments were ablated using laser ablation tomography (LAT) (Hall *et al,* unpublished) to obtain images for anatomical analysis. In brief, LAT is a semi-automated system that uses a laser beam (Avia 7000, 355 nm pulsed laser) to vaporize or sublimate the root at the camera focal plane ahead of an imaging stage. The sample is incremented, vaporized or sublimated, and imaged simultaneously. The cross-section images were taken using a Canon T3i camera (Canon Inc. Tokyo, Japan) with 5X micro lens (MP-E 65 mm) on the laser-illuminated surface. Root images were analyzed using *RootScan,* an image analysis tool developed for analyzing root anatomy (Burton et al., 2012). CCFN was determined from three different images per root segment. CCFN was obtained by counting the cell layers from the epidermis to the endodermis.

Experiment I (GH1)

The aim of this experiment was to assess the relationship between phenotypic variation for CCFN and root respiration. The experiment was laid out as a randomized complete block design (RCBD) replicated three times with time of planting as a blocking factor. A set of 26 genotypes was planted in the mesocosms and water stress was imposed by withholding water starting 14 days after planting. Plants were harvested for root respiration measurements and anatomical analysis 35 days after planting.

Experiment II (GH2) and III (GH3)

Two experiments were conducted, one in Fall 2011 (GH2) and Summer 2012 (GH3) and were laid out using as a randomized complete block design (RCBD), replicated four times with time of planting as a blocking factor. Planting was staggered by seven days. A set of six genotypes contrasting in CCFN was planted in each experiment (Supplemenatal table S1). These genotypes

were selected based on phenotypes from previous experiments (Burton and Lynch, 2010; Chimungu, 2014). In both experiments, the irrigated mesocosms (control) each received 200 ml of water every other day, to replenish water lost by evapotranspiration, and in stressed mesocosms, water application was withheld starting 5 days after planting to allow the plants to exploit residual moisture to simulate terminal drought.. Leaf gas exchange of the third fully expanded leaves was measured with a LI-6400 Infrared Gas Analyzer (LI-COR, Lincoln, NE, USA) using a red-blue light at PAR intensity of 1200 μmol photons m^{-2} s^{-1} and constant CO_2 concentration of 400 ppm 28 days after planting. The measurements were done between 9:00 and 11:00h. Plants were harvested 30 days after planting for root respiration measurements, root growth distribution and shoot biomass. The dry matter of the shoot and root were measured after drying at 70^{o}C for 72 h and root length distribution was determined as described above.

Field experiments

Assessing phenotypic variation of CCFN (MW2011)

The experiment was conducted at Bunda College research farm, Lilongwe, Malawi (33^{o}48'E, 14^{o}10'S,) in 2011 under optimal conditions (i.e. the plots were rainfed but only rarely were they severely moisture stressed). The soil is Lilongwe series sandy clay loam (Oxic Rhodustalf). The experiment was arranged as randomized complete block design (RCBD) with three replications. Each plot consisted of a single 6 m long row with 25 plants. Root crowns were excavated by 'shovelomics' (Trachsel et al., 2010). In brief, roots were excavated by removing a soil cylinder 30-40 cm in diameter with the shoot at its center and a depth of 20-30 cm. The excavated root crowns were shaken briefly to remove a large fraction of the soil adhering to the root crown. The root crowns were immersed in soapy water for 5-10 minutes in order to facilitate removal of the remaining soil. Three 8-cm root segments were collected 10-20 cm from the base of a representative second whorl crown root of each plant, and used to assess CCFN. The segments were preserved in 75% ethanol before being processed as described above.

Utility of CCFN under water stress

In the USA two experiments were conducted in rainout shelters (Supplemental figure S3) located at the Russell E. Larson Agricultural Research Center in Rock Springs, PA, USA (40°42′ N, 77°57′ W,), during the summer of 2011 (PA2011) and 2012 (PA2012). In Malawi experiments were conducted at the Chitala Agriculture Research station of the Ministry of Agriculture, Salima, Malawi (13^{o}28'S, 33^{o}59'E) (CH2012), and the Bunda Research farm of the Lilongwe University of Agriculture and Natural Resources (14^{o}10'S, 33^{o}48'E,) (BU2012). The soil is a Hagerstown silt loam (fine, mixed, mesic Typic Hapludalf) in Rock Springs, and sandy clay loam (Oxic Rhodustalf) both at Chitala and Bunda. The experiments (PA2011, PA2012, CH2012 and BU2012) were arranged as a randomized complete block split plot design with four replications. The main plots were composed of two moisture regimes and the subplots contained genotypes contrasting in CCFN in each experiment. The experiments were hand-planted on 15th June 2011 and 25th June 2012 in Rock Springs and 3rd and 4th September in Bunda and Chitala respectively. In Rock Springs, each subplot consisted of three rows, with each row being 2.5 m long, with 25 cm between plants and 75 cm between rows. In Malawi the experiments were planted in single 6 m row plot with 25 cm and 75 cm spacing between planting stations and rows respectively. The drought treatment was initiated starting 35-40 days after planting using an automated rainout shelter in Rock Springs and by withholding water application in Malawi. The

shelters (10 by 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 non-sheltered control plots were rainfed and drip-irrigated when necessary to maintain the soil moisture close to field capacity throughout the growing season. At each location, the recommended fertilizer rate was applied before planting. Soil water content for both well watered and water stressed treatments was monitored regularly during the experiment (Fig 11). Soil water content was monitored using the TRIME FM system (IMKO Micromodultechnik GmbH, Ettlingen, Germany) at three depths (20, 35 and 50 cm) at six points inside the shelter and three points outside the rainout shelter. Seven readings were taken between 30 to 120 days after planting.

Plant measurements

In all field experiments, midday leaf relative water content (RWC) was measured and used as a physiological indicator of plant water status. To measure leaf RWC, fresh leaf discs (3 cm in diameter) were collected from the third fully expanded leaf for three representative plants per plot 60 days after planting and weighed immediately to determine fresh weight (FW). After which the discs were immediately hydrated to full turgidity (6 h) by soaking them distilled water. Following soaking, the discs were blotted dry and again weighed to determine turgid weight (TW). Discs were then oven dried at 70^{o}C for 72 h, and dry weight (DW) was determined. Leaf RWC was calculated according to the equation: $RWC = 100[(FW - DW)/(TW - DW)]$.

In PA2011 and PA2012 soil cores were collected 80 days after planting to determine root distribution in the soil profile. A soil coring tube (Giddings Machine Co., Windsor, CO, USA) 5.1 cm in diameter and 60 cm long was used for sampling, the core was taken midway between the plants within a row. The cores were sectioned into 6 segments of 10 cm depth increments and washed. Subsequently the washed roots were scanned using a flatbed scanner (Epson, Perfection V700 Photo, Epson America, Inc. USA) at a resolution of 23.6 pixel mm^{-1} (600 dpi) and analyzed using image processing software WinRhizo Pro (Regent Instruments, Québec, Canada). Root distribution in the soil profiles was calculated as described above.

Shoot and roots were evaluated 75 days after planting. To accomplish this, three representative plants in each plot were cut at soil level. The collected shoot material was dried at 70^{o}C for 72 hours and weighed. Root crowns were excavated by 'shovelomics' (Trachsel et al., 2010). Three 8-cm root segments were collected 10-20 cm from the base of a representative second whorl crown root of each plant for anatomical analysis. The segments were preserved in 75% alcohol before being processed as described above. At physiological maturity grain yield was collected each plot.

Soil and plant sampling of $\delta^{18}O$ analysis

In PA2011, soil samples were collected adjacent to plants in the rainout shelter 65 days after plant using 5 cm diameter soil core. Soil cores were taken to the maximum achievable depth of 60 cm. The cores were immediately separated into 10 cm increments; 10, 20, 30, 40 50, and 60 cm. The corresponding maize stems were collected at the same time when soil was sampled, approximately 8-10 cm of the stem was collected just aboveground level and the epidermis was immediately removed. Soil and maize stem samples were put in a snap vials, sealed with parafilm to prevent evaporation, and refrigerated immediately. Cryogenic vacuum distillation

(West et al., 2006; Koeniger et al., 2010) 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 nitrogen, and then both tubes were evacuated by vacuum pump to create a closed U-shape configuration. After that, the tube containing sample was heated, while the collection tube was still immersed in liquid nitrogen to catch the vapor. Samples were weighed and oven dried after extraction to ensure the extraction time was sufficient to vaporize all the water in samples. The water samples were analyzed at the Penn State Institutes of Energy and the Environment (PSIEE). Stable isotopic analyses were performed using a PICARRO L2130-i δD/δ18O Ultra High Precision Isotopic Water Analyzer (PICARRO Inc, CA, USA). Results were expressed as parts per thousand deviations from the Vienna Standard Mean Ocean Water (VSMOW). To determine the percent contribution of soil water at depth to the signature of water within the plant's xylem, 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 plant xylem water signature. The fractional increment was set at 1%, and tolerance at 0.1.

Data analysis

The data from each year were analyzed separately considering that different sets of genotypes were used. For greenhouse data, for comparisons of genotypes, irrigation levels and their interaction effects a two-way analysis of variance (ANOVA) was used. Field data were analyzed as randomized complete block split plot designs to determine the presence of significant effects due to irrigation level, genotype and interaction effects on the measured and calculated parameters. Mean separation of genotypes for the different parameters was performed by a Tukey-HSD test. Unless otherwise noted, $HSD_{0.05}$ values were only reported when the F test was significant at $P \leq 0.05$. Linear regression analysis was used to establish relationships between CCFN and measured and calculated parameters.

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Figure Legends

Figure 1. Cross section images showing genotypic differences in root cortical cell file number (CCFN) in maize: **(A)** 8 cell files and **(B)** 14 cell files. Cross sections are from standard reference tissue collected 10-20 cm from the base of the second nodal crown root at 70 days after planting from field-grown plants. Images obtained from laser ablation tomography.

Figure 2. Genetic variation for root cortical cell file number (CCFN) in maize **(A)** selected IBM lines (GH1) and **(B)** recombinant inbred lines from the Malawi maize breeding program (MW2011). The data shown are standard reference tissue collected from 10-20 cm from the base of the second nodal crown root. In the greenhouse roots were sampled 30 days after planting and in the field at 70 days after planting.

Figure 3. Correlation of root respiration per unit length and cortical cell file number (CCFN) for GH1-NyH ($y = 1.7x-0.31$, $r^2 = 0.46$, $p = 0.009$), GH1-IBM ($y = 1.9x-0.49$, $r^2 = 0.46$, $p = 0.009$), GH2 ($y = 0.8x-4.32$, $r^2 = 0.59$, $p = 0.001$) and in GH3 ($y = 2.11x-3.09$, $r^2 = 0.52$, $p = 0.018$) in the mesocosms 30 days after planting. Each point is the mean of at least three measurements of respiration from the second nodal crown root per genotype.

Figure 4. Correlation of root depth (D_{95}) and cortical cell file number for GH2WS ($y = 113.4-2.4x$, $r^2 = 0.57$, $p < 0.001$), for GH2WW ($y = 120.6 + 0.003x$, $r^2 = 0.003$,ns), GH3WS ($y = 124.9-4.2x$, $r^2 = 0.41$, $p < 0.01$), and GHWW ($y = 138.6 – 2.9x$, $r^2 = 0.10$, ns) in greenhouse mesocosms 30 days after planting. Data include water stressed (WS) and well watered (WW) conditions. D_{95} measures the depth above where 95% of root length is present.

Figure 5. Carbon dioxide exchange rate (A) and stomatal conductance (B) of six genotypes with contrasting CCFN 28 days after planting in well watered (WW) and water stressed (WS) conditions in greenhouse mesocosms (GH3). Bars represent means ±SE of four replicates per treatment. Bars with the same letters are not significantly different ($p<0.05$).

Figure 6. Shoot dry weight of genotypes contrasting in CCFN 30 days after planting in well watered (WW) and water stressed (WS) conditions in mesocosms (**A),** GH2 and (**B),** GH3. Bars show means ±SE of four replicates per treatment. Bars with the same letters are not significantly different within the same panel ($p<0.05$).

Figure 7. Correlation of root depth (D_{95}) and root cortical cell file number in rainout shelters at Rock Springs, PA, USA; for PA2011WS ($y = 66.7 – 1.59x$, $r^2 = 0.59$, $p < 0.01$), PA2012WW ($y = 49.89 + 0.02x$, $r^2 = 0.002$, ns), PA2012 ($r^2 = 0.42$, $p < 0.05$), and PA2012WW ($y = 47.4 + 0.02x$, $r^2 = 0.05$, ns) 80 days after planting. Data include water stressed (WS) and well watered (WW) conditions. D_{95} measures the depth above where 95% of root length is present in the soil profile.

Figure 8. Mean oxygen isotope composition ± S.E. of soil water along the soil profile in the rainout shelters (PA2011). Sampling was done 65 days after planting. Values are the means ±SE of 3 observation points in the rainout shelters.

Figure 9. Performance of maize lines contrasting in CCFN in water stress (WS) and well watered (WW) conditions in rainout shelters at Rock Springs, PA, USA. Leaf relative water content at 60 days after planting (A), PA2011 and (B), PA2012; shoot biomass per plant at 70 days after planting (C), PA2011 and (D), PA2012; and yield per plant (E), PA2011 and (F), PA2012. Bars show means ±SE of four replicates per treatment. Bars with the same letters are not significantly different within the same panel ($p<0.05$).

Figure 10. Performance of maize lines contrasting in CCFN in the field in water stress (WS) and well watered (WW) conditions at two field sites in Malawi. Leaf relative water content at 60 days after planting (A), Bunda and (B), Chitala; shoot biomass per plant at 70 days after planting (C), Bunda and (D,) Chitala; yield per plant (E), Bunda, (F), Chitala. Bars show means ±SE (n=16-18) of four replicates per treatment and trait. Bars with the same letters are not significantly different within the same panel ($p<0.05$).

Figure 11. Effect of drought treatment on soil volumetric water content at 15-, 30-, and 50-cm depths both in well watered (WW) and water stressed (WS) conditions in the rainout shelters at Rock Springs, PA, USA (PA2012). Points are means ±SE of six measurements in the rainout shelter and three measurements in well watered plots. Terminal drought was imposed in WS plots beginning at 30 DAP.

Table Captions

Table 1. Summary of analysis of variance for respiration, root depth (D_{95}), stomatal conductance, carbon dioxide exchange rate and shoot biomass as influenced by soil moisture regime (treatment) and genotype in the greenhouse mesocosms experiments (GH2 and GH3). The associated F-values and probabilities (ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001) are shown

Table 2. Summary of linear models (y = a + bx) of root respiration as predicted by root cortical cell file number (CCFN) and specific root length (SLR) in 15 IBM RILs (GH1-IBM) and 11 NyH RILs (GH1-NyH) under moderate drought in the greenhouse (GH1)

Table 3. Summary of analysis of variance for leaf relative water content (RWC) (%), shoot biomass and yield as influenced by soil moisture regime (treatment) and genotype in the rainout shelters at Rock Springs, PA, USA (PA2011 and 2012). The associated F-values and probabilities (ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001) are shown.

Table 4. Means of $\delta^{18}O$ of xylem water ± SE measured for six genotypes contrasting in CCFN under water stress 65 days after planting. Proportional water use by depth from different soil layers where deep is the aggregate of three deep soil layers (Fig 8) calculated using multi-source mixing model analysis (Phillips et al., 2005).

Table 5. Summary of analysis of variance for root depth (D95), leaf relative water content (RWC) (%), shoot biomass and yield as influenced by soil moisture regime (treatment) and genotype in the field Malawi (Bunda and Chitala). The associated F-values and probabilities (ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001) are shown.

Supplemental figures

Supplemental figure S1. Root length density at different soil depths for genotypes with reduced cortical cell file (FF) and many cortical cell file number (MF) under water stressed (WS) and well watered (WW) conditions in the greenhouse (GH1) (A) and in the field (PA2011) (B) with corresponding D_{95}. D_{95} measures the depth above where 95% of root length is present.

Supplemental figure S2. Greenhouse mesocosms consisting of PVC cylinders 1.5 m in height by 0.15 m in diameter, with plastic liners made of 4-mil (0.116-mm) transparent hi-density polyethylene film, which was used to facilitate root sampling (A) just after planting and (B) 30 days after planting

Supplemental figure S3. Automated rainout shelter facility at Rock Springs, PA, USA. The shelter are covered with plastic mounted on a precipitation-activated rail system, so that at the onset of precipitation each roof shields an area of 10 x 30 m. Adjacent irrigated plots provide unstressed comparisons. (A) well-watered plots (blue arrow) and water stressed plots (red arrow) early the season, (B) shelter covering water stressed just after rain, and (C) well watered plots adjacent to the shelter.

Supplemental tables

Supplemental table S1. Overview of the experiments, treatments, and plant material used. Genotypes were classified as 'reduced CCFN' ≤ 10 cell files and 'many CCFN' ≥13 cell files.

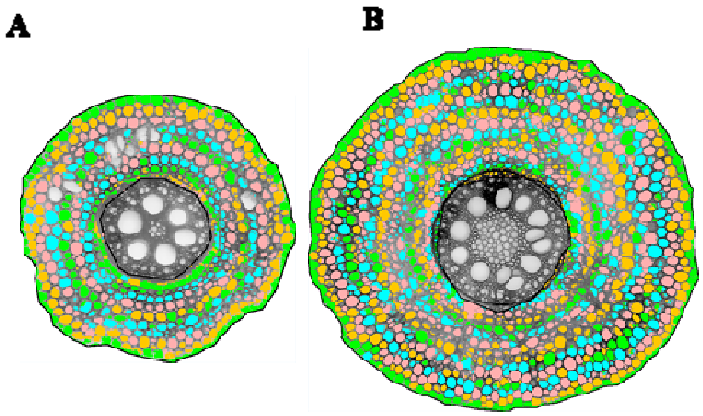

Figure 1. Cross section images showing genotypic differences in root cortical cell file number (CCFN) in maize: **(A)** 8 cell files and **(B)** 14 cell files. Cross sections are from standard reference tissue collected 10-20 cm from the base of the second nodal crown root at 70 days after planting from field-grown plants. Images obtained from laser ablation tomography.

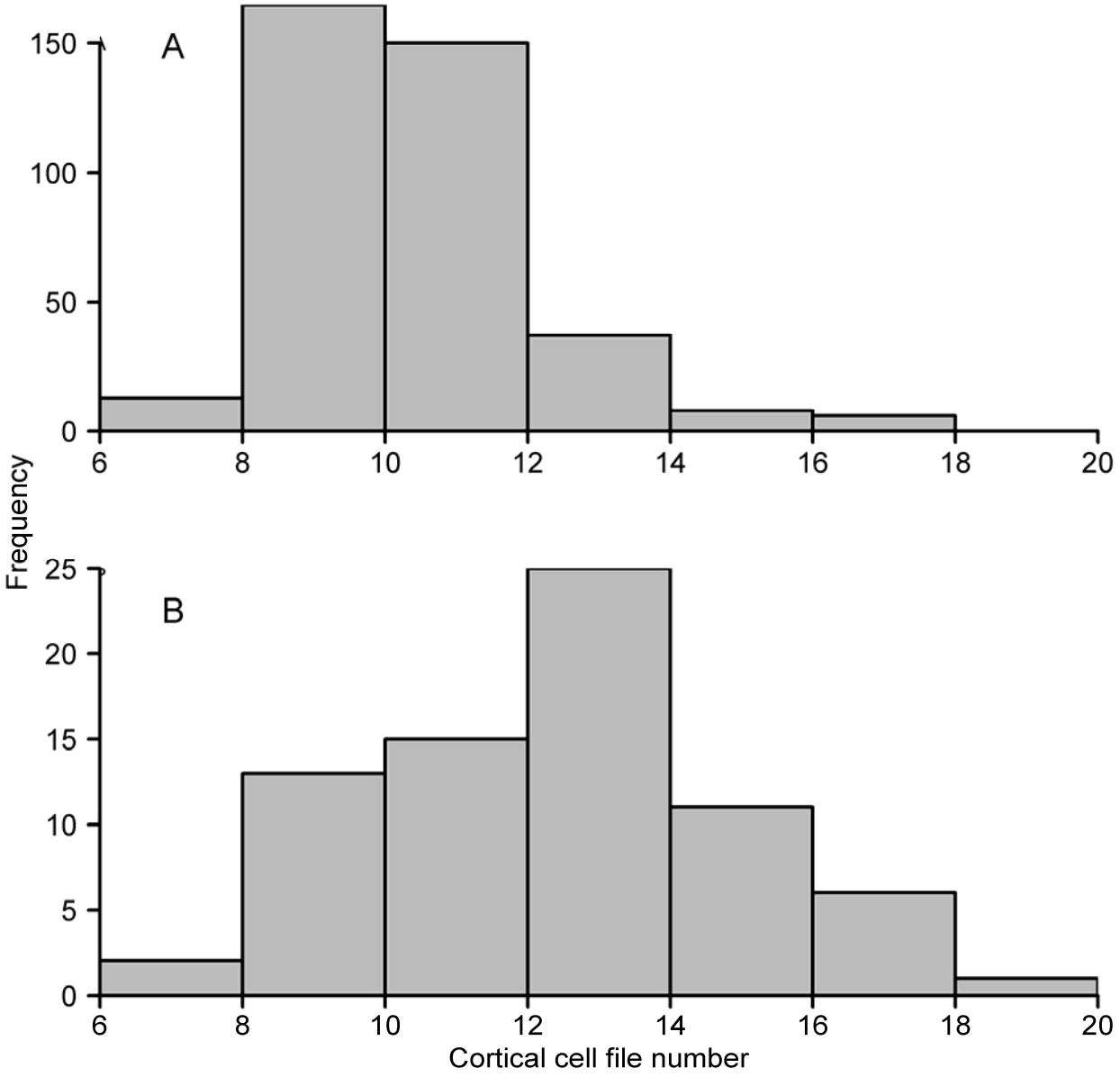

Figure 2. Genetic variation for root cortical cell file number (CCFN) in maize **(A)** selected IBM lines (GH1) and **(B)** recombinant inbred lines from the Malawi maize breeding program (MW2011). The data shown are from standard reference tissue collected from 10-20 cm from the base of the second nodal crown root. In the greenhouse roots were sampled 30 days after planting and in the field at 70 days after planting.

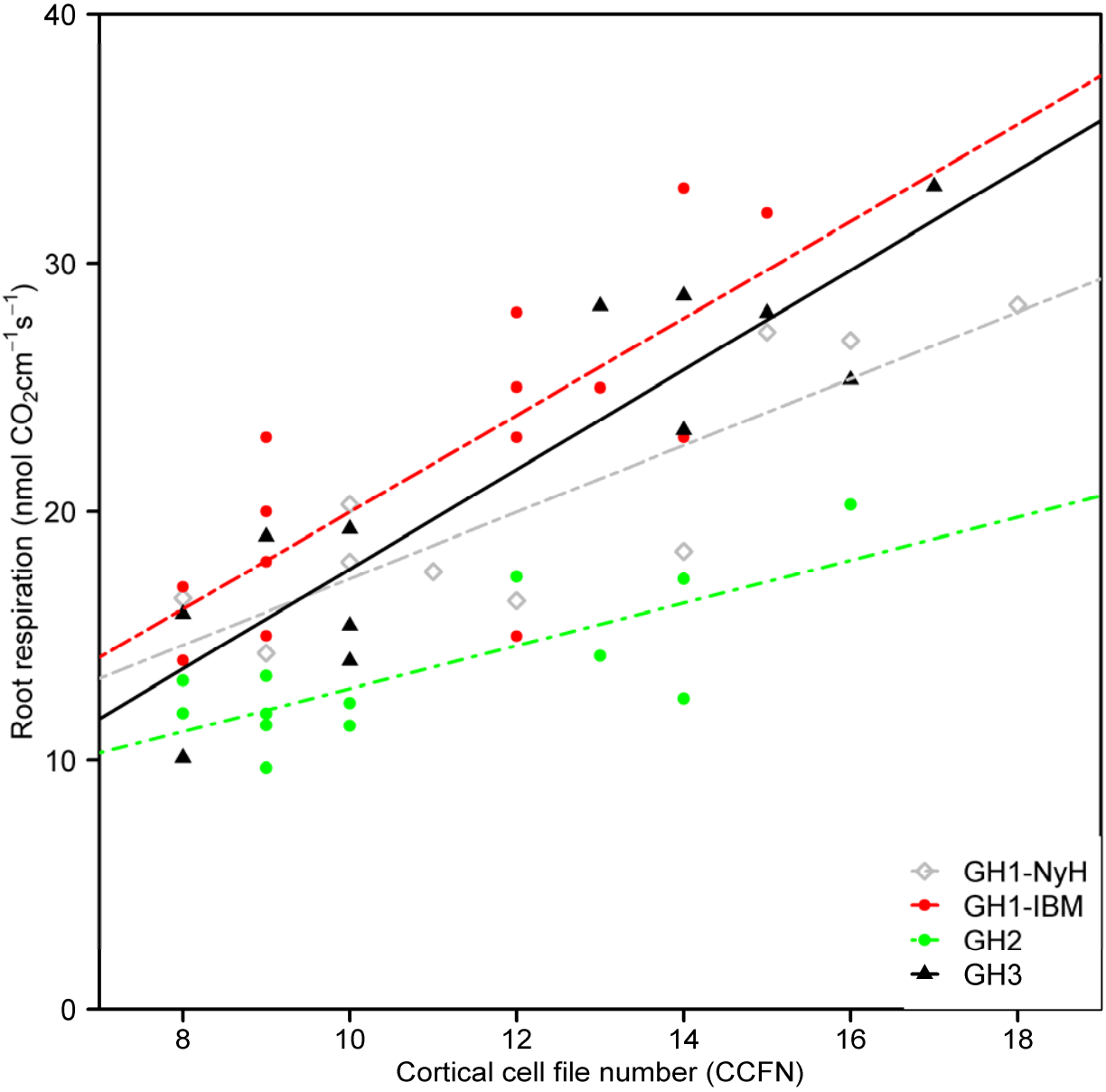

Figure 3. Correlation of root respiration per unit length and cortical cell file number (CCFN) for GH1-NyH (y =1.7x-0.31, r^2 = 0.53, *p* = 0.012), GH1-IBM (y = 1.9x-0.49, r^2 = 0.56, *p* = 0.009), GH2 (y = 0.8x-4.32, r^2 = 0.59, *p* = 0.001) and in GH3 (y = 2.11x-3.09, r^2 = 0.52, *p* = 0.018) in the mesocosms 30 days after planting. Each point is the mean of at least three measurements of respiration from the second nodal crown root per genotype.

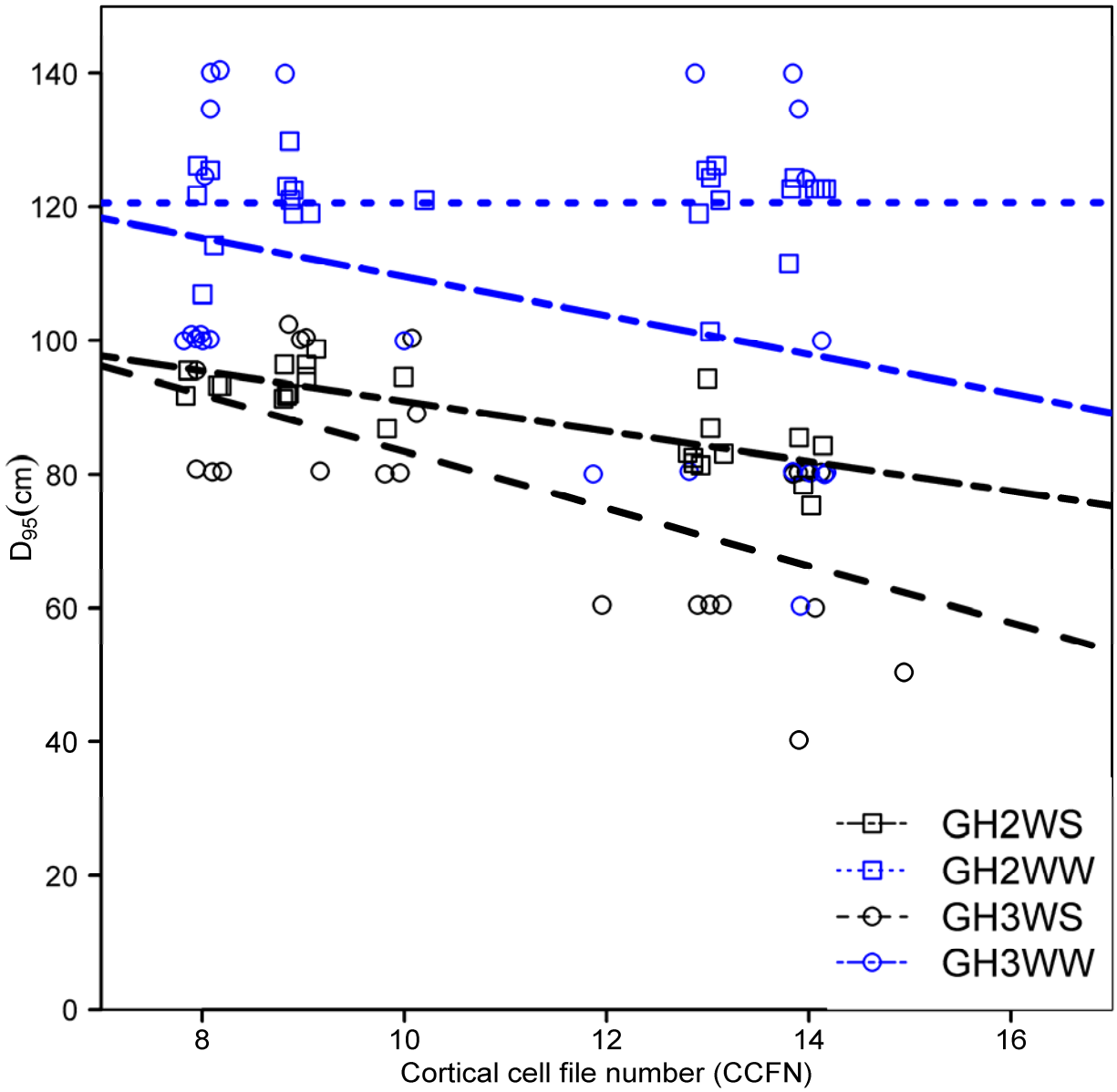

Figure 4. Correlation of root depth (D_{95}) and cortical cell file number for GH2WS (y = 113.4-2.4x, $r^2 = 0.57$, $p < 0.001$), for GH2WW (y = 120.6 + 0.003x, $r^2 = 0.003$,ns), GH3WS (y = 124.9-4.2x, $r^2 = 0.41$, $p < 0.01$), and GHWW (y = 138.6 – 2.9x, $r^2 = 0.10$, ns) in greenhouse mesocosms 30 days after planting. Data include water stressed (WS) and well watered (WW) conditions. D_{95} measures the depth above where 95% of root length is present.

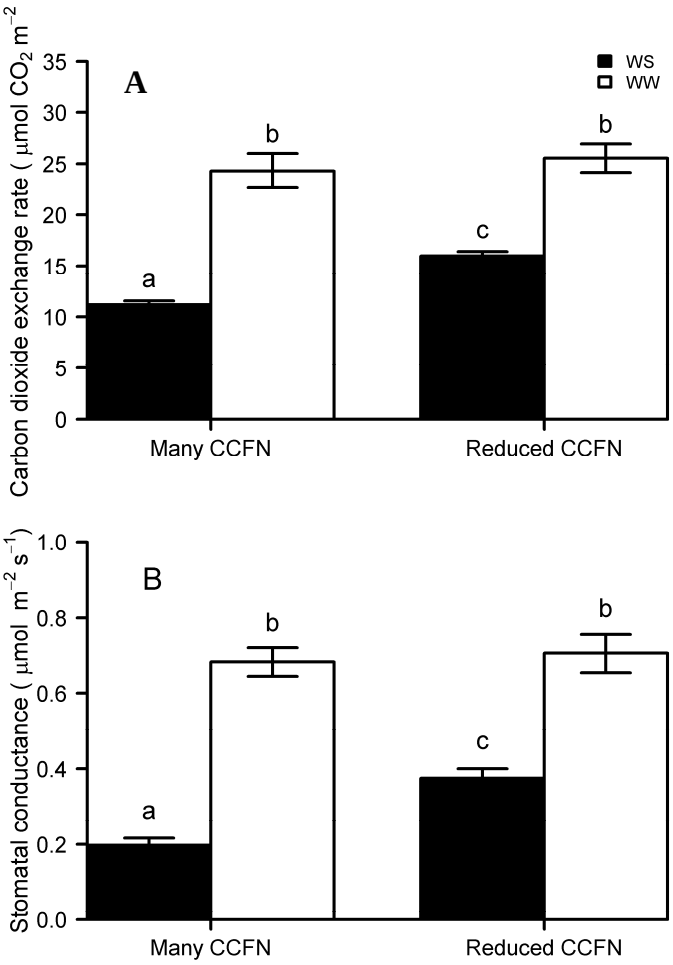

Figure 5. Carbon dioxide exchange rate (A) and stomatal conductance (B) of six genotypes with contrasting CCFN 28 days after planting both in well watered (WW) and water stressed (WS) conditions in the mesocosms (GH3). Bars represent means ±SE of four replicates per treatment. Bars with the same letters are not significantly different ($p<0.05$).

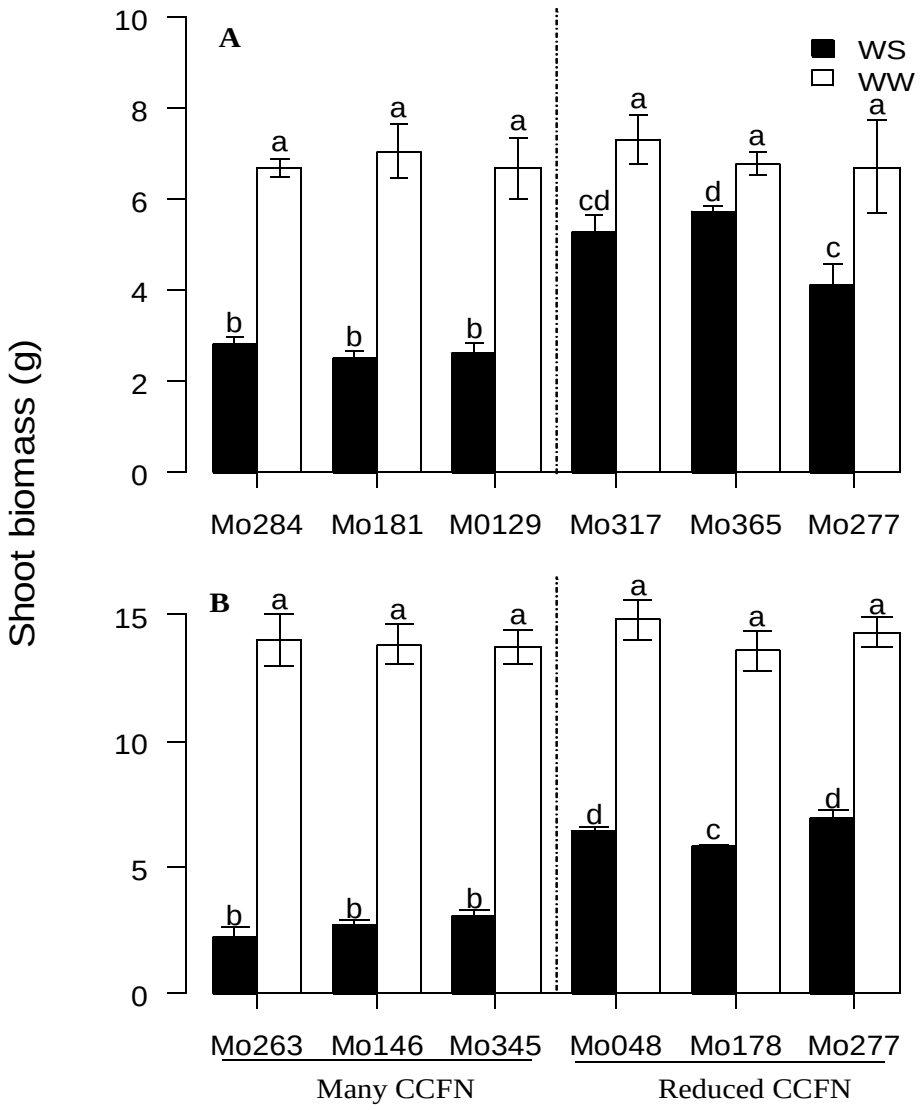

Figure 6. Shoot dry weight of genotypes contrasting in CCFN 30 days after planting in well watered (WW) and water stressed (WS) conditions in greenhouse mesocosms (**A),** GH2 and (**B),** GH3. Bars show means ±SE of four replicates per treatment. Bars with the same letters are not significantly different within the same panel ($p<0.05$).

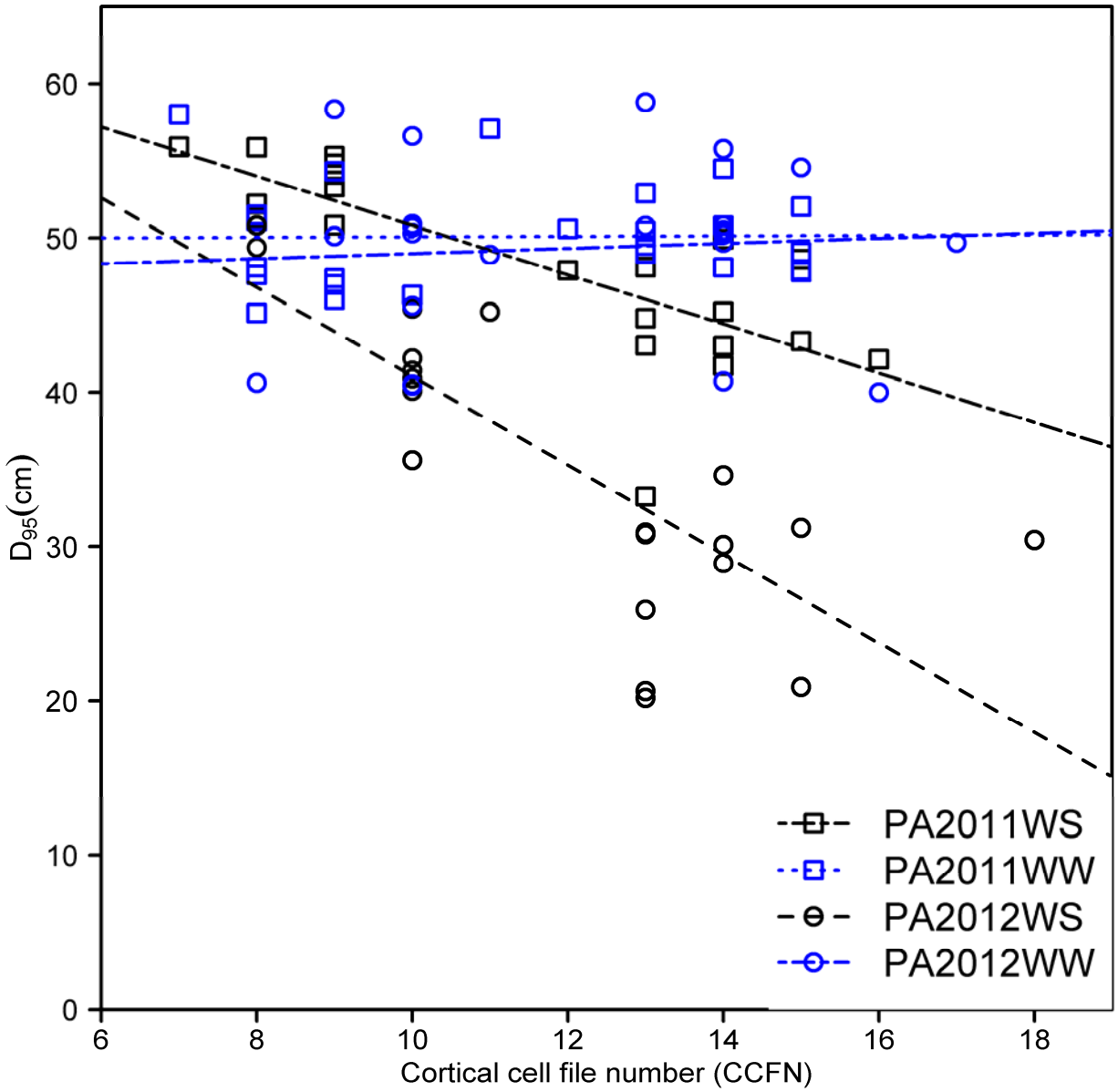

Figure 7. Correlation of root depth (D_{95}) and root cortical cell file number for PA2011WS (y = 66.7 – 1.59x, r^2 = 0.59, p < 0.01), PA2012WW (y = 49.89 + 0.02x, r^2 = 0.002, ns), PA2012 (r^2 = 0.42, p <0.05), and PA2012WW (y = 47.4 + 0.02x, r^2 = 0.05, ns) 80 days after planting. Data include water stressed (WS) and well watered (WW) conditions. D_{95} measures the depth above where 95% of root length is present in the soil profile.

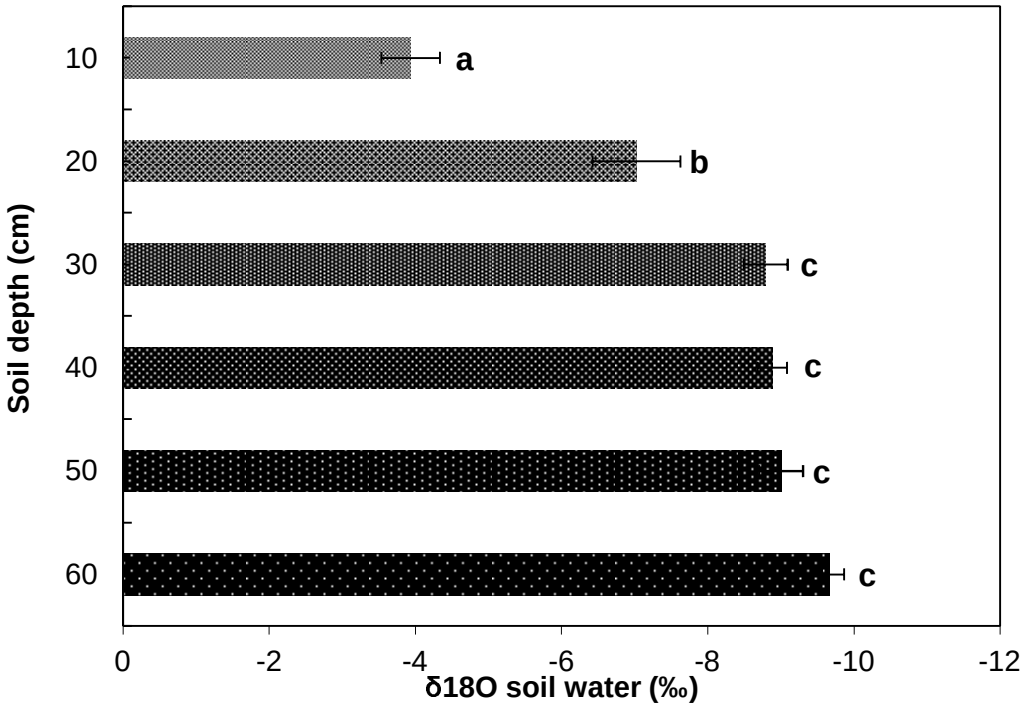

Figure 8. Mean oxygen isotope composition ± S.E. of soil water along the soil profile in the rainout shelters (PA2011). Sampling was done 65 days after planting. Values are the means ±SE of 3 observation points in the rainout shelters.

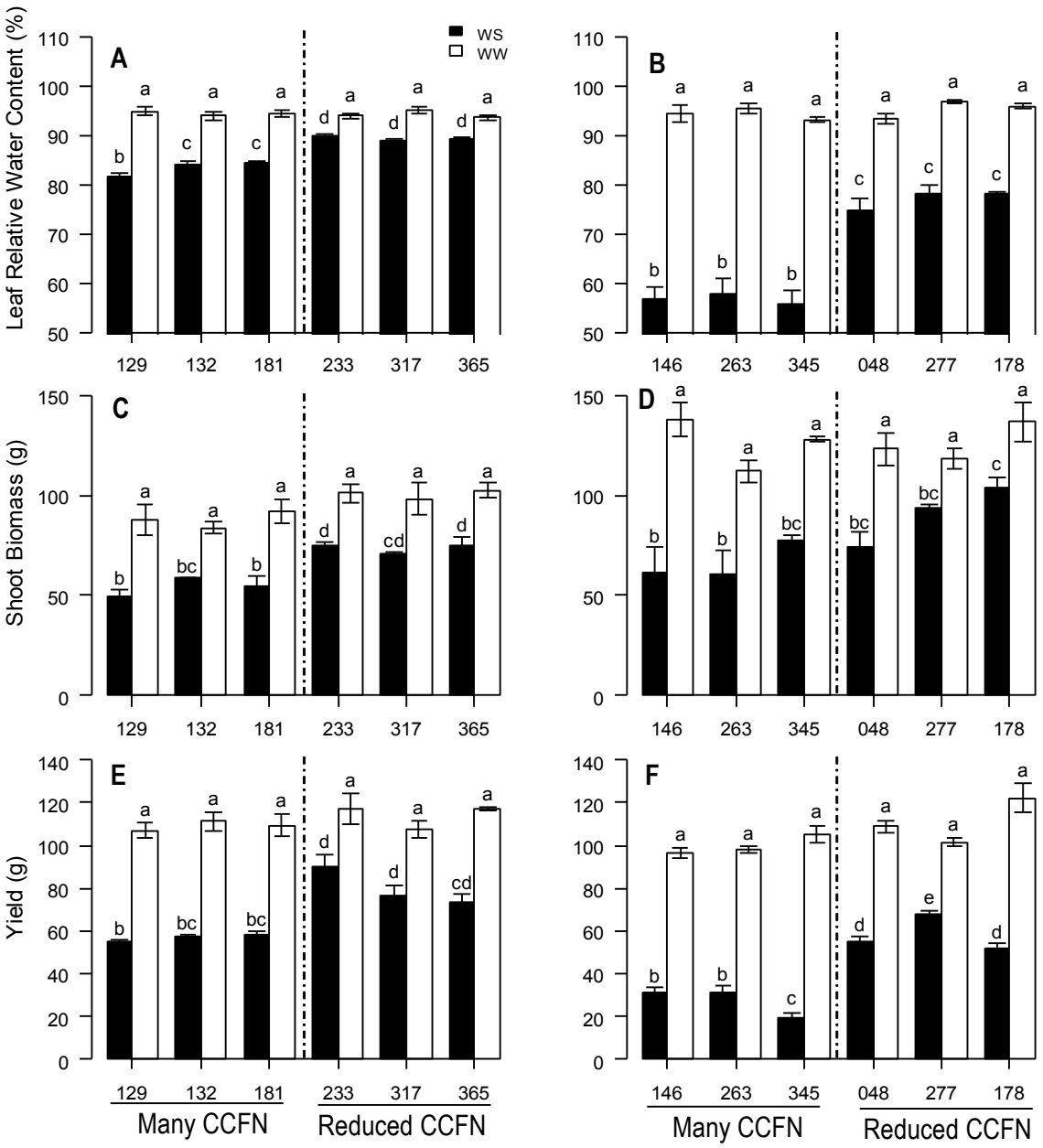

Figure 9. Performance of maize lines contrasting in CCFN in water stress (WS) and well watered (WW) conditions in rainout shelters at Rock Springs, PA, USA. Leaf relative water content at 60 days after planting (A), PA2011 and (B), PA2012; shoot biomass per plant at 70 days after planting (C), PA2011 and (D), PA2012; and yield per plant (E), PA2011 and (F), PA2012. Bars show means ±SE of four replicates per treatment. Bars with the same letters are not significantly different within the same panel ($p<0.05$).

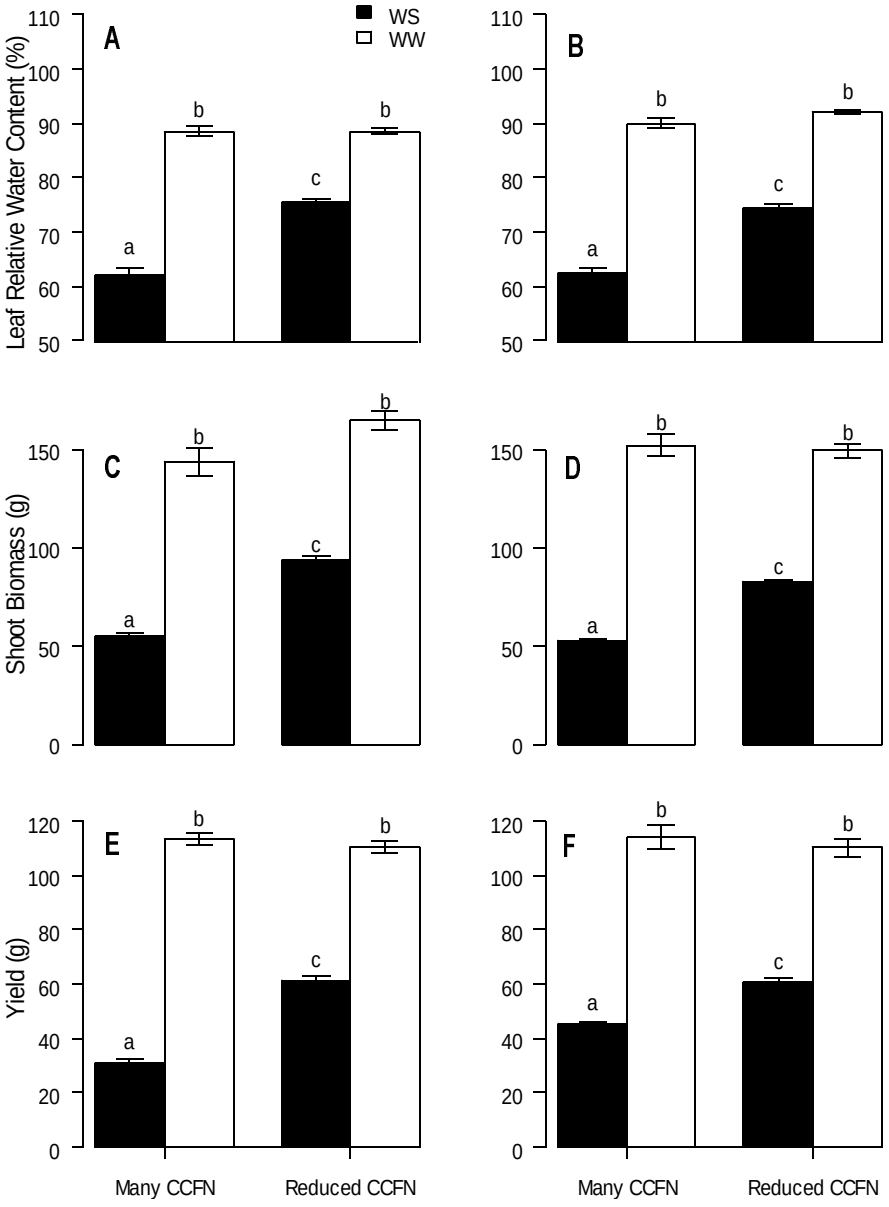

Figure 10. Performance of maize lines contrasting in CCFN in the field in water stress (WS) and well watered (WW) conditions at two field sites in Malawi. Leaf relative water content at 60 days after planting (A), Bunda and (B), Chitala; shoot biomass per plant at 70 days after planting (C), Bunda and (D,) Chitala; yield per plant (E), Bunda, (F), Chitala. Bars show means ±SE (n=16-18) of four replicates per treatment and trait. Bars with the same letters are not significantly different within the same panel ($p<0.05$).

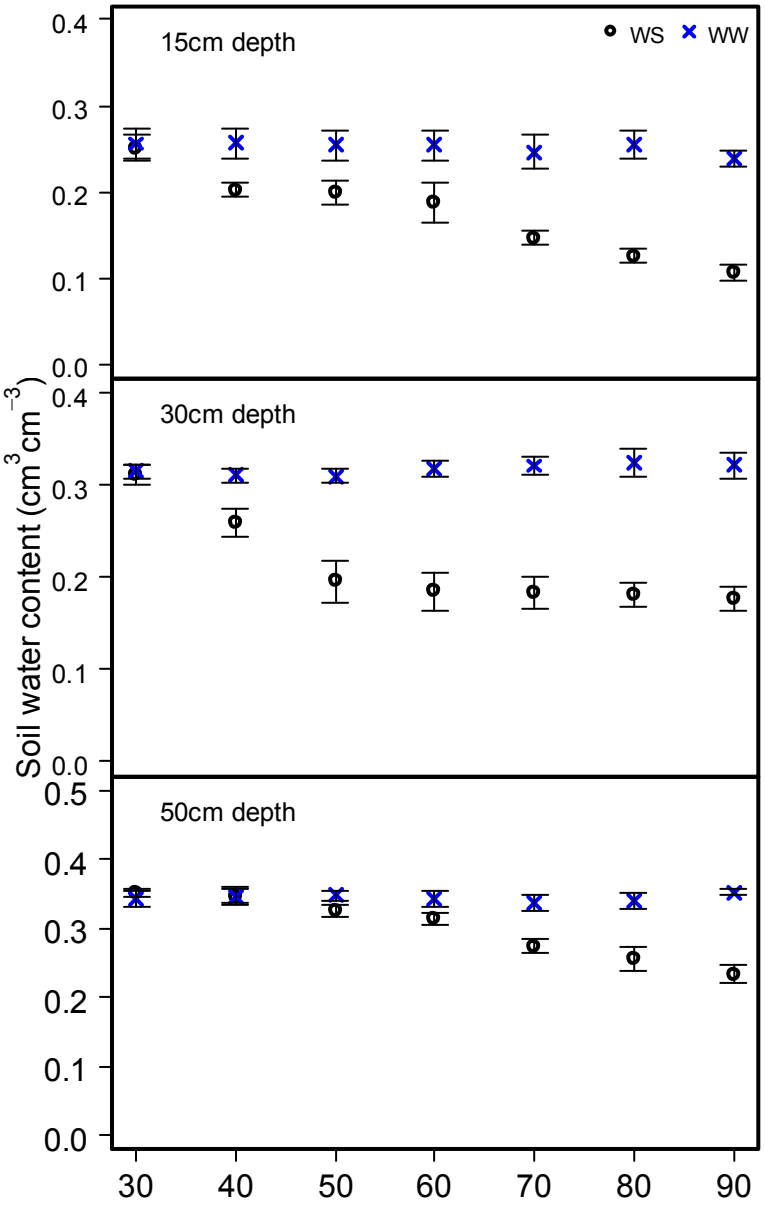

Figure 11. Effect of drought treatment on soil volumetric water content at 15-, 30-, and 50-cm depths both in well watered (WW) and water stressed (WS) conditions in the rainout shelters at Rock Springs, PA, USA (PA2012). Points are means ±SE of six measurements in the rainout shelter and three measurements in well watered plots. Terminal drought was imposed in WS plots beginning at 30 DAP.

Tables

Table 1. Summary of analysis of variance for respiration, root depth (D_{95}), stomatal conductance, carbon dioxide exchange rate (CER) and shoot biomass as influenced by soil moisture regime (treatment) and genotype in the greenhouse mesocosms experiments (GH2 and GH3). The associated F-values and probabilities (ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001) are shown

Source of variation	GH2			GH3				
	Respiration	D_{95}	Biomass	Respiration	D_{95}	CER	Stomatal conductance	Biomass
Treatment (T)	1.1ns	438.6***	134.6***	0.34ns	29.3***	127.4***	115.9***	106.1***
Genotype (G)	6.3***	3.1*	5.5**	49.5***	3.43*	4.3**	5.6*	8.1***
G x T	0.8ns	3.4*	4.4**	0.56ns	1.1ns	4.4**	4.2*	5.1**

Table 2. Summary of linear models (y = a + bx) of root respiration as predicted by root cortical cell file number (CCFN) and specific root length (SLR) in 15 IBM RILs (GH1-IBM) and 11 NyH RILs (GH1-NyH) under moderate drought in the greenhouse (GH1)

	CCFN		SRL	
	GH1-IBM	GH1-NyH	GH1-IBM	GH1-NyH
a	-0.49	-0.3	31.55	32.519
b	1.9	1.7	-0.72	-0.94
R^2	0.56	0.53	0.41	0.38
	$P<0.009$	$P< 0.012$	$P< 0.01$	$P< 0.04$

Table 3. Summary of analysis of variance for root depth (D95), leaf relative water content (RWC) (%), shoot biomass and yield as influenced by soil moisture regime (treatment) and genotype in the rainout shelters at Rock Springs, PA, USA (PA2011 and 2012). The associated F-values and probabilities (ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001) are shown.

Source of variation	PA2011				PA2012			
	D_{95}	RWC	Biomass	Yield	D_{95}	RWC	Biomass	Yield
Treatment (T)	1.80ns	522.10***	119.80***	310.2***	1.88ns	722.4***	108.06***	1341.8**
Genotype (G)	6.90***	14.8***	7.01***	8.00***	6.92***	21.81***	4.21**	33.58***
G x T	6.71***	18.12***	0.75ns	3.64**	6.77***	16.78***	2.49	18.36**

Table 4. Means of $\delta^{18}O$ of xylem water ± SE measured for six genotypes contrasting in CCFN under water stress 65 days after planting. Proportional water use by depth from different soil layers where deep is the aggregate of three deep soil layers (Fig) calculated using multi-source mixing model analysis (Phillips et al., 2005).

Group by phenotype	RIL	$\delta^{18}O$ of xylem water	Proportional water use by depth (%)		
			10 cm	20 cm	Deep
Many CCFN	129	-5.23±0.36	34	66	0
	181	-6.92±0.37	0	69	32
	284	-5.54±0.16	18	82	0
Reduced CCFN	317	-6.56±0.36	0	79	21
	365	-7.71±0.25	0	42	58
	233	-8.43±0.39	0	19	81

Table 5. Summary of analysis of variance for root depth (D95), leaf relative water content (RWC) (%), shoot biomass and yield as influenced by soil moisture regime (treatment) and genotype in the field Malawi (Bunda and Chitala). The associated F-values and probabilities (ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001) are shown.

Source of variation	Bunda			Chitala		
	RWC	Biomass	Yield	RWC	Biomass	Yield
Treatment (T)	76.2 ***	52.8 ***	294.1 ***	814.9 ***	141.1 ***	596.2 ***
Genotype(G)	3.3 **	3.0 ***	4.0 ***	3.8 **	3.1 **	2.1 **
G x T	3.2 ***	2.1 ***	4.6 ***	2.3***	3.4 ***	2.6 ***