Received: 7 August 2019 | Accepted: 1 November 2019

DOI: 10.1002/tpg2.20003

The Plant Genome

ORIGINAL RESEARCH

Genetic control of root anatomical plasticity in maize

Hannah M. Schneider[1] | **Stephanie P. Klein**[1] | **Meredith T. Hanlon**[1] | **Shawn Kaeppler**[2] | **Kathleen M. Brown**[1] | **Jonathan P. Lynch**[1]

[1]Dep. of Plant Science, Pennsylvania State Univ., University Park, PA 16802, USA

[2]Dep. of Agronomy, Univ. of Wisconsin, Madison, WI 53706, USA

Correspondence
Jonathan P. Lynch, Dep. of Plant Science, Pennsylvania State Univ., University Park, PA 16802, USA.
Email: jpl4@psu.edu

Funding information
National Institute of Food and Agriculture, Grant/Award Number: 2014-67013-21572; USDOE ARPA-E ROOTS, Grant/Award Number: DE-AR0000821

Abstract

Root anatomical phenes have important roles in soil resource capture and plant performance; however, their phenotypic plasticity and genetic architecture is poorly understood. We hypothesized that (a) the responses of root anatomical phenes to water deficit (stress plasticity) and different environmental conditions (environmental plasticity) are genetically controlled and (b) stress and environmental plasticity are associated with different genetic loci than those controlling the expression of phenes under water-stress and well-watered conditions. Root anatomy was phenotyped in a large maize (*Zea mays* L.) association panel in the field with and without water deficit stress in Arizona and without water deficit stress in South Africa. Anatomical phenes displayed stress and environmental plasticity; many phenotypic responses to water deficit were adaptive, and the magnitude of response varied by genotype. We identified 57 candidate genes associated with stress and environmental plasticity and 64 candidate genes associated with phenes under well-watered and water-stress conditions in Arizona and under well-watered conditions in South Africa. Four candidate genes co-localized between plasticity groups or for phenes expressed under each condition. The genetic architecture of phenotypic plasticity is highly quantitative, and many distinct genes control plasticity in response to water deficit and different environments, which poses a challenge for breeding programs.

1 | INTRODUCTION

Root anatomical phenes have important implications for plant interactions, nutrient cycling, and soil resource capture, particularly in environments with suboptimal water and nutrient availability (Lynch, 2013, 2018). The identification of root anatomical phenes (*phene* is to *phenotype* as *gene* is to *genotype*) (Lynch, 2011; Pieruschka & Poorter, 2012; York, Nord, & Lynch, 2013) and understanding their genetic architecture is an important step in phene-based or ideotype breeding critical for crop improvement (Lynch, 2013). Unpredictable growth environments due to decreasing freshwater availability, climate change, and the rising costs of N fertilizer demand the development of cultivars that are resilient to abiotic stress (Brisson et al., 2010; Sandhu et al., 2016; Tebaldi & Lobell, 2008; Woods, Williams, Hughes, Black, & Murphy, 2010). Generally, cultivars are selected for uniformity and yield stability within specific environmental and management practices, and phenotypic plasticity is often considered a challenge

Abbreviations: AA, total root cortical aerenchyma area; CCFN, cortical cell file number; CCS, average cortical cell size in the whole root cortex; RCA, root cortical aerenchyma; RXSA, root cross-sectional area; SNP, single nucleotide polymorphism; TCA, total cortical area; TMETVA, total metaxylem vessel area; TSA, total stele area.

(Basford & Cooper, 1998; Cooper, Rajatasereekul, Immark, Fukai, & Basnayake, 1999). The ability of a plant to alter its phenotype in response to the environment is called *phenotypic plasticity* and affects morphology, anatomy, development, or changes in resource allocation (Sultan, 2000). Phenotypic plasticity is under genetic control (e.g., Sandhu et al., 2016) and includes components of genotype × environment interaction, adaptation, and acclimation. Cultivars that can adapt their phenotypic expression in response to environmental signals are potential breeding targets for increasing agricultural productivity (e.g., Nicotra et al., 2010; Topp, 2016), although the fitness impacts of phenotypic plasticity are poorly understood, and it has been proposed that in some cases plasticity may be maladaptive (Lynch, 2013, 2019).

Root phenes affect the temporal and spatial distribution of roots in specific soil domains and therefore the ability of roots to acquire mobile and immobile soil resources (Lynch, 2013, 2018; Lynch & Brown, 2012; Lynch & Wojciechowski, 2015). Mobile nutrients, including NO_3 and water, are generally more available in deeper soil strata with time due to leaching, crop uptake, and evaporation. In contrast, P, an immobile soil nutrient, is more available in the topsoil (Lynch & Brown, 2001). Plants that are able to acquire soil resources at reduced metabolic cost will have increased productivity by permitting greater resource allocation to growth, continued resource acquisition, and reproduction (Lynch, 2013, 2015, 2018, 2019).

Several root anatomical phenes can improve plant performance under edaphic stress by reducing the nutrient and carbon cost of tissue maintenance and construction (Lynch, 2013, 2015, 2018). Root cortical aerenchyma (RCA) are air-filled lacunae that result from programmed cell death (Drew, He, & Morgan, 2000). These air-filled lacunae replace respiring cortical cells and therefore reduce root respiration and nutrient demand (Chimungu et al., 2015b; Saengwilai, Nord, Chimungu, Brown, & Lynch, 2014). The formation of RCA and the subsequent reduction in tissue maintenance costs improves plant growth and yield in environments with suboptimal water and N availability by enabling roots to explore deeper soil domains, thereby improving the capture of water and N (Chimungu et al., 2015b; Jaramillo, Nord, Chimungu, Brown, & Lynch, 2013; Saengwilai et al., 2014; Zhu, Brown, & Lynch, 2010a). Similarly, greater cortical cell size and/or fewer cortical cell files reduce root respiration and nutrient content, thereby improving soil exploration of deep soil domains, enhancing water acquisition and subsequently plant performance and yield in drought environments (Chimungu, Brown, & Lynch, 2014a, 2014b).

Another strategy for increasing plant performance in drought environments is "water banking," involving the conservation of soil water throughout the growth season (Feng, Lindner, Robbins, & Dinneny, 2016). Plants have a variety of strategies to conserve soil water throughout the growth season, and reducing root hair density and/or the diameter of xylem vessels have been proposed as water banking strategies (Wasson et al., 2012). In addition, conserving water throughout the growth season may moderate shoot water stress by increasing shoot water use efficiency. Wheat (*Triticum aestivum* L.) breeding programs selecting for decreased xylem vessel area resulted in 3–11% greater yields in drought environments than in well-watered treatments (Richards & Passioura, 1989).

Depending on the root phene, phenotypic changes as a result of plasticity may be of variable duration. For example, the number and size of cortical cells is established near the root apex, and changes in mature tissues are limited. In contrast, the expression of aquaporins fluctuates in response to water availability (Zargar et al., 2017). Under conditions of sustained edaphic stress, such as low P availability, plasticity that is established in early development, such as the number and size of cortical cells, may be beneficial. However, plasticity of long duration may be maladaptive for stresses that fluctuate on shorter time scales, including drought, by generating a sustained response to ephemeral conditions (Lynch, 2013).

In variable environments, phenotypic plasticity may be advantageous; however, intensive fertilization and greater nutrient availability in high-input environments may cause phenotypic plasticity to be maladaptive. Crop species evolved in environments with abiotic and biotic stresses influencing root growth and function, and therefore strategies for soil resource capture may not have utility in high-input environments in which constraints for soil resource acquisition and plant growth under stress are mitigated (Lynch, 2018). In most agricultural systems, root phenotypes that explore deep soil domains, whether plastic or not, enhance the capture of water and N (Gowda, Henry, Yamauchi, Shashidhar, & Serraj, 2011; Henry, Gowda, Torres, Mcnally, & Serraj, 2011; Manschadi, Christopher, deVoil, & Hammer, 2006).

In maize, root cortical aerenchyma (Chimungu et al., 2015b), cortical cell file number (Chimungu et al., 2014b), and cortical cell size (Chimungu et al., 2014a) improve shoot biomass and yield under drought conditions. High yield stability correlated with high root architectural plasticity in drought and low-P environments in rice (*Oryza sativa* L.) (Sandhu et al., 2016). Plasticity has been observed for RCA in rice in response to water stress, corresponding to greater shoot dry weight and yield (Niones, Suralta, Inukai, & Yamauchi, 2012, 2013). However, root cortical aerenchyma formation in rice did not influence water uptake or shoot biomass in drought (Gicaraya, Cadiz, & Henry, 2017). In soybean [*Glycine max* (L.) Merr.] grown under water stress, metaxylem number increased, thereby improving root hydraulic conductivity, while the total cortical area was reduced, which reduced the metabolic cost of accessing water in deeper soil domains (Prince et al., 2017). Plasticity of xylem vessel diameter and

number and stele diameter has been observed in response to water deficit in wheat and rice (Kadam et al., 2017). It has been speculated that greater phenotypic plasticity in wheat root anatomical traits results in greater stress tolerance compared with rice (Kadam et al., 2017).

Few genetic loci have been associated with root anatomical phenes. Quantitative trait loci (QTL) have been identified for root stele and xylem vessel diameter in rice (Uga et al., 2008, 2010), xylem vessel phenes in wheat (Sharma et al., 2010), RCA in *Zea* species (Mano, Omori, Muraki, & Takamizo, 2005, 2007), and areas of cross section, stele, cortex, aerenchyma, and cortical cells, root cortical aerenchyma, and cortical cell file number in maize (Burton et al., 2014). However, the majority of root QTL studies have focused on young or greenhouse-grown plants grown in artificial media that do not represent the heterogeneous matrix of soil found under field conditions and may be a poor predictor of mature root system architecture (Zhu, Ingram, Benfey, & Elich, 2011).

Root anatomical phenes merit consideration in breeding programs to improve crop performance (Lobet et al., 2019). It is essential to understand individual phenes and their genetic architecture in order to increase abiotic stress tolerance and provide insights into the mechanisms controlling phenotypic variation for root phenes. In this study, we phenotyped a large diversity panel for root anatomical phenes in mature, field-grown maize in multiple environments. The objectives of this research were to test the hypotheses that (a) the responses of root anatomical phenes to water deficit (stress plasticity) and different environments (environmental plasticity) are under genetic control and (b) genetic loci associated with plasticity are distinct from loci controlling phenotypic expression under water-stress and well-watered conditions.

2 | MATERIALS AND METHODS

2.1 | Field conditions, experimental design, and plant materials

Root anatomical phenotypes were measured on the Wisconsin Diversity Association Panel (Hansey et al., 2011), a collection of maize inbred lines that display uniformity and vigor and reach grain maturity in the northern Midwest of the United States. Experiments were conducted at the Apache Root Biology Center (ARBC) in Willcox, AZ (32°153′9.252″ N, 109°49′56.928″ W) under well-watered and water-stressed conditions (Supplemental Table S1) and the Ukulima Root Biology Center (URBC) in Alma, Limpopo, South Africa (24°33′12″ S, 28°7′2584″ E) under non-stress conditions (Supplemental Table S2). At the Arizona site, experiments were conducted from May to August in 2016 on a Grabe loam (a coarse-loamy, mixed, thermic Typic Torrifluvent), and genotypes were grown in two replications per treatment in a randomized complete block design. At the South Africa site, experiments were conducted from January to April in 2010, 2011, and 2012 and from November to February in 2013 on a Clovelly loamy sand (a Typic Ustipsamment). Genotypes were grown in four replications in a randomized complete block design each year. Each line was planted in a single-row plot, consisting of 20 plants. Row width was 75 cm and the distance between plants within a row was 23 cm. Soil nutrient levels and pH were adjusted according to maize production requirements and based on soil tests at the beginning of the season. All experiments were irrigated using a center pivot system. Drought was induced approximately 4 wk after planting and monitored throughout the growth season by PR2 multidepth soil moisture probes (Dynamax). The water-stress treatment was confirmed by ∼20% vegetative biomass growth reduction and 40% yield reduction under water-stressed compared with well-watered conditions.

2.2 | Phenotypic analysis

Root anatomy was phenotyped in all experiments, and evaluations were performed based on the "shovelomics" method (Trachsel, Kaeppler, Brown, & Lynch, 2011) followed by laser ablation tomography (Hall, Lanba, & Lynch, 2019). At anthesis, one representative plant per plot was excavated in a soil monolith using a standard shovel and soaked in water for 15 min to remove soil. Root crowns were washed with low-pressure water to remove the remaining soil. Root anatomy was collected by excising a nodal root from the crown and sampling a 3-cm segment from 5–8 cm of the basal portion of the root. At the South Africa field site, a second whorl crown root was excised, and at the Arizona field site a fourth whorl crown root was excised. The fourth whorl crown roots emerged after drought initiation. The sampled root segment was placed in 75% ethanol for preservation until ablation using laser ablation tomography. In short, a 355-nm pulsed laser (Avia 7000) was used to vaporize the root at the camera focal plane ahead of an imaging stage. The sample was moved into the ablation plane, vaporized, and imaged simultaneously. Root cross-sectional images were captured using a Canon T3i camera with a 53 micro lens (MP-E 65 mm). Root images were analyzed for eight anatomical phenotypes (Table 1) using RootScan2.0 (Burton, Williams, Lynch, & Brown, 2012) and MIPAR (Sosa, Huber, Welk, & Fraser, 2014) software. At anthesis, plant height was measured on three plants per plot in South Africa and shoot dry biomass was collected for one plant per plot in Arizona. Yield was collected at maturity and cobs from three plants per plot. All ears from three representative plants were harvested, and the grain and cobs were weighed.

TABLE 1 Description of anatomical phenes measured at anthesis from cross-sectional images captured from a representative third whorl crown root

Trait	Description
RXSA	root cross-sectional area, mm^2
TCA	total cortical area, mm^2
TSA	total stele area, mm^2
TMETVA	total metaxylem vessel area, mm^2
AA	total root cortical aerenchyma area, mm^2
RCA	root cortical aerenchyma (as a percentage of TCA), %
CCS	average cortical cell size in the whole root cortex, μm^2
CCFN	number of cortical cell files

2.3 | Data analysis

Plasticity in response to water deficit (stress plasticity) was calculated relative to control (no stress) growing conditions for each phene:

$$\text{Stress Plasticity} = \frac{(\text{WS} - \text{WW})}{\text{WW}}$$

where WS is the mean value of the phene under water-stressed conditions for each replication and WW is the mean value of the phene under well-watered conditions for each replication.

Plasticity in response to the environment (environmental plasticity) was calculated as a relative phenotypic value of South Africa growing conditions compared with the Arizona growing conditions for each phene:

$$\text{Environmental Plasticity} = \frac{(\text{SA} - \text{AZ})}{\text{AZ}}$$

where SA is the mean value of the phene in the South Africa environment and AZ is the mean value of the phene in the WW Arizona environment.

Broad-sense heritability and repeatability on an entry mean basis were calculated for each anatomical phene according to Fehr (1993).

Best linear unbiased predictors were calculated for each treatment and environment using a random-effects linear model in the R package lme4 (Bates et al., 2015) and used for subsequent analysis.

$$y_{ijk} = \mu + c_i + g_j + b_{k(i)} + (cg)_{ij} + e$$

where y_{ijk} is the predicted response, μ is the grand mean, c is the effect of year, g is the effect of genotype, b is the effect of replication nested within year, and e is the residual error.

For phenotypes in water-stress and well-watered environments in Arizona, an average of two replications within each treatment was calculated. For phenotypes in well-watered environments in South Africa, an average of four replications over four years was calculated. Stress plasticity was calculated by replication. Environmental plasticity was calculated by year. Residuals were transformed according to Box Cox analysis, and all phenes required transformation. Allometric analysis was performed by logarithmically transforming plant performance metrics (i.e. yield and biomass) and regressing them against a logarithmic transformation of each phene. From these regression analyses, the coefficient of determination (R^2) and the slope of the regression line (α) were determined to reveal the underlying exponential relationship between size traits and biomass or yield.

Anatomical phenes and their stress and environmental plasticity values were used in a multiple-loci linear mixed model for genome-wide association mapping analysis (Zhang et al., 2010) implemented in the FarmCPU R package (Liu, Huang, Fan, Buckler, & Z, 2016). The model used 593,727 single nucleotide polymorphism (SNP) markers for the Arizona dataset and 602,361 SNP markers for the South Africa dataset (Mazaheri et al., 2019), and allelic effects were estimated relative to the minor allele. Significant SNPs were identified based on a genome-wide corrected Bonferroni threshold of $-\log(p) = 7.07$. Q–Q plots suggested a good model fit (Supplemental Figure S1).

R software (version 3.2.4) (R Core Team, 2018), Bioconductor (Bioconductor, 2002), MapMan (Usadel et al., 2009), and MaizeGDB (Lawrence, 2005) were used to annotate genes and compare significant SNPs. Candidate genes identified through significant genome-wide association mapping hits were detected based on the physical position of genes in the version 4 B73 (AGPv4) reference sequence assembly (Jiao et al., 2017). A one-way ANOVA and Tukey's honestly significant difference was used to analyze the differences among phenotypic distributions of phenes in different environment and treatments.

3 | RESULTS

3.1 | Root anatomical phenes demonstrate genetic variability and environmental plasticity

Both stress and environmental plasticity were observed for each phene (Figure 1), and plasticity varied by genotype (Figure 2). Genotype and replication had a significant effect on most root phenes (Supplemental Table S2). A wide range of natural variation for anatomical phenes and their plastic response was observed (Supplemental Figure S2, Supplemental Table S3). Water deficit had a significant effect on root anatomical phenotypes (Supplemental Table S3). On average, root cross-sectional area (RXSA), total stele area (TSA), and total metaxylem vessel area (TMETVA) were reduced 9, 11, and 18%, respectively, under water-stressed conditions compared with well-watered conditions. Cortical phenes including total root cortical aerenchyma area (AA), the percentage

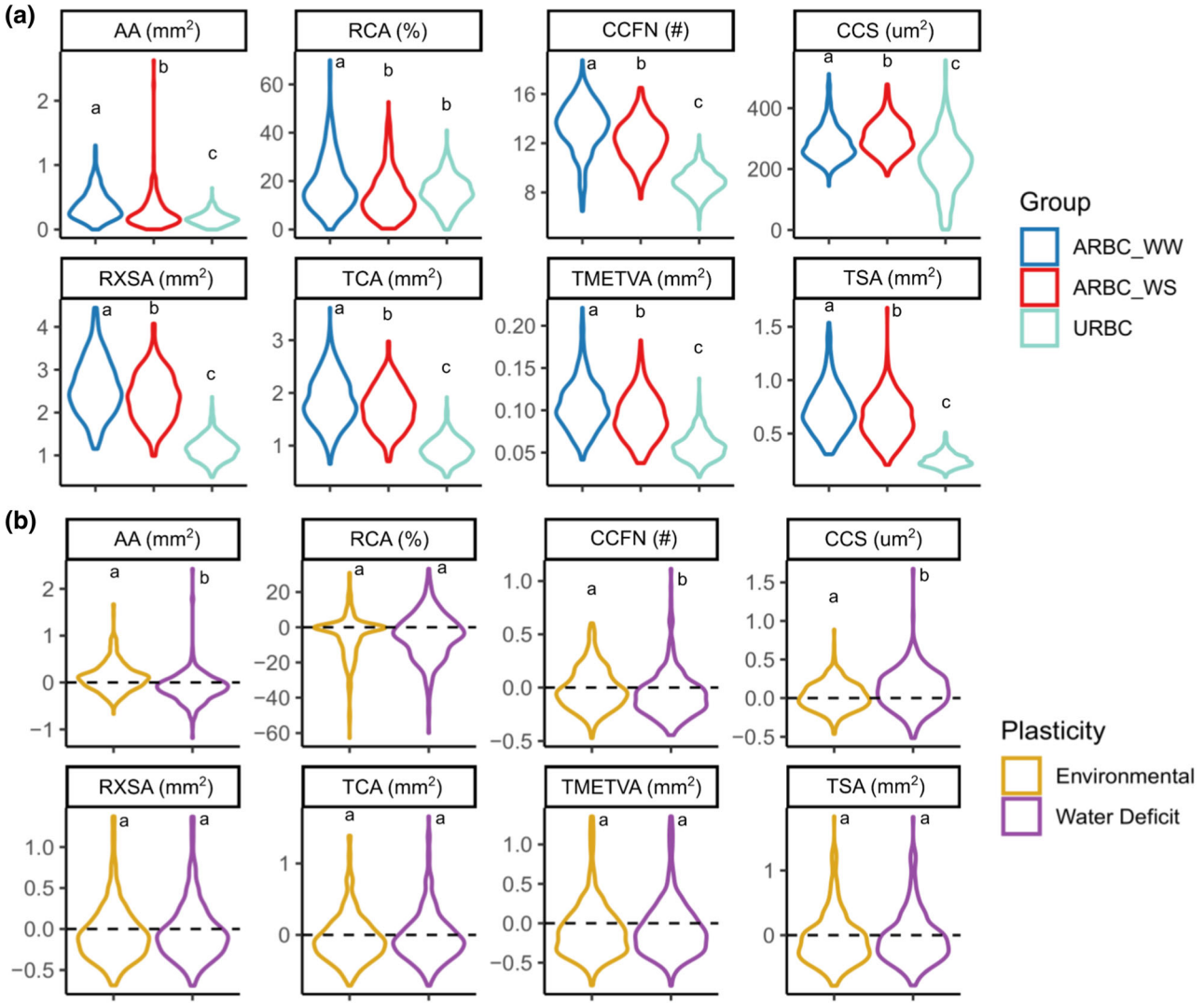

FIGURE 1 (a) Distributions of genotypic means for each phene under well-watered (WW) and water-stress (WS) conditions at the Apache Root Biology Center (ARBC) in Arizona and WW conditions at the Ukulima Root Biology Center (URBC) in South Africa; and (b) distribution of the change in root phene values under drought stress or between environments, where the *y* axis represents the relative difference in phene value between well-watered and water-stress conditions (stress plasticity, change relative to the well-watered treatment) or the relative difference between the two environments environment (environmental plasticity, change relative to the Arizona field site) for each phene. Phenes include aerenchyma area (AA), root cortical aerenchyma (RCA), number of cortical cell files (CCFN), cortical cell size (CCS), root cross-sectional area (RXSA), total cortical area (TCA), total metaxylem vessel area (TMETVA), and total stele area (TSA). Letters above the plots show significant differences between distributions according to a one-way ANOVA and Tukey's honestly significant difference

of the cortex that is root cortical aerenchyma (RCA), the total cortical area (TCA), and cortical cell file number (CCFN) were reduced 27, 25, 7, and 9%, respectively, under water-stressed compared with well-watered conditions. The average cortical cell size (CCS) was 7% greater under water-stress conditions than well-watered conditions (Figure 1), but the response of cortical cells to water stress depended on their position within the cortex (Supplemental Figure S4).

Root phenes under well-watered and water-stress conditions and environmental plasticity were more heritable than plastic responses to drought (Table 2). Repeatability for TMETVA was the greatest under well-watered and water-stress conditions but the smallest in stress plasticity compared with all other phenes. Repeatability for RXSA and TSA were relatively stable across well-watered, water-stress, and stress plasticity. A large number of genes with relatively small effect sizes are associated with these highly quantitative and genetically controlled anatomical phenes (Table 2).

Allometric relationships between root anatomical phenes and yield or vegetative biomass were not significant. The majority of root phenes with significant scaling exponents ($p < .05$) had smaller scaling exponent values than the expected isometric values under well-watered and water-stressed conditions. Therefore, as plant size or yield increases,

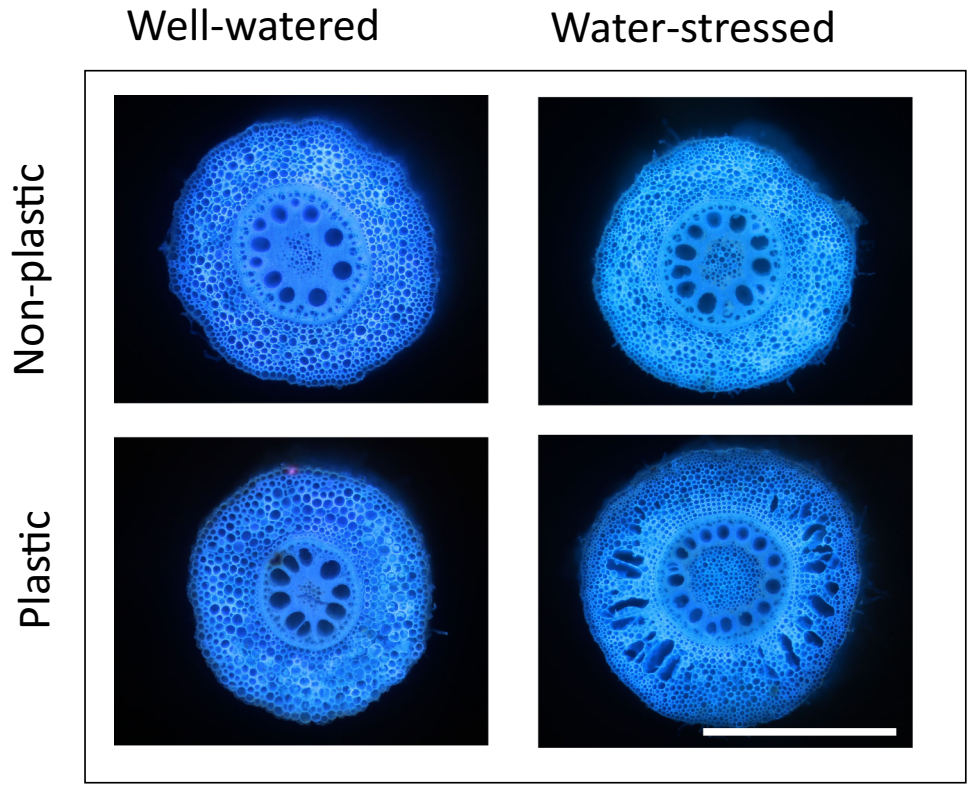

FIGURE 2 Examples of genotypic variation in plastic response to drought and environment. Anatomical images of a non-plastic genotype and a plastic genotype for root cortical aerenchyma (RCA) under well-watered and water-stress conditions. Scale bar represents 1 mm

TABLE 2 Heritability and repeatability of root anatomical phenes in the Wisconsin Diversity Panel. Heritability was calculated for phenes measured in South Africa because data were available for multiple years; repeatability was calculated for phenes measured in Arizona because data were not available for multiple years, only multiple replications. Variation explained by the single nucleotide polymorphisms (SNPs) refers to the phenotypic variance. No significant SNPs were identified for TSA under well-watered conditions or for TMETVA under water-stress conditions

Parameter	Statistic	RXSA	TCA	TSA	TMETVA	AA	RCA	CCFN	CCS
Arizona, well-watered	repeatability	.26	.29	.27	.31	.17	.20	.24	.24
	variation explained by SNPs, %	7.98	9.23	–	11.11	17.68	20.32	12.62	11.07
Arizona, water stress	repeatability	.23	.23	.26	.38	.13	.14	.11	.12
	variation explained by SNPs, %	10.3	11.01	21.92	–	20.31	11.97	8.58	2.05
Stress plasticity	repeatability	.23	.17	.21	.1	.13	.16	.15	.13
South Africa, well-watered	heritability	.38	.37	.56	.55	.5	0.59	.43	.3
	variation explained by SNPs, %	82.33	44.11	29.6	95.01	108.24	12.98	61.37	92.5
Environmental plasticity	heritability	.26	.21	.29	.43	.22	.17	.19	.14

Notes: RXSA, root cross-sectional area; TCA, total cortical area; TSA, total stele area; TMETVA, total metaxylem vessel area; AA, total root cortical aerenchyma area; RCA, root cortical aerenchyma; CCFN, number of cortical cell files; CCS, average cortical cell size.

the proportion of these phenes would be less than that predicted with isometric growth (Supplemental Table S4).

3.2 | Few candidate genes overlapped between phene values and plasticity responses

Genome-wide association mapping identified 158 significant SNPs associated with root anatomy in well-watered and water-stressed plants and their stress and environmental plastic responses using a Bonferroni-corrected genome-wide threshold value of $\log(p) = 7.07$ (Figure 3, Supplemental Figure S3). Candidate genes were identified as gene models containing SNPs above the Bonferroni significance threshold. Significant SNPs were identified in 24 candidate genes associated with stress plasticity and 33 candidate genes associated with environmental plasticity between the two field sites. In addition, 16 and 18 candidate genes were associated

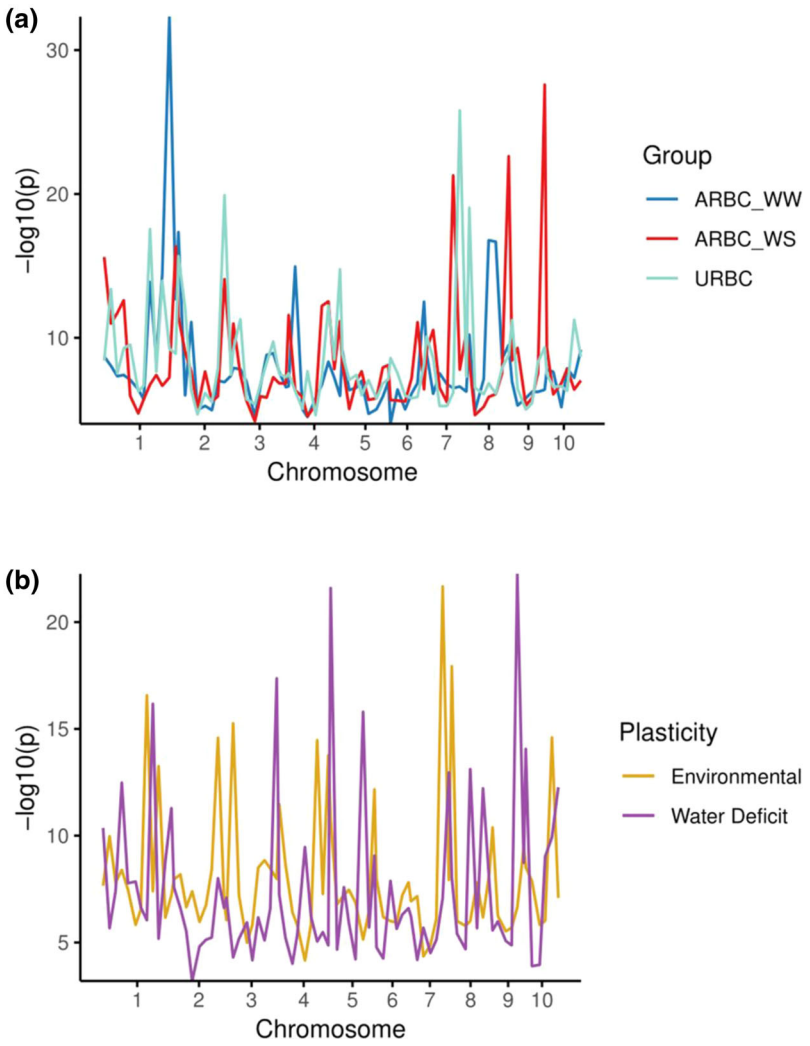

FIGURE 3 Genome-wide association mapping results for root cortical aerenchyma (RCA) in plants grown (a) under well-watered (WW) and water-stress (WS) conditions at the Apache Root Biology Center (ARBC) in Arizona and WW conditions at the Ukulima Root Biology Center (URBC) in South Africa; and (b) their stress and environmental plasticities. See Supplemental Figure S2 for plots of other anatomical phenes

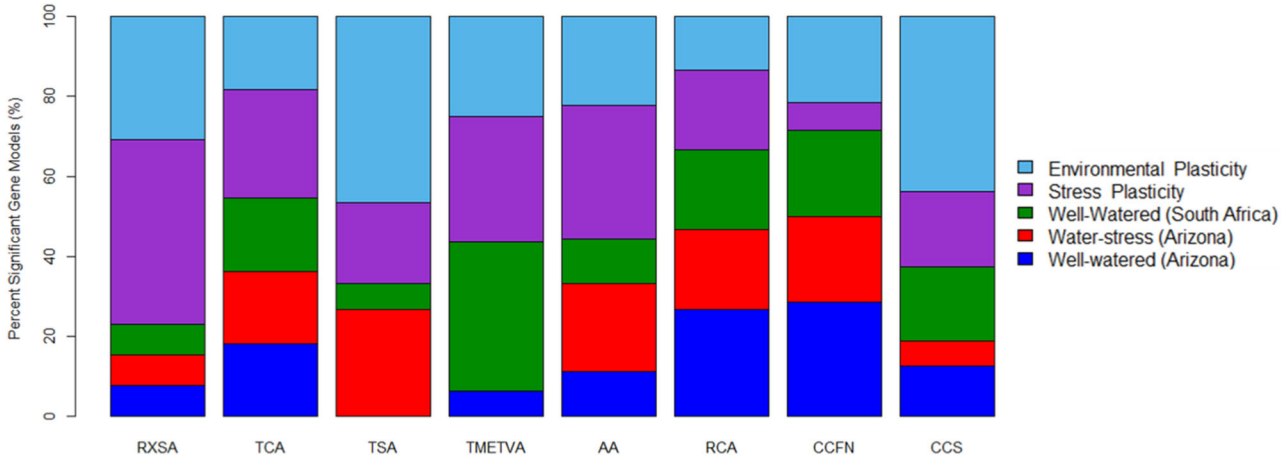

FIGURE 4 The relative proportion of unique gene models associated with well-watered and water-stress conditions and environmental and stress plasticity for root cross-sectional area (RXSA), total cortical area (TCA), total stele area (TSA), total metaxylem vessel area (TMETVA), aerenchyma area (AA), root cortical aerenchyma (RCA), number of cortical cell files (CCFN), and cortical cell size (CCS)

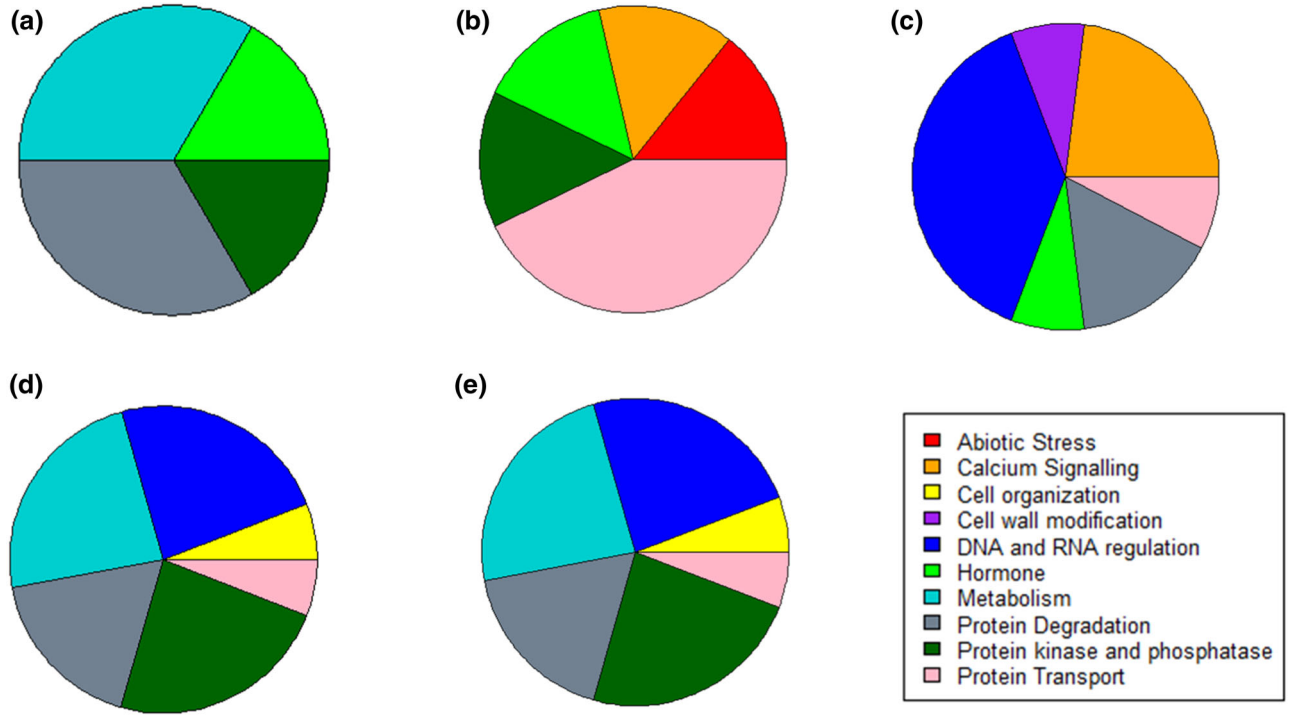

FIGURE 5 Mapman ontogenic categories for annotated gene models associated with significant single nucleotide polymorphisms (a) under water stress in Arizona (44% annotated), (b) well watered in Arizona (50% annotated), and (c) well watered in South Africa (90% annotated), and measures of (d) stress plasticity in Arizona (71% annotated), and (e) environmental plasticity (73% annotated)

with phenes under well-watered and water-stress conditions, respectively, in Arizona and 30 candidate genes were associated with root phenes under well-watered conditions in South Africa (Figure 4). All candidate genes are listed in Supplemental Tables S5, S6, S7, S8, and S9. Approximately 66% of the candidate gene models were annotated for Mapman ontogenic categories (Supplemental Tables S5, S6, S7, S8, and S9). Of the annotated gene models, the water stress group had significantly more genes associated with metabolism and protein degradation than the well-watered and plasticity groups. The Arizona well-watered group had significantly more genes associated with protein transport and the South Africa well-watered group had significantly more genes associated with DNA and RNA regulation compared with the other groups (Figure 5).

A few significant SNPs co-localized between well-watered, water-stressed, and/or stress and environmental plasticity groups. Two sets of significant SNPs co-localized among well-watered, water-stressed, and plasticity expression in response to drought (Supplemental Tables S7, S8, and S9). Interestingly, both genes (Zm00001d003513, abscisic acid synthesis/degradation, and Zm00001d026383, signaling receptor kinase) were found to be associated with TCA. One set of two SNPs co-localized between well-watered and plasticity groups for TMETVA (Zm00001d032659, protein EXECUTER 1; Supplemental Tables S8 and S9). Two co-localized SNPs located in a gene (Zm00001d031445) associated with ethylene signaling were co-localized between environmental plasticity and well-watered groups in the South Africa environment (Supplemental Tables S5 and S6). One SNP for AA was associated with the environmental plasticity response and the other SNP was associated with the RCA percentage under well-watered conditions in South Africa.

Significant SNPs for plasticity were detected for all anatomical phenes measured. Significant SNPs were detected for all phenes except TSA in the well-watered environment and for all phenes except TMETVA in the water-stressed environment. Approximately 27 and 20% of unique gene models were associated with environmental and stress plasticity, respectively, while approximately 26, 15, and 13% of unique gene models were associated with well-watered conditions in South Africa and well-watered and water-stress conditions in Arizona, respectively (Figure 4).

4 | DISCUSSION

Understanding phenotypic plasticity and its genetic architecture has important implications for understanding plant adaptation to edaphic stress and breeding more resilient crops. Root anatomical phenes and their plastic response to drought stress and environment are heritable and demonstrate characteristics of quantitative traits. We observed a large variation in the extent and direction of drought

stress and environmental plasticity of root anatomical phenes (Figure 1). Few significant SNPs co-localized between the plasticity groups or the expression of anatomical phenes within water-stress or well-watered environments (Figure 3, Supplemental Figure S3, Supplemental Tables S5, S6, S7, S8, S9), reflecting the range of adaptation strategies used by the genotypes in this collection of diverse and agronomically relevant inbred lines.

4.1 | Potential adaptive value of phene states and plasticity

In water-stressed conditions, the cortical phenes total cortical area (TCA), aerenchyma area (AA), percent cortex that is root cortical aerenchyma (RCA), and cortical cell file number (CCFN) were smaller on average compared to well-watered conditions, while cortical cell size (CCS) was greater on average in water-stress compared to well-watered conditions. Reduced CCFN and greater CCS improve drought tolerance in maize by reducing the metabolic cost of the root cortex, enabling greater soil exploration, water acquisition, growth, and yield from drying soil (Chimungu et al., 2014a, 2014b). Reducing CCFN and increasing CCS may be an adaptation to improve growth under drought, while greater CCFN may be a more adaptive trait in compacted soils by enabling the penetration of hard soils and potentially enabling greater carbon storage to support further root development.

Under water stress, the average RXSA across the panel was less, but the stele/cortical area ratio remained unchanged compared with well-watered conditions (Supplemental Table S3). A smaller root diameter is beneficial under drought by reducing the metabolic cost of soil exploration. However, drought and mechanical impedance commonly co-occur in agroecosystems and have the potential to overlap in their impacts on plant growth because dry soils are often harder than moist soils. The ability of a root to penetrate hard soil is particularly important under drought, and a smaller diameter may reduce the ability of a root to overcome soil impedance. Stele diameter is correlated with root tensile strength (Chimungu, Loades, & Lynch, 2015a) and therefore reduced TSA under water stress may reduce the ability of a root to penetrate hard, drying soil. In addition, the size of cells in different cortical regions is important for root penetration of hard soil because smaller cortical cells in the outer cortical regions prevent the root from buckling during penetration (Chimungu et al., 2014a). Genotypes with a thicker cortex (TCA) had greater root bending strength (Chimungu et al., 2015a). Although the average CCS in the entire cortex was greater under water stress, cell size in the outer cortical regions was 38% less than that under well-watered conditions (Supplemental Figure S4). Cortical cell size was increased under drought, which may leave the root susceptible to buckling and with a reduced ability to penetrate hard soils (Chimungu et al., 2015a; Striker et al., 2007). However, it is important to note that root segments near the base of the plant were sampled, while the penetration ability of roots is dependent on the cortical phenes near the root tip. Cortical phenes have important implications in reducing root metabolic costs and the ability of roots to penetrate hard soils. This study demonstrates that nodal roots responded to water deficit by decreasing RXSA, which presumably has more value for root foraging and water capture than maintaining thick roots.

Mean values of RCA and AA were significantly reduced by water stress, although there was substantial plasticity in both directions for RCA in response to drought. The majority of genotypes had a reduction in AA and RCA formation under drought; however, decreases in AA and RCA mean values under water deficit are driven by a smaller proportion of genotypes with greater plastic responses. Several studies using fewer genotypes have demonstrated that upregulation of RCA formation by drought stress reduces the cortical metabolic burden to facilitate greater soil exploration (Chimungu et al., 2015b; Jaramillo et al., 2013; Zhu et al., 2010a), although some genotypes in these studies also exhibited reduced RCA under drought (Chimungu et al., 2015b; Jaramillo et al., 2013). Different watering regimes and soil types influence water availability in different soil strata, which could explain the reduction in RCA in some genotypes (17% of genotypes) in this study. For example, due to high evapotranspiration and sandy soil, we used frequent irrigation, which may have caused temporary hypoxia in the topsoil, thereby promoting the formation of RCA, unlike typical rainfed maize production. In this study, RCA formation was generally greater than in other field studies under water-stress and well-watered conditions in silt and sandy loam soils (Chimungu et al., 2015b; Zhu et al., 2010a). Optimal RCA formation most likely varies with the interaction of soil type and drought timing and intensity.

The TMETVA was less under water-stress than well-watered conditions. A smaller xylem vessel area, and therefore reduced axial hydraulic conductivity, can be advantageous under water-limited conditions by conserving soil water throughout the growth season, a strategy known as "water banking" (Feng et al., 2016). Reduced hydraulic conductivity may prevent desiccation of the growing root tip and surrounding soil throughout the growth season, as well as moderating shoot water stress by increasing shoot water use efficiency. We speculate that the plastic response of reduced TMETVA under water stress is an adaptation to improve drought tolerance. While the general trend across all genotypes was reduced TMETVA under water stress, substantial plasticity was observed in both directions under water-stressed compared with well-watered conditions. While reduced TMETVA may be advantageous under drought conditions, reduced hydraulic conductivity may also reduce carbon gain, growth,

and yield, which may be detrimental under well-watered conditions. Deeper roots may represent a carbon cost of the root system at the expense of the growth of other tissues (Tardieu, Draye, & Javaux, 2017) unless deep roots are linked with or caused by root phene states that reduce the metabolic cost of soil exploration (Lynch, 2018). In this case, plasticity for TMETVA may be useful because large conductance is optimal under well-watered conditions and reduced conductance is useful under water stress.

4.2 | Variation of genetic control of anatomical phenes with environment

Our results indicate that root architectural and anatomical phenes and their plastic response to water stress and the environment are genetically controlled and highly quantitative. A total of 121 unique gene models were identified as being associated with root anatomy in well-watered and water-stress environments and with phenotypic plasticity. Many genes with relatively small effect sizes were associated with these phenes (Table 2). Stress plasticity was less heritable than root phene values within water-stress and well-watered conditions. Heritable plasticity responses indicate that root plasticity is genetically controlled. Root phenes are highly quantitative, and plasticity in response to edaphic stress may make breeding for these phenes challenging. Understanding root phenotypic plasticity and its genetic control may permit the selection of lines with optimal plasticity to improve plant growth in specific environments. For example, plants with greater phenotypic plasticity for TMETVA may be more useful in environments with fluctuating drought, while reduced phenotypic plasticity for CCS may be more beneficial in environments with sustained stress throughout the growth season, including low N availability.

While the overall trend was a decrease in vegetative biomass and yield under drought, significant plasticity was observed in both directions (Supplemental Figure S5), that is, genotypes varied in their tolerance to the type of drought imposed. Genotypes may have different strategies to achieve drought tolerance, and the plastic responses of root phenes or phene aggregates to drought may be an adaptive or maladaptive response. Specific root phenes are important in plant stress tolerance; however, root phenes do not function in isolation (Miguel, Postma, & Lynch, 2015; York et al., 2013). Synergisms exist between phene states with a large metabolic cost and phenes that reduce the metabolic cost of the root. For example, RCA is more beneficial in plants with increased lateral branching density since the lateral roots represent a significant cost (Postma & Lynch, 2011). Understanding phene synergisms and their plastic interactions may be an important consideration for breeders.

4.3 | Candidate genes with known associations with stress responses

Several genes were identified for anatomical phenes and plastic responses that were previously associated with low phosphate responses in crops. A gene associated with CCS under well-watered conditions in this study (Zm00001d038608, Supplemental Table S8) and a gene (Zm00001d034241) associated with environmental plasticity of TSA (Supplemental Table S5) were found to be upregulated in maize grown with low P (Su et al., 2014). A gene associated with TSA under well-watered conditions in South Africa (Zm00001d023377, Supplemental Table S6) is homologous with genes associated with phosphate starvation in *Arabidposis* and rice (Cheng, Pittman, Bronwyn, Shigaki, & Hirschi, 2003, 2005). A number of root phenes influence P capture under suboptimal P availability, including the density of lateral branching (Postma, Dathe, & Lynch, 2014; Zhu & Lynch, 2004), RCA (Galindo-Castañeda, Brown, & Lynch, 2018), the number of crown roots (Sun, Gao, & Lynch, 2018), and root hair length (Zhu, Zhang, & Lynch, 2010b). Presumably, genes that are differentially expressed under low-P availability could contribute to controlling root phenes under many edaphic stresses through common signaling pathways such as ethylene.

Fiber protein Fb2 (Zm00001d043911) is differentially expressed in maize tassels and ears under water deficit (Zhuang et al., 2008), and SNPs within that gene were associated with the plastic response of RCA to drought (Supplemental Table S9). Sucrose synthase 2 (Zm00001d047253) is associated with the plastic response to drought, is differently expressed in soybean roots under water stress, and plays a key role in N_2 fixation under drought (Supplemental Table S9). A significant SNP associated with the plastic response of RCA under drought was located in sucrose synthase 2 (Supplemental Table S9). The SNPs within genes associated with the environmental plastic response of CCS (Zm00001d018045, Supplemental Table S5) and TMETVA under well-watered conditions in South Africa (ARF25, Zm00001d011953, Supplemental Table S6) were associated with drought tolerance in maize (Kim et al., 2012; Xu et al., 2014). Many strategies exist for drought tolerance, and presumably many different genes in different pathways control the expression of phenes that optimize plant growth under water deficit.

A gene downregulated in maize leaves upon formation of arbuscular mycorrhizae (Zm00001d051932) (Gerlach et al., 2015) was associated with the environmental plasticity of TMETVA (Supplemental Table S5). A gene associated with the environmental plasticity of RXSA (Zm00001d018342) was downregulated in maize cobs under low N (Pan et al., 2015) (Supplemental Table S5). Fluctuating environmental factors, including N availability and arbuscular mycorrhizae

colonization presumably target some of the same genes or gene pathways as factors including environmental plasticity.

A gene differentially expressed under water-logged conditions (Zm00001d047253) (Arora, Panda, Mittal, & Mallikarjuna, 2017) was associated with the plastic response of RCA (Supplemental Table S9). In addition, a gene associated with cell death (Zm00001d015971) (Gao et al., 2009) was associated with the plastic response of AA (Supplemental Table S9). Hypoxia induces RCA formation via ethylene signaling and programmed cell death (Evans, 2003), and presumably common signaling pathways (e.g. ethylene) induce RCA formation and control the expression of other root phenes under a range of edaphic stresses.

Two significant SNPs associated with TCA co-localized among well-watered, water-stressed, and plasticity expression in response to drought. One SNP was located in Zm00001d003513, which is associated with abscisic acid synthesis and degradation (Supplemental Tables S7, S8, and S9). Abscisic acid has been demonstrated to help maintain shoot and root growth under water deficit and prevent excess ethylene production from tissues under water stress (Sharp & LeNoble, 2002). The other SNP was located in Zm00001d026383, annotated as a signaling receptor kinase (Supplemental Tables S7, S8, and S9). Receptor-like kinases are key regulators of plant architecture and growth behavior and contribute to improving plant performance under drought (Marshall et al., 2012). Genes associated with plant hormone signaling, synthesis, and degradation have large implications in the expression of root phenes under different environmental conditions and their plastic responses.

Of the gene models identified, 66% were annotated. Plasticity may be controlled by many genes in various pathways as indicated by the co-localization of a few SNPs among well-watered, water-stress, and plasticity groups (Figures 4 and 5). The expression of anatomical phenes is highly dependent on environment and abiotic stress factors. Differences in soil texture, photoperiod, and precipitation patterns presumably contributed to environmental plasticity. Generally, anatomical features were smaller in the South Africa environment than the Arizona environment, probably because anatomical phenes were measured on a second whorl crown root at the South Africa field site and on a fourth whorl crown root at the Arizona field site. Crown roots typically become thicker at each successive whorl, and there are allometric relationships between RXSA and other area-based anatomical phenes (Yang, Schneider, Brown, & Lynch, 2019). Differences in phene expression between different whorls may also indicate that different whorls are under distinct genetic control. Consideration of gene networks, phene networks, and dynamic responses may result in stronger associations of phenes with genetic loci and pathways.

Substantial variation was observed for all phenes in well-watered and water-stress environments and for plasticity, that is, phenotypic responses to water availability and/or environment. Root anatomical phenes and their plastic responses are heritable, and their genetic architectures are highly quantitative and complex. Many distinct candidate genes with small effects were associated with plasticity and anatomical phenes per se. However, not only is the genetic control of plasticity in a specific environment complex, but plasticity in response to various abiotic stresses and/or environments is controlled by distinct genes. In the current study, no genes associated with plasticity were similar between the water-stress and environmental plasticity groups. Presumably, plasticity in response to other abiotic stresses, including low N and P availability, are also controlled by a suite of distinct genes.

Highly complex quantitative traits with small effects pose a challenge for breeding programs, particularly single-trait breeding programs using conventional tools like marker-assisted selection. Distinctly genetically controlled plasticity responses to different environmental conditions limits the adaptive value of a plastic genotype, and breeders may need to target a specific plastic response to a specific abiotic stress or environment rather than just generally breeding for a highly plastic cultivar. In this study, genotypes that were highly plastic in response to water deficit were distinct from those with a plastic response to the environment. Breeding efforts to develop cultivars that are plastic for a range of conditions and stresses may be maladaptive in environments with multiple stresses or stresses that fluctuate on a shorter time scale. For example, in environments with sustained P stress, plasticity established early in development, such as CCS, may be beneficial; however, this plasticity of long duration may be maladaptive under drought or N stresses that fluctuate on a shorter time scale (Lynch, 2013). The fitness landscape of plasticity is highly complex yet poorly understood and merits further research to understand how to deploy plasticity in breeding programs. Interactions between root phenes and/or phenotypic plasticity may be synergistic or antagonistic, and further studies are needed to fully understand these interactions.

Identifying genes that control root phenes and their plastic expression will provide tools for breeders to (a) develop crops better adapted to a wide range of environments and management practices or (b) develop lines with defined plasticity to be grown in targeted agroecosystems. Phenotypic plasticity and interactions between phenes will require further research to understand their implications for edaphic stress tolerance.

ACKNOWLEDGMENTS

This research was supported by the USDA National Institute of Food and Agriculture, Award no. 2014-67013-21572, USDOE ARPA-E ROOTS Award no. DE-AR0000821, and Hatch Project no. 4582. This research was also supported by the Howard G. Buffet Foundation. We thank Robert Snyder, Curtis Fredrick, Johan Prinsloo, and Patricio Cid for technical support.

ORCID

Jonathan P. Lynch
https://orcid.org/0000-0002-7265-9790

REFERENCES

Arora, K., Panda, K. K., Mittal, S., & Mallikarjuna, M. G. (2017). RNAseq revealed the important gene pathways controlling adaptive mechanisms under waterlogged stress in maize. *Scientific Reports*, *7*, 1–12.

Basford, K. E., & Cooper, M. (1998). Genotype × environment interactions and some considerations of their implications for wheat breeding in Australia. *Australian Journal of Agricultural Research*, *49*, 153–174.

Bates, D., Machler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using {lme4}. *Journal of Statistical Software*, *67*, 1–48.

Bioconductor. (2002). Bioconductor [Software]. Retrieved from www.bioconductor.org

Brisson, N., Gate, P., Gouache, D., Charmet, G., Oury, F. X., & Huard, F. (2010). Why are wheat yields stagnating in Europe? A comprehensive data analysis for France. *Field Crops Research*, *119*, 201–212.

Burton, A. L., Johnson, J., Foerster, J., Hanlon, M. T., Kaeppler, S. M., Lynch, J. P., & Brown, K. M. (2014). QTL mapping and phenotypic variation of root anatomical traits in maize (*Zea mays* L.). *Theoretical and Applied Genetics*, *128*, 93–106.

Burton, A. L., Williams, M., Lynch, J. P., & Brown, K. M. (2012). RootScan: Software for high-throughput analysis of root anatomical traits. *Plant and Soil*, *357*, 189–203.

Cheng, N-H., Pittman, J., Bronwyn, B., Shigaki, T., & Hirschi, K. D. (2003). The Arabidopsis *cax1* mutant exhibits impaired ion homeostasis, development, and hormonal responses and reveals interplay among vacuolar transporters. *The Plant Cell*, *15*, 347–364.

Cheng, N-H., Pittman, J., Shigaki, T., Lachmansingh, J., LeClere, S., Lahner, B., … & Hirschi, K. D. (2005). Functional association of Arabidopsis CAX_1 and CAX_3 is required for normal growth and ion homeostasis. *Plant Physiology*, *138*, 1194–1206.

Chimungu, J., Brown, K., & Lynch, J. (2014a). Large root cortical cell size improves drought tolerance in maize (*Zea mays* L.). *Plant Physiology*, *166*, 2166–2178.

Chimungu, J., Brown, K., & Lynch, J. (2014b). Reduced root cortical cell file number improves drought tolerance in maize. *Plant Physiology*, *166*, 1943–1955.

Chimungu, J., Loades, K., & Lynch, J. (2015a). Root anatomical phenes predict root penetration ability and biomechanical properties in maize (*Zea mays*). *Journal of Experimental Botany*, *66*, 3151–3162.

Chimungu, J. G., Maliro, M. F. A., Nalivata, P. C., Kanyama-Phiri, G., Brown, K. M., & Lynch, J. P. (2015b). Utility of root cortical aerenchyma under water limited conditions in tropical maize (*Zea mays* L.). *Field Crops Research*, *171*, 86–98.

Cooper, M., Rajatasereekul, S., Immark, S., Fukai, S., & Basnayake, J. (1999). Rainfed lowland rice breeding strategies for Northeast Thailand: I. Genotypic variation and genotype × environment interactions for grain yield. *Field Crops Research*, *64*, 131–151.

Drew, M. C., He, C., & Morgan, P. W. (2000). Programmed cell death and aerenchyma formation in roots. *Trends in Plant Science*, *5*, 123–127.

Evans, D. E. (2003). Aerenchyma formation. *New Phytologist*, *161*, 35–49.

Fehr, W. (1993). *Principles of cultivar development*. New York: Macmillan.

Feng, W., Lindner, H., Robbins, N. E., & Dinneny, J. R. (2016). Growing out of stress: The role of cell- and organ-scale growth control in plant water-stress responses. *The Plant Cell*, *28*, 1769–1782.

Galindo-Castañeda, T., Brown, K. M., & Lynch, J. P. (2018). Reduced root cortical burden improves growth and grain yield under low phosphorus availability in maize. *Plant Cell and Environment*, *41*, 1579–1592.

Gao, M., Wang, X., Wang, D., Xu, F., Ding, X., Zhang, Z., … & Zhang, Y. (2009). Regulation of cell death and innate immunity by two receptor-like kinases in Arabidopsis. *Cell Host and Microbe*, *6*, 34–44.

Gerlach, N., Schmitz, J., Polatajko, A., Schlüter, U., Fahnenstich, H., Witt, S., … & Bucher, M. (2015). An integrated functional approach to dissect systemic responses in maize to arbuscular mycorrhizal symbiosis. *Plant, Cell and Environment*, *38*, 1591–1612.

Gicaraya, V. A. G., Cadiz, N. M., & Henry, A. (2017). Effects of aerenchyma formation on the root porosity, lateral root development, total water uptake and shoot biomass accumulation of global magic rice under seedling-stage drought stress. *Journal of the International Society for Southeast Asian Agricultural Sciences*, *23*, 192–202.

Gowda, V. R. P., Henry, A., Yamauchi, A., Shashidhar, H. E., & Serraj, R. (2011). Root biology and genetic improvement for drought avoidance in rice. *Field Crops Research*, *122*, 1–13.

Hall, B., Lanba, A., & Lynch, J. (2019). Three-dimensional analysis of biological systems via a novel laser ablation technique. *Journal of Laser Applications*, *31*, 022602.

Hansey, C. N., Johnson, J. M., Sekhon, R. S., Kaeppler, S. M., & De Leon, N. (2011). Genetic diversity of a maize association population with restricted phenology. *Crop Science*, *51*, 704–715. https://doi.org/10.2135/cropsci2010.03.0178

Henry, A., Gowda, V. R. P., Torres, R. O., McNally, K. L., & Serraj, R. (2011). Variation in root system architecture and drought response in rice (*Oryza sativa*): Phenotyping of the OryzaSNP panel in rainfed lowland fields. *Field Crops Research*, *120*, 205–214.

Jaramillo, R. E., Nord, E. A., Chimungu, J. G., Brown, K. M., & Lynch, J. P. (2013). Root cortical burden influences drought tolerance in maize. *Annals of Botany*, *112*, 429–437.

Jiao, Y., Peluso, P., Shi, J., Liang, T., Stitzer, M. C., Wang, B., … & Ware, D. (2017). Improved maize reference genome with single-molecule technologies. *Nature*, *546*, 524–527.

Kadam, N. N., Tamilselvan, A., Lawas, L. M. F., Quinones, C., Bahuguna, R. N., Thomson, M. J., … & Jagadish, S. V. K. (2017). Genetic control of plasticity in root morphology and anatomy of rice in response to water-deficit. *Plant Physiology*, *174*, 2302–2315.

Kim, M. J., Park, M.-J., Seo, P. J., Song, J.-S., Kim, H.-J., & Park, C.-M. (2012). Controlled nuclear import of the transcription factor NTL6 reveals a cytoplasmic role of SnRK2.8 in the drought-stress response. *Biochemical Journal*, *448*, 353–363.

Lawrence, C. J. (2005). The maize genetics and genomics database: The community resource for access to diverse maize data. *Plant Physiology*, *138*, 55–58.

Liu, X., Huang, M., Fan, B., Buckler, E. S., & Zhang, Z. (2016). Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLOS Genetics*, *12*, e1005767.

Lobet, G., Paez-Garcia, A., Schneider, H., Junker, A., Atkinson, J. A., & Tracy, S. (2019). Demystifying roots: A need for clarification and extended concepts in root phenotyping. *Plant Science*, *282*, 11–13.

Lynch, J. P. (2011). Root phenes for enhanced soil exploration and phosphorus acquisition: Tools for future crops. *Plant Physiology*, *156*, 1041–1049.

Lynch, J. P. (2013). Steep, cheap and deep: An ideotype to optimize water and N acquisition by maize root systems. *Annals of Botany*, *112*, 347–357.

Lynch, J. P. (2015). Root phenes that reduce the metabolic costs of soil exploration: Opportunities for 21st century agriculture. *Plant Cell & Environment*, *38*, 1775–1784.

Lynch, J. P. (2018). Rightsizing root phenotypes for drought resistance. *Journal of Experimental Botany*, *69*, 3279–3292.

Lynch, J. P. (2019). Root phenotypes for improved nutrient capture: An underexploited opportunity for global agriculture. *New Phytologist*, *223*(2), 548–564.

Lynch, J. P., & Brown, K. M. (2001). Topsoil foraging: An architectural adaptation of plants to low phosphorus availability. *Plant and Soil*, *237*, 225–237.

Lynch, J. P., & Brown, K. M. (2012). New roots for agriculture: Exploiting the root phenome. *Philosophical Transactions of the Royal Society, Series B*, *367*, 1598–1604.

Lynch, J. P., & Wojciechowski, T. (2015). Opportunities and challenges in the subsoil: Pathways to deeper rooted crops. *Journal of Experimental Botany*, *66*, 2199–2210.

Mano, Y., Omori, F., Muraki, M., & Takamizo, T. (2005). QTL mapping of adventitious root formation under flooding conditions in tropical maize (*Zea mays* L.) seedlings. *Breeding Science*, *55*, 343–347.

Mano, Y., Omori, F., Takamizo, T., Kindiger, B., Bird, R. M., Loaisiga, C. H., & Takahashi, H. (2007). QTL mapping of root aerenchyma formation in seedlings of a maize × rare teosinte "*Zea nicaraguensis*" cross. *Plant and Soil*, *295*, 103–113.

Manschadi, A. M., Christopher, J., deVoil, P., & Hammer, G. L. (2006). The role of root architectural traits in adaptation of wheat to water-limited environments. *Functional Plant Biology*, *33*, 823.

Marshall, A., Aalen, R. B., Audenaert, D., Beeckman, T., Broadley, M. R., Butenko, M. A., … Smet, I. D. (2012). Tackling drought stress: RECEPTOR-LIKE KINASES present new approaches. *The Plant Cell*, *24*, 2262–2278.

Mazaheri, M., Heckwolf, M., Vaillancourt, B., Gage, J. L., Burdo, B., Heckwolf, S., … & Kaeppler, S. M. (2019). Genome-wide association analysis of stalk biomass and anatomical traits in maize. *BMC Plant Biology 19*, 45.

Miguel, M. A., Postma, J. A., & Lynch, J. (2015). Phene synergism between root hair length and basal root growth angle for phosphorus acquisition. *Plant Physiology*, *167*, 1430–1439.

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., … Kleunen, M. V. (2010). Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, *15*, 684–692.

Niones, J. M., Suralta, R. R., Inukai, Y., & Yamauchi, A. (2012). Field evaluation on functional roles of root plastic responses on dry matter production and grain yield of rice under cycles of transient soil moisture stresses using chromosome segment substitution lines. *Plant and Soil*, *359*, 107–120.

Niones, J. M., Suralta, R. R., Inukai, Y., & Yamauchi, A. (2013). Roles of root aerenchyma development and its associated QTL in dry matter production under transient moisture stress in rice. *Plant Production Science*, *16*, 205–216.

Pan, X., Hasan, M. M., Li, Y., Liao, C., Zheng, H., Liu, R., & Li, X. (2015). Asymmetric transcriptomic signatures between the cob and forets in the maize ear under optimal- and low-nitrogen conditions at silking, and functional characterization of amino acid transporters ZmAAP4 and ZmVAAT3. *Journal of Experimental Botany*, *66*, 6149–6166.

Pieruschka, R., & Poorter, H. (2012). Phenotyping plants: Genes, phenes and machines. *Functional Plant Biology*, *39*, 813–820.

Postma, J. A., Dathe, A., & Lynch, J. (2014). The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. *Plant Physiology*, *166*, 590–602.

Postma, J. A., & Lynch, J. P. (2011). Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiology*, *156*, 1190–1201.

Prince, S. J., Murphy, M., Mutava, R. N., Durnell, L. A., Valliyodan, B., Shannon, J. G., & Nguyen, H. T. (2017). Root xylem plasticity to improve water use and yield in water-stressed soybean. *Journal of Experimental Botany*, *66*, 2027–2036.

R Core Team. (2018). *R: A language and environment for statistical computing. Version 3.3.1*. Vienna: R Foundation for Statistical Computing.

Richards, R. A., & Passioura, J. B. (1989). A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. *Australian Journal of Agricultural Research*, *40*, 943–950.

Saengwilai, P., Nord, E. A., Chimungu, J. G., Brown, K. M., & Lynch, J. P. (2014). Root cortical aerenchyma enhances nitrogen acquisition from low-nitrogen soils in maize. *Plant Physiology*, *166*, 726–735.

Sandhu, N., Raman, K. A., Torres, R. O., Audebert, A., Dardou, A., Kumar, A., & Henry, A. (2016). Rice root architectural plasticity traits and genetic regions for adaptability to variable cultivation and stress conditions. *Plant physiology*, *171*, 2562–2576.

Sharma, S., Demason, D. A., Ehdaie, B., Lukaszewski, A. J., & Waines, J. G. 2010. Dosage effect of the short arm of chromosome 1 of rye on root morphology and anatomy in bread wheat. *Journal of Experimental Botany*, *61*, 2623–2633.

Sharp, R. E., & LeNoble, M. E. (2002). ABA, ethylene and the control of shoot and root growth under water stress. *Journal of Experimental Botany*, *53*, 33–37.

Sosa, J. M., Huber, D. E., Welk, B., & Fraser, H. L. (2014). Development and application of MIPAR^{TM}: A novel software package for two- and three-dimensional microstructural characterization. *Integrating Materials and Manufacturing Innovation*, *3*, 10.

Striker, G. G., Insausti, P., Grimoldi, A. A., & Vega, A. S. (2007). Trade-off between root porosity and mechanical strength in species with different types of aerenchyma. *Plant, Cell & Environment*, *30*, 580–589.

Su, S., Wu, L., Liu, D., Lu, Y.-L., Lin, H.-J., Zhang, S.-Z., … Gao, S. (2014). Genome-wide expression profile of maize root response to phosphorus deficiency revealed by deep sequencing. *Journal of Integrative Agriculture*, *13*, 1216–1229.

Sultan, S. E. (2000). Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*, *5*, 537–542.

Sun, B., Gao, Y., & Lynch, J. (2018). Large crown root number improves topsoil foraging and phosphorus acquisition. *Plant Physiology*, *177*, 90–104.

Tardieu, F., Draye, X., & Javaux, M. (2017). Root water uptake and ideotypes of the root system: Whole-plant controls matter. *Vadose Zone Journal*, *16*, 1–10.

Tebaldi, C., & Lobell, D. B. (2008). Towards probabilistic projections of climate change impacts on global crop yields. *Geophysical Research Letters*, *41*, 1–14.

Topp, C. N. (2016). Hope in change: The role of root plasticity in crop yield stability. *Plant Physiology*, *172*, 5–6.

Trachsel, S., Kaeppler, S. M., Brown, K. M., & Lynch, J. P. (2011). Shovelomics: High throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil*, *341*, 75–87.

Uga, Y., Okuno, K., & Yano, M. (2008). QTLs underlying natural variation in stele and xylem structures of rice root. *Breeding Science*, *58*, 7–14.

Uga, Y., Okuno, K., & Yano, M. (2010). Fine mapping of Sta1, a quantitative trait locus determining stele transversal area, on rice chromosome 9. *Molecular Breeding*, *26*, 533–538.

Usadel, B., Poree, F., Nagel, A., Lohse, M., Czedik-Eysenberg, A., & Stitt, M. (2009). A guide to using MapMan to visualize and compare Omics data in plants: A case study in the crop species, maize. *Plant, Cell and Environment*, *32*, 1211–1229.

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. V. S., Rebetzke, G. J., … Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, *63*, 3485–3498.

Woods, J., Williams, A., Hughes, J. K., Black, M., & Murphy, R. (2010). Energy and the food system. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*, 2991–3006.

Xu, J., Yuan, Y., Xu, Y., Zhang, G., Guo, X., Wu, F., … Lu, Y. (2014). Identification of candidate genes for drought tolerance by whole-genome resequencing in maize. *BMC Plant Biology*, *14*, 1–15.

Yang, J., Schneider, H., Brown, K., & Lynch, P. (2019). Genotypic variation and nitrogen stress effects on root anatomy in maize are node-specific. *Journal of Experimental Botany*, *70*, 5311–5325.

York, L. M., Nord, E. A., & Lynch, J. P. (2013). Integration of root phenes for soil resource acquisition Integration of root phenes for soil resource acquisition. *Frontiers in Plant Science*, *4*, 355.

Zargar, S. M., Nagar, P., Deshmukh, R., Nazir, M., Wani, A. A., Masoodi, K. Z., … Rakwal, R. (2017). Aquaporins as potential drought tolerance inducing proteins: Toward instigating stress tolerance. *Journal of Proteomics*, *169*, 233–238.

Zhang, Z., Ersoz, E., Lai, C-Q., Todhunter, R. J., Tiwari, H. K., Gore, M. A., … Buckler, E. S. (2010). Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics*, *42*, 355–360.

Zhu, J., Brown, K. M., & Lynch, J. P. (2010a). Root cortical aerenchyma improves the drought tolerance of maize (*Zea mays* L.). *Plant, Cell & Environment*, *33*, 740–749.

Zhu, J., Ingram, P. A., Benfey, P. N., & Elich, T. (2011). From lab to field, new approaches to phenotyping root system architecture. *Current Opinion in Plant Biology*, *14*, 310–317.

Zhu, J. M., & Lynch, J. P. (2004). The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays*) seedlings. *Functional Plant Biology*, *31*, 949–958.

Zhu, J., Zhang, C., & Lynch, J. P. (2010b). The utility of phenotypic plasticity of root hair length for phosphorus acquisition. *Functional Plant Biology*, *37*, 313–322.

Zhuang, Y. L., Ren, G. J., Zhu, Y., Hou, G. H., Qu, X., Li, Z. X., … Zhang, J. R. (2008). Transcriptional profiles of immature ears and tassels in maize at early stage of water stress. *Biologia Plantarum*, *52*, 754–758.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Schneider HM, Klein SP, Hanlon MT, Kaeppler S, Brown KM, Lynch JP. Genetic control of root anatomical plasticity in maize. *Plant Genome*. 2020;e20003. https://doi.org/10.1002/tpg2.20003