GETTING TO THE ROOT CAUSE: THE GENETIC UNDERPINNINGS OF ROOT SYSTEM ARCHITECTURE AND RHIZODEPOSITION IN SORGHUM

Farren Smith
West Virginia University, fs0034@mix.wvu.edu

Follow this and additional works at: https://researchrepository.wvu.edu/etd

Part of the Agricultural Science Commons, Agriculture Commons, Agronomy and Crop Sciences Commons, Biodiversity Commons, Bioinformatics Commons, Biology Commons, Botany Commons, Computational Biology Commons, Developmental Biology Commons, Evolution Commons, Food Biotechnology Commons, Genetics Commons, Genomics Commons, Horticulture Commons, Molecular Biology Commons, Molecular Genetics Commons, Other Ecology and Evolutionary Biology Commons, Other Food Science Commons, Other Plant Sciences Commons, Plant Biology Commons, and the Plant Breeding and Genetics Commons

Recommended Citation
Smith, Farren, "GETTING TO THE ROOT CAUSE: THE GENETIC UNDERPINNINGS OF ROOT SYSTEM ARCHITECTURE AND RHIZODEPOSITION IN SORGHUM" (2022). Graduate Theses, Dissertations, and Problem Reports. 11438.
https://researchrepository.wvu.edu/etd/11438

This Thesis is protected by copyright and/or related rights. It has been brought to you by the The Research Repository @ WVU with permission from the rights-holder(s). You are free to use this Thesis in any way that is permitted by the copyright and related rights legislation that applies to your use. For other uses you must obtain permission from the rights-holder(s) directly, unless additional rights are indicated by a Creative Commons license in the record and/or on the work itself. This Thesis has been accepted for inclusion in WVU Graduate Theses, Dissertations, and Problem Reports collection by an authorized administrator of The Research Repository @ WVU. For more information, please contact researchrepository@mail.wvu.edu.
GETTING TO THE ROOT CAUSE: THE GENETIC UNDERPINNINGS OF ROOT SYSTEM ARCHITECTURE AND RHIZODEPOSITION IN SORGHUM

Farren Smith

Thesis submitted to the Eberly College of Arts and Sciences at West Virginia University in partial fulfillment of the requirements for the degree of Master of Science in Biology

Jennifer S. Hawkins, Ph.D., Chair
Jonathan Cumming, Ph.D.
Edward Brzostek, Ph.D.

Department of Biology

Morgantown, West Virginia
2022

Keywords: Sorghum, root system architecture, rhizodeposition, exudates, morphology, metabolic, quantitative trait loci, domestication, root phenotyping, genetic mapping, recombinant inbred lines

Copyright 2022 Farren Smith
Plants are some of the most diverse organisms on earth, consisting of more than 350,000 different species. To understand the underlying processes that contributed to plant diversification, it is fundamental to identify the genetic and genomic components that facilitated various adaptations over evolutionary history. Most studies to date have focused on the underlying controls of above-ground traits such as grain and vegetation; however, little is known about the “hidden half” of plants. Root systems comprise half of the total plant structure and provide vital functions such as anchorage, resource acquisition, and storage of energy reserves. The execution of these key functions via root system architecture and root exudation directly determines plant performance, and thus reproductive fitness. Despite the significance of roots, the genetic controls contributing to their variation have gone understudied due to the technical difficulties associated with below-ground phenotyping.

Domesticated plants provide an excellent framework for studying the genomic underpinnings of phenotypic diversity due to the telescoped evolutionary time frame under which artificial selection took place. The rapid evolution of domesticates from their extant antecedent affords direct observations of derived traits and their underlying genetic controls. *Sorghum*, a globally important domesticated grass species with a small diploid genome, few genetic repeats, and a wide variety of adaptations, serves as a good model for studying selection during domestication. Domesticated *S. bicolor* is an annual accession with large seeds and flowering organs compared to its wild relative, *S. propinquum*. Comparatively, *S. propinquum* is perennial with dense rhizomes and small flowering panicles. Due to their distinctly opposing root systems, a recombinant inbred line (RIL) population formed from a cross between *S. bicolor* and *S. propinquum* was used to identify the specific root morphological and metabolic adaptations that derived from domestication. The RIL population was phenotyped using high-throughput image analysis to locate the underlying genomic factors controlling derived traits via high-density Quantitative Trait Loci (QTL) mapping. Nine novel QTL influencing root morphology were identified. No QTL were identified for metabolic exudation; however, crown root growing angle was found to be a statistically significant predictor of the percentage of carbon and nitrogen in the rhizosphere. The relationship between steep growing angles and increased rhizosphere carbon and nitrogen suggests that increased
exudation was derived during domestication. Candidate genes and pathways were identified including those that encode meristem transcription factors, plant hormone receptors, and actin trafficking. These findings advance our understanding of the underlying genomic factors controlling root system architecture (RSA) and root exudation that were selected during the domestication of *Sorghum*. The results of this study can be integrated into breeding programs for the establishment of elite root lines used to mitigate the effects of current and future environmental challenges of croplands in a sustainable manner.
# Table of Contents

ii. Abstract  
iii. Table of Contents  
1. Chapter 1: Overview and Objectives ................................................................. pg. 1  
2. Chapter 2: Background ......................................................................................... pg. 2  
   2.1. Domestication  
   2.2. Roots  
   2.3. Root Morphology  
   2.4. Root Exudation  
   2.5. Sorghum as a Model  
   2.6. QTL Mapping  
   2.7. Phenotyping  
      2.7.1. Morphological Quantification  
      2.7.3. Exudation Quantification  
   2.8. Research Aim 1  
   2.8.1. Hypothesis  
   2.9. Research Aim 2  
   2.9.1. Hypothesis  
3. Works Cited ............................................................................................................ pg. 12  
   4.1. Introduction ...................................................................................................... pg. 19  
   4.2. Materials and Methods ................................................................................... pg. 20  
      4.2.1. Plant Material  
      4.2.2. Experimental Conditions and Excavation  
      4.2.3. High-throughput Imaging  
      4.2.4. Rhizosphere and Biomass Collection  
      4.2.5. Image Analysis of Crown Root Angle and Root Color  
      4.2.6. High-throughput Image Analysis  
      4.2.7. Rhizosphere Pre-processing and Analysis  
      4.2.8. Statistical Analyses  
         4.2.8.1. Percent Error  
         4.2.8.2. Radial Symmetry  
         4.2.8.3. Least-square Means  
         4.2.8.4. Trait Correlations and Regression Analyses  
   4.2.9. Genetic Map Construction and QTL Analysis  
   4.2.10. Candidate Genes and Expression  
   4.3. Results ............................................................................................................. pg. 25  
      4.3.1. Morphological Factors of Root Domestication
4.3.1.1. QTL Identified as Important Regulators of Morphology
4.3.2. Metabolic Factors of Root Domestication
   4.3.2.1. RSA was Identified as a Likely Regulator of Exudation
4.3.3. Above-ground Model Predictions of RSA Traits

4.4. Discussion
   4.4.1. Root Morphology QTL
   4.4.2. Carbon Exudation
      4.4.2.1. Metabolic Relationship with Morphology
   4.4.3. Domestication of Sorghum
      4.4.3.1. Domestication and Root Morphology
      4.4.3.2. Domestication and Carbon Exudation
      4.4.3.3. The Relationship between Above- and Below-ground Traits

4.5. Conclusion
   4.5.1. Future Directions
   4.5.2. Implications

5. Acknowledgements

6. Works Cited

7. Tables

8. Figures
Chapter 1

Overview and Objectives

Root system architecture (RSA) and root exudation are essential for the exploration and acquisition of soil resources. Therefore, these traits directly influence agronomic yield. There is limited understanding, however, of the genomic underpinnings controlling these traits due to the challenges of observing phenes underground.

Domestication through artificial selection led to accelerated phenotypic diversification wherein the domesticated descendants often naturally coexist with their extant antecedents. Therefore, comparisons between domesticated species and their wild relatives provide an excellent framework for the identification of genes and biological pathways controlling traits that contribute to plant diversification over evolutionary time.

To identify the molecular mechanisms that contribute to diversity in RSA and rhizodeposition, novel methodologies for phenotyping the root systems of an advanced Sorghum recombinant inbred line (RIL) population will be employed. Phenotypic measurements and whole-genome sequences of each RIL, formed from a cross between domesticated S. bicolor and wild S. propinquum, were utilized in high-density QTL mapping to answer the following questions:

1. What are the genomic underpinnings that predict RSA in addition to those that regulate carbon exudation in Sorghum?

2. Do loci that influence RSA colocalize with those that influence exudation, suggesting a relationship and shared biological pathway?

3. What are the candidate genes within significant loci that may influence the respective phenotypes?

4. Can above-ground phenotypes predict below-ground phenotypes, suggesting a shared biological pathway that was selected during domestication?

5. Are there key root traits indicative of artificial selections during Sorghum domestication?

This work will aid in identifying the genomic underpinnings of morphological development and rhizodeposition of root systems in a globally important grain crop. Further, this study lays the groundwork for the exploration of polymorphisms controlling RSA that can be used for introgressive breeding of elite-lines with improved root traits in agroeconomically important grain species. Because of Sorghum’s close evolutionary relationship with other important grain crops, our findings are likely to be applicable to related species such as maize, sugarcane, and millet.
Chapter 2

Background

Plants are some of the most diverse eukaryotic organisms on earth, consisting of more than 350,000 different species with more than 75 percent being flowering plants (Christenhusz, et al., 2016). To understand the underlying process that contributed to plant diversification and speciation, it is imperative to identify the genetic and genomic components that were selected over evolutionary history. Although such processes are challenging to identify due to the extinction of antecedents and intermediary descendants, the rapid evolutionary descent of domesticated species through artificial selection often permits the coexistence of descendants and antecedents. Thus, domesticated models afford an excellent framework to directly observe derived traits and to dissect the genomic components of diversification.

Domestication

Agronomic domestication began over 10,000 years ago with the anthropogenic selection of plants and plant traits that influenced the societal shift from hunter-gatherer to organized communities. The cultivation of selected plants with desirable traits allowed human populations to establish sustainable permanent settlements through agricultural production systems (Bellwood, 2004). Archeological records indicate that many phenotypic variations of crops that humans largely depend on today, such as maize, wheat, and rice, arose from neolithic selection pressures. Compared to wild progenitors, many common domesticated traits exist across important agricultural species, referred to as the “domestication syndrome” (Hammer, 1985). This suite of traits includes larger seeds and flowering organs, a reduction in the number of tillers, loss of seed dispersal, and loss of photoperiod sensitivity (Doebley, et al., 2006; Pickersgill, 2007). Many of these above-ground traits have been studied to identify the genomic underpinnings that resulted in the derivation of domesticated phenotypes and the overall loss of genetic diversity (Doebley, et al., 2006; Gross, et al., 2010; Flint-Garcia, 2013). The derived below-ground phenotypic root traits and their respective genomic underpinnings, however, are largely understudied.

Roots

The foundation of healthy plant vigor starts with roots which provide water and nutrients for cellular growth and a reservoir for energy reserves (Surówka, et al., 2020). The structure of the roots directly determines the extent of the main functions; absorption, storage, anchorage, and conduction, as well as inter- and intra-specific
interactions with nearby plant and microbial species (Moore, et al., 2021; Slovak, et al. 2016). The structure of roots is further classified by the shape, size, distribution, and spatial arrangement termed root system architecture (RSA) (Khan, et al., 2016; Ye, et al., 2018). Monocotyledonous plants (monocots) have fibrous root systems that are composed of primary roots that form from the seed during embryogenesis, adventitious (crown and brace) roots that form from the stem, and lateral roots which form as offshoots during maturation. The internal structure of a monocotyledonous root consists of the outermost layer, termed the epidermis, inwardly followed by the cortex. The epidermis is responsible for nutrient and water absorption that is diffused through the cortex toward the endodermis. The endodermis contains the Casparian strip, which regulates the passage of solutes through a semipermeable plasma membrane, and the pericycle which is responsible for lateral root initiation. Inside of the endodermis lies the vascular tissues, xylem, and phloem. Xylem is responsible for the upward transport of water and phloem is responsible for the downward transport of metabolic sugars (Wachsman, et al., 2015; Bellairs, et al., 2021).

Most of the new growth and development occurs in the root tip and is composed of three generalized zones. The zone of maturation contains the beginning of the differentiation of specialized cells such as the xylem and phloem as well as root hairs. The zone of elongation is where primary tissues begin forming from the procambium and increase in length. Lastly, at the farthest tip of the root, the zone of division contains active locations of mitosis: the root cap, root apical meristem (RAM), and procambium (Figure 1). Growth is regulated by the synthesis of auxin (IAA) and cytokinin (CK) which are influenced by the plant’s developmental age and environmental conditions. IAA is transported to root tips from shoots and/or generated in the root tip where it is redirected upward in root epidermal cells, creating a gradient. IAA synthesis, transport, and signaling mutants have been shown to influence the capacity to initiate, emerge, and/or elongate lateral root growth (Fukaki, et al., 2009). Similarly, CK is synthesized in root tips and transported upward through plasmodesmata. CK, however, negatively regulates root growth and development by modifying the number of dividing cells in the RAM (Werner, et al., 2003; Aloni, et al., 2006). Although it is evident that IAA and CK gradients control RSA development, it is still unclear how cells decode this information.

The two most limiting nutrients, phosphates and nitrates, reside in spatially opposing soil layers. Inorganic and immobile phosphates accumulate in the top soil layers, whereas mobile nitrates leach with water and accumulate in deeper soil horizons, illustrating the need for root plasticity. Root systems that are optimally adapted will be efficient in both water and nutrient uptake, which is influenced by two important traits, morphology and exudation. Root morphology and exudation directly dictate compatibility to the surrounding soil environment and thus promote adequate vegetative growth and optimal crop yield (Sharma S, et al., 2021; de la Fuente Cantó, et al., 2020).
**Root Morphology**

Plant roots are extremely variable in their morphology and influenced by myriad factors such as physiology, developmental age, genetic makeup, and environmental cues. Though plants are sessile, morphological root modifications allow resistance to biotic and abiotic stressors. For instance, during nitrate deficiency, primary root elongation is promoted to reach deeper soil layers and new lateral root initiation is halted (Saengwilai, et al., 2014). Contrastingly, root architecture shifts from primary root growth to lateral root initiation during phosphate deficiency. An increase in the expression of an auxin receptor (TIR1) promotes auxin sensitivity during phosphate deficiency, leading to degradation of auxin response modules and thus, lateral root initiation (Pérez-Torres, et al., 2008). This modification, observed in *Arabidopsis*, leads to a shallow root system ideal for phosphate foraging (Gruber et al., 2013).

Groundwater is typically stored in deeper soil layers and plants that are structured to develop deeper root systems subsequently will be more efficient in water acquisition. This is extremely important during drought conditions especially as global temperatures rise due to climate change. Deep root development is likely controlled via abscisic acid (ABA). Mutant *Arabidopsis* with drought tolerance was shown to display high sensitivity to ABA and strong inhibition of lateral rooting (Xiong et al., 2006). Further, Rowe et al. (2016) documented that ABA inhibited auxin-responsive *PIN1* expression during osmotic stress which reduced auxin transport and lateral root formation.

**Root Exudation**

The regulation of nutrient acquisition through adjustments in carbon exudation allows sessile plants to obtain resources while conserving energy used during morphological root modification (Wen, et al., 2002). Root exudates are organic substances that are secreted by the root systems of plants into the surrounding soil interface, termed the rhizosphere (Koo, et al., 2004). Plants contribute between 20-40 percent of their fixed carbon to root exudation, consisting predominantly of primary metabolites; amino acids, organic acids, and sugars (Pétriacq, et al., 2017; Canarini, et al., 2019). These metabolites are known to play a distinct role in microbial interactions and supply, signal transduction, and solubilization of inactive nutrients. As roots interact with the soil substrate for anchorage and acquisition of nutrients, they also begin to alter the rhizosphere both chemically and physically.

Organic acids such as malic (C₄H₆O₅) and citric (C₆H₈O₇) acid have been documented to increase in the rhizosphere when plants were grown in phosphorus-deficient environments, indicating that they either play roles in the mobility of inorganic phosphorus or in the signaling of root expansion (Koo, et al., 2004; Canarini, et al.)
During phosphorus deficiency, RSA is optimized by reducing primary root growth and initiating lateral root growth, spreading surface area to scavenge nutrients in the subsoil layer (Imai et al., 2010). Researchers found that malate, the conjugate base of malic acid, plays a role in the inhibition of mitotic division at the terminal root meristem of *Arabidopsis* (*STOP1*, a transcription factor; and *ALMT1*, a malate transporter), thus promoting the proliferation of lateral root growth (Mora-Macías, et al., 2017).

Carbohydrates such as fructose (C$_6$H$_{12}$O$_6$) and sucrose (C$_{12}$H$_{22}$O$_{11}$) are essential to mycorrhizal fungi root colonization. Higher levels of carbohydrates exuded into the rhizosphere stimulate the growth and germination of symbiotic mycorrhizal fungi, exploiting a larger volume of soil for nutrient/water uptake and defense against root pathogens without contributing large metabolic outputs towards RSA modification (Dighton, J., 2009; Carvalhais, et al., 2011).

Primary metabolites, amino acids, organic acids, and sugars, exuded by roots flow through efflux carriers and channels (Badri et al., 2008). Protein-mediated rhizodeposition can occur either by ambiguous transporters such as ABC transporters, or by compound-unique transporters such as organic acid MATE transporters (Vives-Peris, et al., 2020). Thus, exudate levels can be adjusted through up- and down-regulation of gene expression and/or post-translational modification of efflux facilitators (Badri et al., 2008). For example, SWEET and UMAMIT transporters have been characterized for cellular efflux of amino acid and carbohydrate exudates in *Arabidopsis*. However, little is known about what genes are responsible for their synthesis nor what environmental and/or nutrient demands influence their transcription (Canarini, et al. 2019). The rhizosphere, as outlined, is highly dynamic. Despite the fact that exudation aids in ecological interactions and acquisition of limited mineral resources which contributes to overall plant vitality and optimal crop yield, the process is poorly understood.

**Sorghum as a Model**

*Sorghum* is a globally important agricultural cereal grain and biofuel crop and is closely related to other important domesticated species such as maize, sugarcane, and millet. Domestication and regional selection pressure over time, followed by human-directed introgression of landraces for the improvement of crop lines, has led to diverse phenotypic traits among *Sorghum* cultivars (Smith, et al., 2019). Domestication from ancestral sorghum, *i.e.*, *Sorghum propinquum*, has led to annual landraces with agronomically desirable traits such as reduced tillering, compacted height, and increased grain yield, such as that seen in *Sorghum bicolor* (Lai, et al, 2018). The human-selected growth pattern of domesticated cultivars is primarily focused on quick nutrient acquisition for reproduction rather than on the establishment of energy reserves to sustain yearly vegetation (Zheng, et al., 2020; Jugpret, 2014).
All cereal grain crops are members of the *Poaceae* (grass) family which is one of the largest plant families that evolved from a common ancestor over 100 million years ago (mya) (Wang, et al., 2015). Across the major cereal domesticates (in respective order of global production) maize, wheat, rice, barley, sorghum, and millet, gene order is conserved across orthologous chromosomal regions (Paterson, A. H., Schertz, et. al. 1995; Awika, 2011). Sixty percent of annotated genes are syntenic and conserved among maize and sorghum which derived from a common ancestor 12 mya (Schnable, 2015). Syntenic genes are likely consistent in expression and retain ancestral function (Davidson et al., 2012; Zheng, et. al., 2020). However, unlike maize, sorghum has a comparatively small, ~772 megabase, diploid genome (2n=20) (Paterson, et al., 2009). Thus, having a small simple genome that shares synteny and gene order with other closely related and economically important grain crops, sorghum serves as a good model for the identification and functionality of genes among other related *Poaceae* species (Zhang, et al., 2018).

**QTL Mapping**

QTL mapping using recombinant inbred lines (RILs) is a powerful approach for identifying the genomic localizations that predict phenotypic variations, such as root morphology and exudation. QTL mapping is a genome-wide analysis that infers genomic loci, including effect and interaction, of phenotypic traits through correlation statistics (Zeng, 2001). RILs are created by repeated selfing of progeny from a cross between parental varieties/species that have opposing phenotypes. Each resulting progeny is an inbred line with a unique mosaic genome of parental alleles. Due to repeated sexual selfing, alleles become fixed at functional linkage groups, which when coupled with QTL mapping, are informative of functionality (Broman, 2005).

**Phenotyping**

A greater amount of genetic information due to the improvements in genotyping and sequencing technology has provided researchers the capability to generate an increased number of markers for use in QTL mapping. High-density markers offer greater genomic coverage and facilitate fine-mapping of loci that control phenotypic variation (Bekkering, et al., 2020; Furbank, Tester, 2011). However, many variations of plant roots have gone unnoticed and understudied because they are challenging to observe underground. The laborious complexity of excavation and measurement methodologies needed for phenotyping “the hidden half” has historically been the limiting factor in RSA QTL studies, leading to what is termed the “phenotyping bottleneck” (Paez-Garcia, Ana, et al., 2015; Furbank, Tester, 2011). Progression of multidisciplinary phenotyping methods and software analyses have emerged
accordingly, each with trade-offs in functionality, in an effort to widen the bottleneck. (Furbank, Tester, 2011).

**Morphological Quantification**

The first challenge encountered during root phenotyping is accessing the “hidden half” (Handakumbura, et al., 2021; Downie, et al., 2015). Multiple approaches have been taken ranging from manual excavation and cleaning in the field (termed “shovelomics” (Colombi, et al., 2015)), to controlled settings in pots or rhizo-boxes, to soilless growing media such as agar or hydroponics. Shovelomics of field-grown plants remains a relevant approach; however, as the name suggests, this technique is labor and time-intensive and requires large tracts of land (Trachsel, et al., 2011). Furthermore, shovelomics bestow difficulties when undergoing sizeably large genetic studies that require phenes to be captured at the same developmental stage. Contrastingly, controlled greenhouse settings cannot replicate abiotic environmental factors that roots will experience in applied field settings, but are useful for easy excavation of immature plant systems. Rhizo-boxes are excellent tools for collecting measurements of phenes that do not require excavation. The omittance of excavation eliminates disturbances and damage to roots experienced during the extraction and cleaning process; however, rhizo-boxes have a high financial overhead for material supplies and setups. Further, soilless media such as agar is not replicative of compressive organic media, and hydroponics systems require a water flow, washing roots of exudates/mucilage, and altering morphology. However, similar to rhizo-boxes, the use of soilless media eliminates the need for excavation and cleaning.

Additional challenges outside of the environmental setting involve the capture of root phenes. Current phenotyping methods predominantly rely on pipelines starting with imaging. Imaging allows multiple phenes of replicates to be captured succinctly at the same developmental stage, which is ideal for large QTL studies containing hundreds of replicates. Two general approaches used for imaging are (1) in-situ phenotyping which employs instruments such as X-ray and computed tomography (CT) scanning, and (2) high-throughput phenotyping which employs RGB imaging (Bodner, et al., 2018). In-situ approaches are non-destructive and are best at capturing the exploratory architecture of roots in media. Technological in-situ instruments, however, are expensive and require long processing times. In addition, in-situ instruments have difficulty discerning dead tissue, fine laterals, and root hairs (Teramoto, et al., 2020). High-throughput approaches are the least expensive, capable of discerning laterals and tissue color, but require destructive excavation and cannot adequately capture dense root entanglements (Danilevicz, et al., 2021; Teramoto, et al. 2020).

Following the acquisition of root images, the phenotyping pipeline requires the undertaking of image analysis (segmentation and quantitative measurement). Many software developments have been made for image analysis over the last decade but
are usually limited to specific experimental designs (Lobet, et al, 2013). Variations of segmentation software differ in the interface, cost, platform, imaging modalities, and segmentation process, among others (Lobet, et al, 2013). Software requiring the purchase of a license allows for more sophisticated measurements whereas open-source/freeware allows for greater accessibility and collective contributions from the scientific community. However, open-source software often requires the knowledge of coding languages. Software programs differ in the extent of automation of thresholding and skeletonization, ranging from fully automatic to fully manual (Cai, et al., 2015). Thresholding is the process in which color contrasted root tissue pixels are segmented from background pixels. Skeletonization is the process in which foreground regions are reduced to single-pixel lines, distinguishing lateral from primary roots based on pixel continuation and width. Fully automatic thresholding and skeletonization operations are useful for researchers that lack experience with graphical alterations and/or machine learning but limit the flexibility of inputs if modifications to the algorithm are needed. The need for a specific operating system can be an additional constraint when choosing an appropriate segmentation software, whereas web-based platforms offer operating system flexibility but are impractical if restricted data access is desired.

In summary, a “one size fits all” root phenotyping methodology without a negative tradeoff is improbable. RSA investigations must be narrowed to the methodology most appropriate for accessing and measuring predetermined root traits. Though phenotyping technology is catching up with the advancements made in the field of genomics, the experimental designs of RSA QTL studies still have many limitations, and thus specific genes regulating root morphological development are largely unidentified.

**Exudation Quantification**

Although carbon exudation has a clear relationship with RSA and nutrient acquisition, the question of what genes determine deposition and what role RSA configuration has in the regulation of deposition remains largely unanswered. This is primarily due to the inaccessibility of underground root structures and the methodologies of exudate collection. Investigations of exudate profiles of plants grown in soil are difficult to quantify due to the chemical heterogeneity of soil and microbial deposition (Oburguer, 2018). Because of this difficulty, researchers typically employ hydroponics, cuvette extraction, and/or in-vitro synthetic cultures for exudate collection. Hydroponic systems (static and percolating) provide a medium that limits the impedance of microbial consumption. However, the hydroponic system does not provide mechanical resistance to root structures which alters root architecture and exudation rates. Synthetic media, i.e., agar or filter paper, are suitable techniques for small spatial capture of exudates but hinder the identification of unknown exudate profiles. Cuvette extraction involves the excavation and cleaning of a fine root, followed by extraction with
a sterile cuvette over a period of days. The cuvettes are then vacuum flushed to remove and collect exudates from the site. This approach allows for in-vitro studies with natural microbial influence; however, it is extremely labor and time extensive (Phillips, et al., 2008; Vives-Peris, et al., 2020).

Following collection, mass profiling such as GC-MS and LC-MS (gas or liquid chromatography coupled with mass spectrometry) is used for the identification of unknown exudate compounds (Dundek, et al., 2011). Studies aiming to understand ecological carbon sinks and microbial consumption typically employ isotope-labeling and stable isotope probing to quantify total carbon exudation and microbial assimilation. Plants are grown in soil media and soil is extracted in-situ. Carbon isotopes from the extraction can be measured with an elemental analyzer coupled with a gas-isotope ratio mass spectrometer. This methodology relies on soil particles to be of a different carbon isotope than the carbon that is exuded. Isotope labeling can be completed through stem-/leaf-feeding or growing plants in a growth chamber that is injected with $^{13}$CO$_2$. This methodology is difficult to establish in-vitro, runs the risk of soil contamination, and/or is restricted to specific plant families and plant ages (Wichern, et al., 2008). Additionally, the expense of analytical instruments and isotope supplies can be limiting for large genomic studies.

In summary, typical investigations either take a carbon isotope probing (quantitative) approach or aim for the identification of unknown exudate chemical compositions (qualitative). However, all current methodologies for collecting and analyzing rhizodeposition have limitations and/or negative tradeoffs.

**Research Aim 1: Identification of genomic loci controlling root morphology that will provide the framework for understanding phenotypic variation and adaptation of RSA**

The modification of root morphology contributes to the optimization of absorption, conduction, storage, and stability, which increases plant fitness and thus agronomic yield. Plants are able to modify root morphology through cellular pathways (i.e., transcription factors and hormone signaling) if genetically predisposed. To delineate the genetic foundations that have contributed to derived root traits, a domesticated grass model under controlled environmental conditions was employed. The work outlined in Chapter 3 aimed to identify the genomic loci that control root system architecture, ascertain if below-ground traits can be inferred from above-ground traits, and identify key phenes of *Sorghum bicolor* that are indicative of traits that were artificially selected during domestication. A RIL population generated from a cross between *S* bicolor and *S* propinquum was grown in sand media, capitalizing on *Sorghum*’s natural drought tolerance and rigid crown roots to eliminate the complications of many phenotyping methodologies. A low-cost RGB imaging system coupled with high-throughput, open-source graphical user interface (GUI) software was used to achieve the highest
resolution and most accurate quantification of root morphology. Phenotypic measurements were used for high-density QTL mapping to locate predictive genomic loci. Correlations between above-ground morphology and RSA traits were calculated, and strong trait correlations were regressed to determine statistical prediction models indicative of a shared cellular pathway. Finally, candidate genes within identified RSA QTL were highlighted.

Hypothesis:

I hypothesized that *S. bicolor* alleles would positively segregate with the total number of roots and growth angle with candidate genes encoding AUXIN F-BOX PROTEINS (AFBs) and WD40 domains due to its crown root system, and *S. propinquum* alleles contrastingly would positively segregate with biomass-related traits due to its rhizomatous morphology. I also expected root system depth and width to be negatively correlated due to a metabolic conservation tradeoff in phosphorus and nitrate acquisition at different soil profiles.

I further hypothesized that above-ground height and tiller values would be predictive of root system depth and the number of crown roots similar to that observed by Canarini, et al. (2019), wherein tiller and root growth were co-regulated by transcription factors (SWEET genes) in Arabidopsis.

Research Aim 2: Identification of the genomic loci controlling carbon exudation that will provide the framework for understanding regulation and adaptation of rhizodeposition

Plants acquire nutrients from different soil profiles through adjustments of metabolic carbon exudation. Exudation solubilizes inactive soil minerals through soil priming and aids in symbiotic microbial recruitment, thus increasing overall plant vigor and agronomic yield. To determine what genetic predispositions contribute to variations in exudation, a non-targeted metabolic quantification of carbon and nitrogen in the rhizosphere was employed. The work outlined in Chapter 3 aimed to delineate the genomic loci that predict carbon exudation and identify relationships between rhizodeposition and root morphology. The use of hydrophilic silica sand allowed natural microbial-root interactions during growth and easy capture of water-soluble metabolites during collection, eliminating the need for synthetic culture media or hydroponics. Furthermore, due to the temporal collection of both metabolic profiles and RSA traits, both traits can be statistically compared to identify relationships. Candidate genes within identified exudation QTL were highlighted.

Hypothesis:
I hypothesized that exudation QTLs with candidate genes encoding proteins for efflux facilitation and/or phloem development would colocalize with QTLs for rooting angle, width, volume, and convex area as a coupled strategy for nutrient foraging. I further hypothesized that higher levels of carbon metabolites would be exuded by RILs that have less exploratory root systems (i.e., reduced depth, volume, and width) to attract microbial symbionts for nutrient acquisition.
Works Cited


Flint-Garcia, Sherry A. “Genetics and Consequences of Crop Domestication.” *Journal of Agricultural and Food Chemistry*, vol. 61, no. 35, Sept. 2013, pp. 8267–76. DOI.org (Crossref), https://doi.org/10.1021/jf305511d.


Chapter 3

Getting to the Root Cause: Genomic Regions Controlling Morphological and Metabolic Root Traits in *Sorghum*

Farren Smith, Rajanikanth Govindarajulu, Melissa Lehrer, Jennifer Hawkins
Department of Biology, West Virginia University, Morgantown, WV

Author contributions:
F.S. and J.H. designed experiments; J.H. and R.G. generated the RIL population; F.S. and J.H. managed the project; F.S. and M.L. carried out the experiments and data collection; R.G. generated the genetic bin-map; F.S. carried out the data analysis; and F.S. wrote the manuscript with edits by J.H.

Introduction

Morphological development and metabolic regulation of root systems are key to plant vigor and agronomic yield. Root system architecture (RSA) is defined as the shape, size, distribution, spatial arrangement, and type of growth (tap, crown, adventitious, etc.) of a plant’s root structure. Optimization of RSA can directly influence water, nitrogen, and phosphorus acquisition in opposing soil profiles (Sharma S, et al., 2021). For example, long crown roots can reach deep water tables and water-soluble nitrates, whereas wide lateral roots can allow greater exploration in phosphorus-rich subsoil (Saengwilai, et al., 2014; Gruber, et al., 2013; York, 2013). In addition, rhizodeposition and microbial association can have further effects on plant growth, nutrition, and health. Rhizodeposition promotes the acquisition of nutrients either through the recruitment of symbiotic microbes or through the direct alteration of soil chemistry. For instance, plants may exude organic acids into the soil which reduces pH levels and thus solubilizes previously inaccessible phosphorus. Comparably, the exudation of carbohydrates into the soil can recruit the colonization of growth-promoting mycorrhizal fungi and rhizobium bacteria (McNear, 2013). Though root morphology and rhizodeposition are clearly indicative of a plant's adaptability and fitness, genes and biological pathways controlling root system development and regulation have gone largely unnamed. Despite advancements in genotyping technology and the ability to generate large numbers of genetic markers, inaccessibility to underground root systems and a lack of phenotyping technology have hindered the identification of responsible genomic loci.

*Sorghum* is a globally important cereal grain and biofuel staple that is closely related to other important crop species such as maize, wheat, and millet (Saarela, et al., 2018). *Sorghum* originated in Eastern Africa and was first domesticated in Ethiopia and Sudan (Wendorf, et al., 1992). Domesticated *Sorghum* acquired many of the same cultivated
traits as other agricultural species including loss of seed dispersal and increased grain quantity, as well as an innate drought tolerance due to the arid conditions of the geographical location of domestication (Lai, et al., 2018; Wendorf, et al., 1992). *Sorghum propinquum*, indigenous to SE Asia, is the wild progenitor to derived *Sorghum bicolor*. *Sorghum propinquum* has a rhizomatous, perennial root system that propagates asexually (Rooney et al. 2007; Jessup et al. 2017). Conversely, *S. bicolor* acquired a crown root (also known as nodal root) system and dense brace roots (roots originating from the stem). The contrasting root systems displayed by ancestral *S. propinquum* and domesticated *S. bicolor* provide an excellent framework to dissect the underlying genetic controls that dictate variations in RSA.

In this study, a recombinant inbred line (RIL) population, generated from a cross between *S. bicolor* (L.) Moench ‘Tx7000’ and *S. propinquum*, was phenotyped using novel methodologies. Phenotypes were further genetically mapped to identify the underlying quantitative trait loci (QTL) that (1) regulate RSA development and (2) regulate carbon exudation. Eleven QTL for eight RSA phenotypes were identified. We observed that enlarged RSA traits (i.e., root depth, and biomass) were controlled by *S. bicolor* (L.) Moench ‘Tx7000’ (*S. bicolor*) alleles, suggesting artificial selection for these traits during domestication. No QTL were identified for carbon exudation; however, steep rooting angles, controlled by *S. bicolor* alleles, were predictive of carbon and nitrogen levels in the rhizosphere.

**Materials and Methods**

**Plant Material**

A recombinant inbred line (RIL) population was established from an interspecific cross between *Sorghum propinquum* (female parent) and *Sorghum bicolor* (L.) Moench ‘Tx7000’, as described in Govindarajulu et al. (2021) via repeated selfing using the single seed descent method. The advancement of several RILs trailed in comparison due to photoperiod sensitivity at some loci, resulting in a total of nine F$_3$, 19 F$_4$, 138 F$_5$, and two F$_6$ RILs (n=168).

**Experimental Conditions and Excavation**

Plants were grown at the West Virginia University Evansdale Greenhouse in 5 x 5 x 25 cm plastic tree pots filled with #4 silica sand and topped with 30 mL of Metro-mix 852 soil for the purposes of seed germination (Sun Gro Horticulture, Agawam, MA, USA). Four replicates of each of the 168 RILs (n= 672) were sown within the soil layer, and pots were randomly positioned in 24 trays that were grouped to generate two large rectangular plots (Figure 2). The plots were covered with cellophane for five days to
trap humidity and promote germination. Growth conditions were 27/23 °C (day/night) and 75% humidity. Plants were watered daily to saturation with tap water and fertilized once a week with Jacks All Purpose Plant Food (20-20-20 NPK) at an 80-ppm injection rate (J.R. Peters Inc., Allentown, PA, USA).

Plants were grown for 35-37 days. Above-ground height and tiller number measurements were recorded on days 33 and 34, respectively. To facilitate effective root cleaning during excavation, watering was discontinued three days before phenotyping. Plants were extracted and root images were taken on days 35-37. To excavate the root systems without damage to the plants, the 30 ml soil plug was removed from the pots with a portable air compressor (similarly to the CREAMD pipeline used by Zheng et al. (2020)). Roots were then removed from the pot and sand, shaken vigorously to remove any excess sand, and then stripped of fine roots that inhabited the soil plug.

**High-throughput Imaging**

After excavation, whole root systems were positioned inside an imaging stage consisting of a black felt background and an overhead white cloth to mitigate variation in lighting. Each image was recorded with a Nikon d1150 camera under standard operating settings with flash deactivated and positioned at a height of 52.5 cm. The camera was held by an anchored tripod and operated via a connected laptop running digiCamControl (Serge, 2021) (Figure 3).

Three images were taken of each root system to reduce planar overlap and to capture expected radial variances, as observed in Zheng, et al. (2020). The first image was taken of the root system without manipulation (Image A). In the second image, plants were rotated 90 degrees to capture potential differences in radial symmetry (Image B). For the third image, roots were manually separated and outstretched on the background to reduce dense root overlap (Image C) (Figure 4). Data for maximum root system width (width), maximum root system depth (depth), convex hull area (convex hull), angle, and perimeter were acquired from Images A and B. Measurements for crown root volume and surface area, lateral root volume and surface area, the median number of roots (number of roots), and red root pigment (color) were acquired from Image C.

**Rhizosphere and Biomass Collection**

Immediately following imaging, roots were submerged in a 50 mL conical tube containing 20 mL of Milli-q water and one drop of 10 mM NaOH to increase the solubility of phenolic compounds. The rhizosphere, deemed as the surface area of the roots and any sand particles that adhered to the roots after shaking, was collected up to 2.5 cm
below the soil plug line to minimize residual soil contamination (Figure 5). Roots remained in the solvent for 10 seconds for collection, after which the rhizosphere collections were stored at 4 °C until filtration.

After the rhizosphere was extracted, roots were excised at the crown. Above-ground and below-ground tissues were bagged separately for biomass measurements and dried for ~30 days at 65°C.

**Image analysis of Crown Root Angle and Root Color**

Images A and B were used to investigate the radial symmetry of the replicate root systems for crown root growing angle. Angle measurements were processed in ImageJ using the angle tool (Schneider, et al., 2012; Schindelin, et al., 2012). To assess the adaptability of crown roots to explore the soil in response to stimuli, the growing angle was measured as the widest point between the left crown root and the right crown root (Figure 6).

Pigmentation of root systems, ranging from light pink to deep red-purple, was manually ranked from 0 to 4, with 0 being indicative of no pigment and 4 being indicative of pigmentation on the entire root system (Figure 7).

**High-throughput Image Analysis**

To reduce the time needed for machine learning and manual skeletonization (the process in which foreground regions are reduced to single-pixel lines) all images (A-C for each replicate) were analyzed using RhizoVision Explorer (RVE) (Seethepalli, et al., 2020). To rectify minor variations in environmental lighting and camera positioning that were experienced during imaging, each distinct tray of plants (1-24) was batch analyzed independently in RVE. Image distances were converted from pixels to millimeters in ImageJ (Schneider, et al., 2012; Schindelin, et al., 2012) using an average of four random images from each batch.

Images were imported to RVE in RGB JPEG format. The binary threshold level was adjusted manually after inversion depending on lighting disparity (between ~130-190). The region of interest (ROI) was established as the rectangular black felt background. Processing settings were specified to keep the largest component of whole root systems. Two root dimensions were designated: dimension one for lateral roots (0-0.78 mm) and dimension two for crown roots (0.78+ mm). To reduce the invalid classification of lateral roots due to surface irregularities during skeletonization, edge smoothing was selected as a 1.0-pixel distance threshold and the root pruning was selected as a 3-pixel threshold for possible lateral branching length. An additional batch analysis was run, adding a 3rd dimension (2.78+ pixels) to Image C replicates, duplicating all other settings, to facilitate the capture of surface area and volume.
measurements in regions of dense root overlap. Roots that were unavoidably overlapped resulted in a classification of one thick crown root instead of a mass of laterals during the skeletonization process. The addition of the 3rd dimension allowed the volume and surface area of the overlapped roots to be added to lateral root measurements (noting that this may have caused an under-estimation of crown root surface area and volume but negligible over-estimation of lateral root surface area and volume). Lastly, processed images were visually inspected for proper segmentation, and incorrect outputs were individually reanalyzed after adjusting the threshold level. If threshold adjustments were not corrective, the replicate was excluded from further analyses.

**Rhizosphere Pre-processing and Analysis**

Three samples from each genotype were vacuum filtered through a 0.22 µm polyvinylidene difluoride membrane (PVDF hydrophilic Durapore®) to remove debris and microorganisms and then stored at 4 ºC until lyophilization. Replicate filtrates were frozen at -20 ºC for more than 24 hours and then loaded onto a Labconco© Freezone 6 system for lyophilization, designated at -40 ºC and 0.13 mBar, until dry (~48 hours). Freeze-dried samples were then resuspended with 100 µL of Milli-q water and transferred into microcentrifuge tubes to form pellets on an Eppendorf 5301 VacuFuge at room temperature (~4 hours). Lastly, pellets were weighed using an ultra-micro balance and then loaded into pressed tin capsules for carbon analysis. Due to the partial loss of samples during the pre-processing and concentration stages of analysis (lyophilization, transfers, and sample loading), documented concentrations of citric acid were processed to determine the percent recovery of the described methodology. The percentage of carbon and nitrogen present in the rhizosphere was analyzed using a Carlo Erba NA 1500 CNS Analyzer. Each processing cycle included six Acetanilide standards to calculate the percent error, respectively.

**Statistical Analyses**

**Percent Error:**

The percent error of standards was calculated for each processing cycle on the CNS Elemental Analyzer and runs that had an error greater than two percent were excluded from further analyses. Samples weighing less than 0.75 mg yielded the greatest variation error when the percentage of carbon was regressed against sample weight, and thus were also excluded from further analyses (RMSE\text{reducedmodel}= 0.75, RMSE\text{fullmodel}= 2.37). Lastly, any genotype with only one replicate remaining was excluded, leaving a total of 181 replicates across 107 genotypes.
Radial Symmetry:
Angle measurements calculated from Image A and B were transformed for normality (Table 1). To identify possible deviation of radial symmetry, Image B measurements were compared to a 95 percent confidence interval of Image A measurements for each biological replicate. The deviation was deemed significant if Image B was measured outside of the Image A confidence interval.

Least-square Means:
Rhizovision explorer (RVE) outputs obtained from Images A and B (width, depth, convex hull, and perimeter) were averaged together for technical replication. One-way analysis of variances (ANOVA) for all traits, including above-ground traits (biomass, tiller number, and height) were performed in RStudio version 1.4.1717 (RStudio Team, 2021) using packages carData (Fox, et al., 2020), emmeans (Lenth, 2021), and bestNormalize (Peterson, 2021). Assumptions for ANOVAs, such as normal distribution, common variance, and independence, were tested using a Shapiro-Wilk test, mean residual, and Levene test, respectively. Models were box-cox transformed as needed and the resulting least squared means (LSM) for each RIL was used for QTL mapping (Table 1).

Statistical models were evaluated for edge effects of the experimental plot, spatial placement of seeds, and soil contamination of rhizosphere collections using a two-way ANOVA for each dependent variable (alpha cutoff of 0.05). The extraneous variables did not add statistical significance to any of the models.

Trait Correlations and Regression Analyses:
To determine trait relationships, correlations were performed and plotted in RStudio (RStudio Team, 2021) using package corrplot (Wei, et al., 2021). Regressions were performed using the lm() function in RStudio. A p-value of <0.05 was used for the significance level.

Genetic Map Construction and QTL Analysis
The RIL population was established as described in Govindarajulu, et al. (2021) but with the addition of 20 new F5-F6 advanced lines. High-quality nuclear DNA was isolated from the additional lines and sequenced at ~2x depth. Genetic map construction was performed following the methodology described in Govindarajulu, et al. (2021). In brief, sequence reads were aligned with the S. bicolor ver. 3.1 reference genome for SNP calling and then ABH formatted using GenosToABHPlugin in Tassel ver. 5.0 (Bradbury et al., 2007). A combined map of all breakpoints was generated via SNPbinner (Gonda
et al., 2019) across all RILS using a 10 kb bin size. Markers were ordered based on the physical positions in the reference genome.

QTL analysis was performed in RStudio (RStudio Team, 2021) using package *qtl*, version 1.48-1, described in Broman, et al. (2003; 2009). Phenotypic LSM data from each RIL was merged with respective genetic markers and parsed into R/qtl. Data was converted to a selfed population to remove all heterozygous bins. The kosambi map function was used to calculate centimorgan distances after removing duplicate markers (49), markers missing more than 60 individuals (1), and internal chromosomal markers resulting in the highest decrease of chromosome length (1 from each chromosome). Markers indicative of double-crossovers were highlighted by the calc.errorlod() function to identify the likelihood of erroneous genotypes and then further investigated using the original data file. Nine markers were apparent double crossovers and hence excluded from the remaining analyses.

Each of the 14 phenotypic measurements was analyzed using Haley Knott multiple QTL mapping (MQM) regression with 256 imputations because the genetic map contained high-density markers and relatively complete genotyping (Broman, et al., 2009). QTL found from scanone() and scantwo() functions (3000 permutations), a genotypic probability error of 0.001, and a genome-wide logarithm of the odds (LOD) threshold significance level of <0.05 were used to generate the MQM models. After MQM positions were refined, predictors outside of the 95% LOD significance level were dropped from the model. Lastly, trait means and standard errors of QTL were calculated for each parental allele using the effectplot() function.

**Candidate Genes and Expression**

The supporting genomic region for each identified QTL was recorded by a genome-wide 95 percent confidence level and a 1 LOD interval. Confidence intervals were expanded to markers and identified using the bayesint() and lodint() functions, respectively. Genes within the 1 LOD interval were identified using the *Sorghum bicolor* ver. 3.1.1 genome in Phytozome (Goodstein, et al., 2012; McCormick, et al., 2017). Enrichment analysis was used to assess candidate genes using the SEA tool and the *S. bicolor* v2.1 background in AgriGO v2.0 (Tian, et al., 2017). Candidates were further delimited by literature review and documented co-expression in *Sorghum* root tissue. Expression profiles were obtained using experimental groups, GeneAtlas v2 and EPICON Root, in Phytozome (Goodstein, et al., 2012; McCormick, et al., 2017).

**Results**

Overall, substantial genomic coverage was obtained from the high-density QTL genetic map, as evidenced by 85.2 percent of 2170 markers being genotyped over 10
chromosomes. Of the genotyped markers, 42.4 percent segregated as *S. propinquum* alleles and 57.6 percent segregated as *S. bicolor* alleles. Chromosomes 2 and 5 were the only chromosomes that contained a higher average of *S. propinquum* alleles at the majority of the chromosomal markers, as was similarly documented in Govindarajulu et al. (2021). (Figure 8)

**Morphological Factors of Root Domestication:**

The rigid nature of *Sorghum*’s crown roots reduced root system displacement during imaging which aided in the capture of the phenotypic variation of the RIL’s root systems. A range of phenotypic variations in the RILs was observed including the amount of lateral root branching, root system width, and root system depth (Figure 9; Table 2). RVE software accurately skeletonized root images as evidenced by the correct segmentation of lateral and crown roots in the analyzed output graphics (Figure 10). Further, all RSA regression on genotype models had statistical significance, indicating that each root phene was strongly influenced by genetic factors and would therefore be amenable to QTL analysis (Table 3).

**QTL Identified As Important Regulators of Morphology**

Novel QTL models predicting nine morphological root traits and root pigmentation were identified. Root traits and the corresponding QTL will be abbreviated as follows; dried below-ground biomass (below-ground biomass) (qBgb2), crown root volume (qV.2), maximum root system depth (depth) (qWd4), maximum root system width (width) (qWd4), crown root growing angle (angle) (qA4), lateral root volume (qV5), root system convex hull area (convex hull) (qC8). Root traits with QTL that have multiple loci will be as follows: median number of roots (number of roots), additive QTL on chromosome 2 (qNr.2), and additive QTL on chromosome 8 (qNr.8); root pigment (color), additive QTL on chromosome 1 (qCL1), additive QTL on chromosome 9 at peak position 7.3 cM (qCL9.1), and additive QTL on chromosome 9 at peak position 25.4 cM (qCL9.2).

**QTL Colocalized on Chromosome 4**

QTL for root angle, width, and depth colocalized on chromosome 4. Width and depth QTL shared a LOD peak position at 89.1 cM (Figure 11) and had a very strong statistical relationship. Depth predicted 99.86 percent of the variation observed for width ($r^2_a$) (Table 4). The QTL models explained 11.57 percent of the phenotypic variation (PVE) for depth and 11.79 percent PVE for width. Root depth and width were both greater in RILs with *S. bicolor* alleles at respective loci (Table 5).
A deviation from radial symmetry was not found in rooting angles from Image A and Image B, unlike that reported in Zheng, et al. (2020). One QTL for root angle had a LOD peak position at 94.5 cM and RILs containing *S. propinquum* alleles at this locus had wider angles (Figure 11). The QTL model explained 8.32 percent of the phenotypic variation for angle (Table 5).

Candidate genes within the QTL for depth and width included those that encode auxin-induced proteins, phloem proteins, and MATE effluxes. Candidate genes within the QTL for angle include those that encode actin and ENTH/VHS family proteins (Supplemental Table 1). All candidate genes have been shown to be expressed in *Sorghum* root tissues. (Goodstein, et al., 2012; McCormick, et al., 2017)

**QTL Colocalized on Chromosome 2**

QTL for belowground biomass, crown root volume, and qNr.2 (QTL from the additive model for the number of roots) colocalized on chromosome 2. The QTL for crown root volume had a LOD peak position at 38.8 cM, the QTL for below-ground biomass had a LOD peak position at 43.7 cM, and the QTL, qNr.2, had a LOD peak position at 39.5 cM (Figure 11). The QTL models explained 11.37 percent, 9.12, and 12.39 percent of the phenotypic variation, respectively. The traits that mapped to chromosome 2 increased in RILs with *S. bicolor* alleles at respective loci (Table 5). There were strong correlations among these traits, as documented in Table 6.

Candidate genes at the colocalized locus included genes encoding WD40 domains, CESA8-cellulose synthases, aldehyde dehydrogenases, and receptor kinases, all of which have been shown to be expressed in *Sorghum* root tissue (Tanaka, et al., 2005; Ou, et al., 2021;). (Goodstein, et al., 2012; McCormick, et al., 2017) (Supplemental Table 1)

**QTL Located on Chromosome 8**

The convex hull QTL and the QTL, qNr.8 (from the additive QTL model for number of roots), colocalized on chromosome 8. The convex hull QTL had a LOD peak position at 46.9 cM and the QTL, qNr.8, had a LOD peak position at 30.2 cM (Figure 11). The individual QTL models explained 8.48 percent and 10.80 percent of the phenotypic variation, respectively. Both traits increased in RILs with *S. bicolor* alleles at respective loci. (Table 5)

Candidate genes for convex hull included genes encoding calcium-dependent protein kinases, UDP-glycosyltransferases (UGTs), and cyclins. None of these genes have been found to be expressed in *Sorghum* roots. Candidate genes for qNr.8 included genes encoding actin and WD-40 domains, both of which have been shown to be expressed in *Sorghum* roots. (Goodstein, et al., 2012; McCormick, et al., 2017) (Supplemental Table 1)
QTL on Chromosome 5

One QTL for lateral root volume had a LOD peak position on chromosome 5 at 46.1 cM (Figure 11). The QTL model explained 9.03 percent of the phenotypic variation. RILs that had *S. bicolor* alleles at this locus had an increased volume of lateral roots (Table 5).

Many genes were of interest at this location. Candidate genes included genes encoding auxin response factors, ROOTHAIRLESS 1 (gene controlling initiation and growth of root hair), and PTR2 (gene found in root hair epidermal cells). Many of the candidate genes were found to be expressed, co-expressed, or weakly expressed in *Sorghum* root tissue. (Goodstein, et al., 2012; McCormick, et al., 2017) (Supplemental Table 1)

Surface Area and Perimeter

No QTL with significant LOD scores were identified for the surface area of lateral roots, the surface area of crown roots, or root system perimeter; however, all had statistical significance when regressed on genotype.

These traits were correlated and significantly predicted by other mapped RSA traits (Table 4 and 6). Lateral root volume explained 93.01 percent of the phenotypic variation of lateral surface area, crown root volume explained 33.42 percent of the phenotypic variation of crown root surface area, and convex hull explained 46.34 percent of the phenotypic variation of perimeter (r²a) (Table 4).

Red Root Pigment

Three additive QTL were identified for the presence of red pigmentation of root systems (color). One mapped to chromosome 1 with a peak LOD score at 5.7 cM (qCL1), and two to chromosome 9 with a peak LOD score at 7.3 cM (qCL9.1) and 25.4 (qCL9.2), respectively (Figure 11). The total additive QTL model explained 28.78 percent of the phenotypic variation: 12.05 percent PVE by qCL1, 11.62 percent PVE by qCL9.1, and 10.17 PVE by qCL9.2. RILs with *S. bicolor* alleles at qCL1 and qCL9.1 had a reduction of pigment, but an increase of pigment at qCL9.2 (Table 5). Candidate genes were associated with leucine-rich repeats (LRRs) (Goodstein, et al., 2012; McCormick, et al., 2017) (Supplemental Table 1).

Metabolic Factors of Root Domestication:

Sample collection and concentration via lyophilization methodologies were effective in capturing the variation of carbon and nitrogen in the rhizosphere (Table 2). The recovery of samples after concentration was between 73 and 78%.

The variation of the percentage of carbon (%C) in the rhizosphere ranged from 5.29 to 16.12% and the percent of carbon to nitrogen ratio (%C:N) ranged from 3.06 to
17.26%. Genotype significantly predicted %C (p=2.8e-3) and %C:N (p<2.2e-16), indicating that exudation was strongly influenced by genetic factors, as documented in Table 7.

**RSA was Identified as a Likely Regulator of Exudation**

No QTL with significant LOD scores were identified for %C or for %C:N. However, uni- and multi-variate regression analyses revealed significant relationships between root morphology and rhizodeposition. Plant height and angle were most strongly correlated (negatively) with %C:N. Angle and genotype were significant predictors and explained 0.99 percent more of the %C:N phenotypic variation than genotype alone (r²a) (Table 6, and 7). Crown root volume and angle were most strongly correlated with %C. Angle and genotype were significant predictors of %C and explained 4.01 percent of the phenotypic variation than genotype alone (r²a) (Table 6, and 7).

**Above-ground Model Predictions of RSA Traits:**

Plant height was most correlated (positively) with lateral root volume and crown root volume of the mapped RSA traits. The number of tillers (tiller number) was most correlated, though weakly, with crown root volume. Dried above-ground biomass (above-ground biomass) was most correlated with below-ground biomass. (Table 6)

Plant height and genotype significantly predicted crown root volume and explained 1.18 percent more of the phenotypic variation than genotype alone (r²a). Above-ground biomass and genotype significantly predicted below-ground biomass and explained 4.19 percent of the phenotypic variation than genotype alone (r²a). (Table 8)

**Discussion**

Plant roots are essential for the acquisition of soil resources, such as nutrients and water. Resource acquisition is dictated by two key root traits: morphology and carbon exudation. Root morphology can directly influence the absorption of water and minerals, as nitrates and phosphates reside in spatially opposing soil layers (Saengwilai, et al., 2014; Gruber, et al., 2013; York, 2013). Comparatively, exudation can also influence nutrient uptake; however, this is achieved indirectly, via chemical and physical alterations of the soil as well as by microbial recruitment (McNear, 2013). This study aimed to uncover the genomic factors that contributed to changes in RSA and exudation during *Sorghum* domestication. The influence of alleles from domesticated *S. bicolor* at nine QTL models controlling root morphology were identified. In addition, the relationship between steep rooting angles, positively controlled by *S. bicolor* alleles, and increased carbon and nitrogen in the rhizosphere was observed.
**Root Morphology QTL**

The identified QTLs explained a minimum of eight percent of the observed phenotypic variation for each root trait, indicating that these loci significantly contribute to root morphology.

**QTL Colocalized on Chromosome 2**

QTLs for biomass-related traits colocalized on chromosome 2 (below-ground biomass, number of roots, and crown root volume). Similarly, syntentic QTL responsible for root mass, maturity, and aboveground development were previously identified at this locus; such as days to flowering (Mace, et al., 2013b), fresh stem biomass (Shiringani, et al., 2011), root dry weight/biomass (Beckel, et al., 2014; Moghimi, et al., 2019), and root morphology (Parra-Londono, et al., 2018).

In summary, these findings suggest that the colocalized QTL on chromosome 2 are responsible for accelerated maturity that leads to increased biomass of both above- and below-ground organs, and were selected during domestication. Candidate genes indicate that metabolically induced cellulose production is responsible for the observed increase in biomass.

**QTL Colocalized on Chromosome 4**

QTL found for root depth and width both mapped to the same location on chromosome 4 and were positively correlated. Candidate genes within this QTL include those that encode for auxin-induced proteins, phloem proteins, and MATE effluxes (involved in metabolic transport). These results suggest that a shared auxin-responsive pathway induces sugar transport to both lateral (width) and crown (depth) roots for cellular proliferation and elongation. Additionally, increased root width and depth can be explained by an up-regulation of growth rates that were selected during domestication to produce high grain yield. Rapid growth requires both deeper roots for water acquisition and thicker/wider root systems for stability and phosphate acquisition (Qi, et al., 2012).

A QTL for angle was also detected on chromosome 4, and candidate genes included those that encode for actin and ENTH/VHS proteins. Actin has been shown to control root plasticity through auxin transport in response to gravitropism and phototropism (Uga, et al., 2013, Garcia-González, et al., 2021), and Zouhar, et al. (2014) identified ENTH/VHS as key proteins during endocytosis and endomembrane trafficking, such as that needed to transport auxin to root tips. In summary, these findings suggest that exploratory modifications to crown roots in response to soil minerals may be a similar cellular response to that of gravitropism and phototropism.

We suspect that the width measurement in this study corresponds to the rooting angle discussed in other literature (Ramalingam, et al., 2017; An, et al., 2017; Alahmad,
et al., 2019). For instance, Ramalingam, et al. (2017), reported that steep rooting angles predicted deeper rooting depth. The results presented here, however, showed a weak correlation between depth and angle but a very strong correlation between depth and width. Further, because width and angle are colocalized and are similar phenes, we suspect that these genomic loci are paralogs.

**QTL Located on Chromosome 8**

A QTL responsible for predicting the convex hull mapped to chromosome 8. This locus is syntenic with those previously shown to be associated with emergence rate (Fiedler, et al., 2012), stem and leaf weight (Guan, et al., 2011), and leaf carbon assimilation rate (Ortiz, et al., 2017). None of the candidate genes have been shown to be expressed in *Sorghum* roots (Goodstein, et al., 2012; McCormick, et al., 2017). The lack of expression of the candidate genes in root tissues and the fact that syntenic loci are responsible for above-ground traits suggest this locus is primarily responsible for controlling above-ground growth. Increases in above-ground biomass would require an influx of nutrients and therefore presumably signals a soil exploration response below-ground. Hence, we suspect that convex hull area is a reflection of an increase in whole plant growth.

The second QTL found on chromosome 8 was part of the additive QTL model for the number of roots (qNr8). This locus had an overlapping confidence interval with the convex area QTL, but the traits were not strongly correlated. The candidate gene within qNr8 encodes a WD-40 repeat domain protein. These results suggest that the QTL predicting the number of roots expresses WD-40 proteins for the onset of development that further activates the transcription of genes on chromosome 2 (qNr.2) responsible for cellulose production. Interestingly, qNr8 also contains a syntenic locus associated with tiller number (Zhao, et al., 2016). Thus, a shared transcriptional factor, comparable to the SWEET transcriptional factors in *Arabidopsis*, is likely controlling both tiller number and root number in *Sorghum* as hypothesized.

**QTL on Chromosome 5**

One QTL predicting lateral root volume mapped to chromosome 5. Many genes at this locus warranted candidacy, including those with roles in root meristem maintenance (Zhou, et al., 2010), auxin response, root hair growth (Lee, et al., 2008), and water uptake (Choi, et al., 2020). This QTL is syntenic with previously identified loci that were also shown to control root traits such as; root biomass, the median number of roots, and root angle (Mace, et al., 2012; Moghimi, et al., 2019; Parra-Londono, et al., 2018), as well as those associated with overall plant and panicle growth (Fiedler, et al., 2014; Zhao, et al., 2016; Nagaraja, et al., 2013). Thus, these results suggest that increased lateral root growth is explained via auxin-responsive kinases and/or the inhibition of
sulfotransferase at the root meristem. Further, since many QTL for root hair formation and root hair growth colocalized with lateral root volume, we suspect that root hair and lateral roots share a developmental pathway.

**Red Root Pigment**

Root color was an unexpected phene, first noticed on some replicates during image acquisition. Three additive QTL were identified for this trait, two on chromosome 9 and one on chromosome 1. Although the origin of this phene is unidentified, it may be indicative of a pathogen infection such as red root rot (*Pythium* and/or *Fusarium*). Candidate genes encoding leucine-rich repeats support this speculation but further investigation into the trait identity is needed.

**Surface Area and Perimeter**

QTL models (perimeter, and surface areas of lateral and crown roots) that did not have statistically significant LOD scores had high correlation coefficients with other mapped traits. Root perimeter was statistically predicted by convex area, and surface area of lateral and crown roots was statistically predicted by volume of lateral and crown roots, respectively. These findings signify that surface area and perimeter phenes were more accurately captured by other measurements.

**Carbon Exudation**

Carbon exudation is important for the symbiotic relationship with mycorrhizal fungi and rhizobacteria. Root systems transfer fixed carbon to microbes in exchange for nutrients, such as nitrogen and phosphorus. The symbiotic relationship allows plants to increase nutrient absorption in a greater volume of soil without modification to root morphology (Bucking, et al., 2012; Bonfante, et al., 2010).

The hypothesis that carbon exudation would colocalize with root morphology traits cannot be confirmed as QTL with significant LOD scores were not identified for the percentage of carbon (%C) or for the percentage of carbon to nitrogen ratio (%C:N) present in the rhizosphere. However, root angle did explain variation in both %C and %C:N.

**Metabolic Relationship with Morphology**

RSA and carbon exudation can positively influence plant growth and nutrition and are often coupled when modeling a plant’s economic strategy of below-ground resource acquisition (Wen, 2022). The results of this study further illustrate how morphology and exudation interact to govern nutrient capture. Root angle is a statistically significant predictor of and is negatively correlated with %C and %C:N, meaning that steeper, less plastic angles predict higher levels of carbon and higher levels of nitrogen per carbon in
the rhizosphere. Interestingly, the locus we identified for root angle on chromosome 4 is syntenic with the locus controlling exudates in *Sorghum* identified by Shehzad, et al. (2020). These findings suggest that higher percentages of carbon may be exuded as a means to attract symbiotic microbes for nitrogen acquisition. This is further supported by recent findings in *Poaceae* host plants, wherein rhizobacteria and mycorrhizal fungi have not only been shown to transfer phosphates from the soil but also ammonium and nitrates (Govindarajulu et al., 2005; Van Deynze et al., 2018, Moreau, et al., 2019).

**Domestication of Sorghum**

The domestication of *Sorghum bicolor* first occurred in northeast-central Africa whereby genotypes with loss of seed shattering, fewer tillers, reduced height, increased biomass, and enlarged panicles were artificially selected (Burgarella, et al., 2021; Lai, et al., 2018). After initial domestication, *S. bicolor* migrated to various regions and evolved into landraces with numerous regional adaptations (Wendorf et al., 1992; Mace et al., 2013; Morris et al., 2013). *Sorghum* accession 'Tx7000' was derived from the Kafir landrace which predominates in South Africa, a drier climate in contrast to that of northeast-central Africa where domestication began, and significantly drier than that of *S. propinquum*’s origin in south-east Asia (Xu, et al., 2000; Evans, et al., 2013; Morris, et al., 2013). The genetic bottleneck and diverse habitats illustrate the various selective pressures the *Sorghum* root system experienced over a relatively short evolutionary time scale.

*S. propinquum* displays rhizomes that contribute to perennialism, whereas *S. bicolor* contrastingly displays an annual crown root system (Rooney et al. 2007; Jessup et al. 2017). Dissection of root traits corresponding to parental alleles from the recombinant cross between *S. propinquum* and *S. bicolor* (L.) Moench ‘Tx7000’ used in this study provides insight into the traits that facilitated the transition to annuality during *Sorghum* domestication. For reference, a well-known noxious weed, *S. halepense* (johnsongrass), is an allopolyploid with *S. bicolor* and *S. propinquum* parentage. Johnsongrass typically has a rhizomatous morphology (though variable across ecotypes) of various lengths (~inches to ~feet) that form a dense tangled sod within the topsoil that further extends sparse deep vertical roots (Wiggins, 1980; Hauser, et al., 1958).

**Domestication and Root Morphology**

All morphological traits, except root angle, were increased when *S. bicolor* alleles were present at the respective QTL, illustrating that selective pressures for increased vegetative and reproductive yield had an effect on changes in RSA. QTL identified on chromosome 2 were associated with biomass-related traits. At this locus, Sobic.002G058600 has been recognized as a targeted gene during sorghum
domestication and improvement (Mace, et al., 2013b). Sobic.002G058600 expresses an F-box/WD40/YVTN repeat-like-containing domain which functions in transcriptional regulation. Across many domesticated grain species, phenotypic adaptations that arose from artificial selection typically are due to alterations in transcriptional regulation, gene duplication, and/or loss of function to transcription factors (Doebley, et al., 2006; Olsen, et al., 2013). The findings on chromosome 2 suggest that domestication likely targeted the WD40 gene at this gene-dense cluster for transcription amplification of genes responsible for root biomass. Increased transcription of genes controlling root biomass would facilitate rapid nutrient accumulation, and thus higher-yielding vegetative growth.

A major aim of this study was to identify root QTL positively influenced by S. propinquum alleles, suggestive of genomic underpinnings that facilitated the shift from perennialism to annualism. Surprisingly, the initiation of rhizome buds was not observed in this population as is displayed in S. halepense species 18-37 days after seeding (Anderson, et al., 1960; McWhorter, 1961). This could partly be due to the whole genome duplication and polyploidy of S. halepense unlike the RIL population of this study (Ramalingam, et al., 2017). Paterson, et al. (1995) noted that a cross between S. propinquum and S. bicolor produced quantitative rhizome traits across nine genomic locations in progeny, some of which were associated with tillers. Of those QTL identified by Paterson, many were identified as orthologous in Oryza (Hu, et al., 2003). However, Hu, et al. (2003) noted that the genes associated with rhizomes and/or tillers were lost over time during the domestication of Oryza through recessive or loss-of-function genes. Further, Gizmawy, et al. (1985) reported that axillary node buds were susceptible to a transformation from rhizome to tiller depending on internal and external factors. The results of our study suggest that this RIL population has either modified expression to initiate tillers instead of rhizomes at axillary nodes or has accrued a loss of function to genes that are responsible for rhizome formation. The disappearance of rhizomes in derived S. bicolor is logical when considering that enhanced seed yield was selected during domestication. Selection pressures on seed yield likely directed energy reserves from rhizome carbon sinks to seed carbon sinks, thus shifting reproduction towards sexual rather than asexual reproduction and propagation.

Red root phenotypes, which are speculated to be due to a fungal pathogen, were reduced in the presence of S. bicolor alleles in two of the three influencing QTL. This may be explained by the genetic bottleneck experienced during domestication. Many ancestral traits were lost during domestication including those associated with disease resistance, explaining why the QTL on chromosome 9 had a reduction in color with the presence of ancestral S. propinquum alleles (Dillon, et al., 2007). However, due to breeding of elite lines such as ‘Tx7000’, some cultivated Sorghum accessions have increased biotic stress resistance, further explaining why two of the influencing QTL had a reduction in color with the presence of S. bicolor alleles. Fungal infection of roots.
typically result in cellular death and may have affected other root traits; however, no correlations were seen between root color and other RSA phenes (Table 6).

**Domestication and Carbon Exudation**

Root angle and plant height were negatively correlated with the percentage of carbon (\%C) and carbon:nitrogen (\%C:N) in the rhizosphere and could be significantly predicted by root angle. Since root angle is negatively influenced by *S. bicolor* alleles, these results suggest that *S. bicolor* exudes carbon into the rhizosphere at an elevated rate compared to *S. propinquum*. This is further supported by the negative correlation between height and exudation, as a reduction in plant height resulted from domestication and has been documented to occur in the presence of *S. bicolor* alleles at height-associated QTL (Hostetler, et al., 2021; Govindarajulu, et al., 2021).

Domestication of *Sorghum*, specifically of the Kafir landrace, occurred in arid environments with an annual rainfall of less than 1200 mm (DataAfrica, 2022; Elischer, et al., 2015). Nitrogen is often the most limiting nutrient in arid environments and has been shown to radiate from soil as nitrogen gas at high soil temperatures (McCalley, et al., 2009). *Poaceae* species displaying rapid growth and increased biomass that inhabit arid, nitrogen-limited environments, are documented to be resource-acquisitive species, which commonly release exudates to accelerate nitrogen cycling in the rhizosphere (Wen, et al., 2020). Consistent with Wen, et al. (2020), the results of our study illustrate selections for nitrogen and water acquisition of resource-limited soils during domestication events of *Sorghum*. Steep growing angles that are influenced by *S. bicolor* alleles and the correlation with increased carbon in the rhizosphere suggest *S. bicolor* is associated with increased carbon exudation. This may either be due to growth signals passively lost from root tips or as a means to attract microbial symbionts. Concerning domestication of *Sorghum* in arid/droughted environments, steeper angles in *S. bicolor* is logical. Steeper angles are associated with greater rooting depth (a trait that was also influenced by *S. bicolor* alleles), allowing access to resource-limited water at lower soil profiles. Increased carbon exudation that promotes symbiotic nutrient cycling could either be an indirect effect of steep crown root angles in which carbon is passively lost during growth or an independently selected trait. Targeted selection of symbiotic activity during domestication is also logical as increased photosynthetic capacity and faster growth rates require the acquisition of environmentally-limited nitrogen. Similar results were seen in maize, wherein increased absorption of nitrogen was greater in maize in comparison to its wild progenitor, teosinte (Perkins, et al., 2021). Perkins, et al. theorized this is due to a larger number of seminal roots in maize than teosinte; however, it could also be related to symbiotic rhizodeposition as evidenced in this study.
The Relationship between Above- and Below-ground Traits

Though the effects of domestication on root systems have been understudied in comparison to above-ground organs, root morphology has likely evolved in conjunction with above-ground organs, as they are often in systematic equilibrium (Bloom, et al., 1985). Multiple regression analyses revealed that above-ground biomass was a statistically significant predictor of below-ground biomass (positive correlation) and plant height was a statistically significant predictor of crown root volume (positive correlation). These results suggest a biological stoichiometry of above- and below-ground plant organs. The observed stoichiometry is explained by the need for a mutual assimilation rate of water and carbon dioxide for photosynthesis and cellular respiration, wherein above-ground vegetation is responsible for carbon accumulation and below-ground is responsible for water accumulation. Further, below-ground biomass and above-ground biomass may be regulated by a shared biological pathway containing homeobox transcription factors that promote cell proliferation proportionately. Root and shoot apical meristems have been documented by Stahl, et al. (2010) to be controlled by homologous homeobox transcription factors, and the locus influencing below-ground biomass is syntenic with the QTL identified by Hostetler, et al. (2021) for dead above-ground biomass of control plants.

Tiller number was not statistically predictive nor correlated with any below-ground traits in this population as previously shown in Arabidopsis (Canarini, et al., 2019). However, the QTL influencing the number of roots on chromosome 8 did colocalize with the locus controlling the number of tillers in Zhao, et al. (2016).

We hypothesized that above-ground traits would be correlated with similar below-ground traits such as plant height to root depth, and tiller number to root number. This was hypothesized to be due to shared pathway regulation by transcriptional factors and hormones that had been selected during domestication. The results of this study suggest the hypothesis to be partly correct; though tiller number was not predictive of any below-ground traits in this population, a proportionate growth rate between above- and below-ground organs was observed.

Additionally, we expected S. propinquum alleles to predict larger root systems due to its rhizome and lateral propagation, and S. bicolor alleles to predict an increased number of crown roots and shallower angles to forage more efficiently since it lacked carbon reserves in rhizomes. However, all RSA QTL had an increased effect in the presence of S. bicolor alleles with the exception of root angle, illustrating that S. bicolor displays overall larger root systems with enlarged mass after five weeks compared to S. propinquum. A similar result was also reported in two other cultivated species, maize and common bean (P. vulgaris L.), wherein derived varieties had enlarged root biomass compared to ancestral relatives, respectively (Araújo, et al., 1997, Perkins, et al., 2021). Domesticated species, such as S. bicolor, were derived via selections for quick,
vegetative growth and enhanced reproductive yield. We suspect that larger root systems with rapid growth rates are reflective of plant systems that require greater influx of water and minerals to sustain the overall accelerated plant growth.

Conclusion

Future Directions

Though the rhizosphere collection methodology was successful, as indicated by statistically significant genotypic segregation, QTL may have gone undetected due to a small sample size. A beneficial solution for future studies would be to allot longer durations of root “steeping” during collection, thus adding technical replication and reducing the percent error of analyses. Further, to parse out why steep crown root angles are correlated to increased carbon deposition, a metagenomics analysis of the rhizosphere of individuals expressing shallow and steep roots needs to occur.

The reproductive shift of derived Sorghum species to an annual growth pattern was suggested to be due to selection pressures on seed and sexual propagation but should be further defined by exploring genes within the root angle QTL on chromosome 4. Investigations should specifically target the gene controlling the number of tillers identified in Zhao, et al. (2016) as well as the candidate genes for angle traits that are highlighted in Supplemental Table 1.

The knowledge gap of the biological mechanisms that lead to root development and architectural fate in Sorghum has been narrowed. However, to ascertain the variation of allelic expression, this study should be replicated at field sites to determine if the identified genomic locations are responsible for controlling the same phenes in field conditions. Candidate genes at each locus should be further explored to pinpoint polymorphisms that control RSA in Sorghum and other orthologous Poaceae species such as rice, maize, and millet.

Implications

The ideal root system architecture allows for stability and adequate capture of biological resources. Carbon exudation promotes the colonization of symbiotic microbes and further enhances the acquisition of soil nutrients. Optimization of both root morphology and metabolic exudation is imperative to reach full-potential growth and agronomic yield. The identification of evolutionary adaptations in domesticated Sorghum and the genetic mapping of eight novel root loci in this study, emphasizes the value of this RIL population for understanding the biological processes regulating root development and rhizodeposition.

The groundwork has been laid by this study for future breeding efforts of crop varieties with improved root traits in agro-economically important grain species. Breeders can use this information to establish elite root lines cataloged for specific land
needs, such as deep roots for arid environments or steep-angled roots for nitrogen-limited soil. Root morphology and metabolic effluxes suited for specific growing conditions will ensure optimal production yield and mitigate current and future environmental challenges of croplands in a sustainable manner.
Acknowledgments

Melissa Lehrer for research collaboration and support; Jasmine Freeman, Kristin Ratliff, Ryan Percifield, Emmelia Braun, and Emel Kangi for help in data collection; West Virginia University Evansdale Greenhouse for supplying space; West Virginia Genomics Core Facility for DNA sequencing; Jennifer Hawkins for endless patience, advice, knowledge, wisdom, imparted confidence, and encouragement; Edward Brzostek and Jonathan Cumming for respective insights, knowledge, wisdom, and patience over countless meetings and debates; and many family and friends for the emotional support and encouragement along the way.
Works Cited


Fiedler, Karin, et al. “Genetic Dissection of the Temperature Dependent Emergence Processes in Sorghum Using a Cumulative Emergence Model and Stability


Zhao, Jing, et al. 'Genome-Wide Association Study for Nine Plant Architecture Traits in Sorghum'. *The Plant Genome*, vol. 9, no. 2, July 2016. DOI.org (Crossref), https://doi.org/10.3835/plantgenome2015.06.0044.


Table 1. Normality Assumptions and Transformations for Traits. A table of the calculated regression assumptions and transformations of measured traits. A Shapiro-Wilk test was used to calculate residual and model normality.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Transformation</th>
<th>Residual normality p-value</th>
<th>Residual mean</th>
<th>Normality p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGB</td>
<td>sqrt(y)</td>
<td>0.01</td>
<td>1.13E-18</td>
<td>0.02</td>
</tr>
<tr>
<td>SA 1</td>
<td>sqrt(y)</td>
<td>0.025</td>
<td>5.37E-16</td>
<td>0.56</td>
</tr>
<tr>
<td>Vol. 1</td>
<td>sqrt(y)</td>
<td>0.149</td>
<td>8.90E-16</td>
<td>0.07</td>
</tr>
<tr>
<td>Width</td>
<td>sqrt(y)</td>
<td>1.95E-05</td>
<td>-7.76E-18</td>
<td>0.13</td>
</tr>
<tr>
<td>Convex hull</td>
<td>sqrt(y)</td>
<td>7.80E-03</td>
<td>-1.18E-16</td>
<td>0.66</td>
</tr>
<tr>
<td>Angle</td>
<td>y^(-0.768)</td>
<td>0.067</td>
<td>4.81E-20</td>
<td>6.70E-03</td>
</tr>
<tr>
<td>SA 2</td>
<td>y^(-0.4848)</td>
<td>2.20E-16</td>
<td>-9.45E-21</td>
<td>1.00E-07</td>
</tr>
<tr>
<td>Num. roots</td>
<td>NA</td>
<td>1.00E-01</td>
<td>1.09E-16</td>
<td>0.7</td>
</tr>
<tr>
<td>Depth</td>
<td>NA</td>
<td>3.05E-05</td>
<td>5.60E-16</td>
<td>0.06</td>
</tr>
<tr>
<td>Perimeter</td>
<td>NA</td>
<td>4.96E-02</td>
<td>2.81E-14</td>
<td>0.8</td>
</tr>
<tr>
<td>Vol. 2</td>
<td>NA</td>
<td>2.50E-01</td>
<td>2.32E-01</td>
<td>9.15E-14</td>
</tr>
<tr>
<td>Color</td>
<td>NA</td>
<td>5.24E-06</td>
<td>3.96E-04</td>
<td>-2.04E-17</td>
</tr>
<tr>
<td>%C</td>
<td>NA</td>
<td>0.475</td>
<td>-4.35E-17</td>
<td>5.94E-02</td>
</tr>
<tr>
<td>%C:N</td>
<td>NA</td>
<td>1.19E-02</td>
<td>-8.20E-18</td>
<td>2.01E-08</td>
</tr>
<tr>
<td>AGB</td>
<td>sqrt(y)</td>
<td>4.24E-01</td>
<td>-1.56E-18</td>
<td>9.35E-02</td>
</tr>
</tbody>
</table>

Abbreviations:
- below-ground biomass (BGB), lateral root surface area (SA 1), lateral root volume (Vol. 1), root system maximum width (width), root system convex hull area (convex hull), crown root growing angle (angle), crown root surface area (SA 2), median number of roots (Num. roots), root system maximum depth (depth), root system perimeter (perimeter), crown root volume (Vol. 2), root pigment (color), percentage of carbon in the rhizosphere (%C), percentage ratio of carbon to nitrogen in the rhizosphere (%C:N), above-ground biomass (AGB)
Table 2. Trait Mean, Maximum, and Minimum Values. Quantitative trait means, minimum, and maximum values of raw data. Values illustrate respective trait variations and ranges.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGB (g)</td>
<td>0.25</td>
<td>0.74</td>
<td>0.03</td>
</tr>
<tr>
<td>SA 1 (mm2)</td>
<td>20,378.69</td>
<td>57,481.58</td>
<td>2,612.22</td>
</tr>
<tr>
<td>Vol. 1 (mm3)</td>
<td>12,783.40</td>
<td>62,432.35</td>
<td>573.74</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>83.70</td>
<td>195.61</td>
<td>33.58</td>
</tr>
<tr>
<td>Convex area (mm2)</td>
<td>19,301.77</td>
<td>39,753.17</td>
<td>6,548.85</td>
</tr>
<tr>
<td>Angle (*)</td>
<td>100.45</td>
<td>360.00</td>
<td>42.63</td>
</tr>
<tr>
<td>SA 2 (mm2)</td>
<td>33,370.08</td>
<td>63,410.70</td>
<td>4,166.96</td>
</tr>
<tr>
<td>Num. roots</td>
<td>18.84</td>
<td>36.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Depth (mm)</td>
<td>323.52</td>
<td>430.25</td>
<td>215.55</td>
</tr>
<tr>
<td>Perimeter (mm)</td>
<td>7,593.22</td>
<td>13,194.53</td>
<td>2,392.97</td>
</tr>
<tr>
<td>Vol. 2 (mm3)</td>
<td>12,872.35</td>
<td>25,835.28</td>
<td>1,293.42</td>
</tr>
<tr>
<td>Color</td>
<td>1.71</td>
<td>4.00</td>
<td>0.00</td>
</tr>
<tr>
<td>%C (%)</td>
<td>9.94</td>
<td>16.12</td>
<td>5.29</td>
</tr>
<tr>
<td>%C:N (%)</td>
<td>7.20</td>
<td>17.26</td>
<td>3.06</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>13.38</td>
<td>23.80</td>
<td>7.30</td>
</tr>
<tr>
<td>Tiller num</td>
<td>0.88</td>
<td>8.00</td>
<td>0.00</td>
</tr>
<tr>
<td>AGB (g)</td>
<td>0.60</td>
<td>1.26</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Abbreviations:
below-ground biomass (BGB), lateral root surface area (SA 1), lateral root volume (Vol. 1), root system maximum width (width), root system convex hull area (convex hull), crown root growing angle (angle), crown root surface area (SA 2), median number of roots (Num. roots), root system maximum depth (depth), root system perimeter (perimeter), crown root volume (Vol. 2), root pigment (color), percentage of carbon in the rhizosphere (%C), percentage ratio of carbon to nitrogen in the rhizosphere (%C:N), plant height (height), number of tillers (tiller num), above-ground biomass (AGB)
Table 3. Genotype Regression Models and Statistical Significance. Statistical significance and coefficient of determinations from RSA trait regression on genotype. Each trait was significantly predicted by genotype.

<table>
<thead>
<tr>
<th>Genotype RSA Regression Models</th>
<th>r2a</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Num Roots ~ Genotype</td>
<td>0.1779</td>
<td>2.99E-07</td>
</tr>
<tr>
<td>Vol. 2 ~ Genotype</td>
<td>0.3425</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>Depth ~ Genotype</td>
<td>0.2053</td>
<td>5.61E-09</td>
</tr>
<tr>
<td>Perimeter ~ Genotype</td>
<td>0.2140</td>
<td>1.42E-09</td>
</tr>
<tr>
<td>BGB ~ Genotype</td>
<td>0.3001</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>SA 1 ~ Genotype</td>
<td>0.3238</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>Vol. 1 ~ Genotype</td>
<td>0.2870</td>
<td>2.32E-15</td>
</tr>
<tr>
<td>Width ~ Genotype</td>
<td>0.2052</td>
<td>5.68E-09</td>
</tr>
<tr>
<td>Convex Hull ~ Genotype</td>
<td>0.2374</td>
<td>2.91E-11</td>
</tr>
<tr>
<td>Color ~ Genotype</td>
<td>0.3505</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>Angle ~ Genotype</td>
<td>0.1351</td>
<td>5.95E-05</td>
</tr>
<tr>
<td>SA 2 ~ Genotype</td>
<td>0.2687</td>
<td>9.07E-14</td>
</tr>
</tbody>
</table>

(167+476 df)

Abbreviations:
- below-ground biomass (BGB), lateral root surface area (SA 1), lateral root volume (Vol. 1), root system maximum width (width), root system convex hull area (convex hull), crown root growing angle (angle), crown root surface area (SA 2), median number of roots (Num. roots), root system maximum depth (depth), root system perimeter (perimeter), crown root volume (Vol. 2), root pigment (color)
Table 4. RSA Trait Predictions and Statistical Significance. RSA trait predictions of other RSA traits with the respective statistical significance and coefficient of determinations. Volume of lateral and crown roots statistically predicted lateral and crown root respectively. Convex hull statistically predicted perimeter. Depth was strongly correlated and predictive of width.

<table>
<thead>
<tr>
<th>RSA Trait Regression</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>r2a</td>
<td>p value</td>
</tr>
<tr>
<td>SA 1 ~ Vol. 1</td>
<td>0.93</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>SA 2 ~ Vol. 2</td>
<td>0.3342</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>Depth ~ Width</td>
<td>0.9986</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>Perimeter ~ Convex Hull</td>
<td>0.4634</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>(1+642 df)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations:
- lateral root volume (Vol. 1), lateral root surface area (SA 1), root system maximum width (width), root system convex hull area (convex hull), root system maximum depth (depth), crown root volume (Vol. 2), crown root surface area (SA 2), root system perimeter (perimeter)
Table 5. QTL LOD scores and Respective Allelic Means. QTL model logarithm of the odds scores (LOD) and allelic means. The LOD significance level column lists the QTL model significance cutoffs and the LOD peak score column lists the highest LOD score for the individual QTL. The columns of allelic means compile the QTL mean of RILs with respective parental alleles. The latter two columns compile the percentage of phenotypic variation explained by the QTL model/s.

<table>
<thead>
<tr>
<th>Trait</th>
<th>LOD significance level</th>
<th>LOD peak score</th>
<th>S. bicolor allele mean</th>
<th>S. pinpinum allele mean</th>
<th>QTL percent PVE</th>
<th>Additive model percent PVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGB (transformed)</td>
<td>&gt;3.15</td>
<td>3.49</td>
<td>26.67 ± 3.2 g</td>
<td>22.49 ± 7.7 e g</td>
<td>9.11</td>
<td>NA</td>
</tr>
<tr>
<td>Vol. 1 (transformed)</td>
<td>&gt;3.21</td>
<td>3.45</td>
<td>116.64 ± 3.28 mm³</td>
<td>100.25 ± 2.44 mm³</td>
<td>9.03</td>
<td>NA</td>
</tr>
<tr>
<td>Width (transformed)</td>
<td>&gt;3.14</td>
<td>4.57</td>
<td>18.11 ± 0.049 mm</td>
<td>17.71 ± 0.08 mm</td>
<td>11.79</td>
<td>NA</td>
</tr>
<tr>
<td>Convex hull (transformed)</td>
<td>&gt;3.11</td>
<td>3.23</td>
<td>140.83 ± 0.90 mm²</td>
<td>135.59 ± 0.97 mm²</td>
<td>8.48</td>
<td>NA</td>
</tr>
<tr>
<td>Angle (transformed)</td>
<td>&gt;3.06</td>
<td>3.17</td>
<td>0.0293 ± 0.0003 degrees</td>
<td>0.0314 ± 0.0004 degrees</td>
<td>8.32</td>
<td>NA</td>
</tr>
<tr>
<td>Num. roots (split-2)</td>
<td>&gt;3.10</td>
<td>5.31</td>
<td>19.77 ± 0.30</td>
<td>17.83 ± 0.33</td>
<td>12.39</td>
<td>20.95</td>
</tr>
<tr>
<td>Num. roots (%NR.8)</td>
<td>&gt;3.10</td>
<td>4.67</td>
<td>19.69 ± 0.30</td>
<td>17.96 ± 0.33</td>
<td>10.8</td>
<td>20.95</td>
</tr>
<tr>
<td>Depth</td>
<td>&gt;3.13</td>
<td>4.48</td>
<td>328.19 ± 1.70 mm</td>
<td>314.33 ± 2.87 mm</td>
<td>11.57</td>
<td>NA</td>
</tr>
<tr>
<td>Vol. 2</td>
<td>&gt;3.13</td>
<td>4.44</td>
<td>139.35 ± 3.09±2 mm³</td>
<td>117.06 ± 3.54 ±2 mm³</td>
<td>11.37</td>
<td>NA</td>
</tr>
<tr>
<td>Color (gCL1.3)</td>
<td>&gt;3.20</td>
<td>5.71</td>
<td>1.308 ± 0.107</td>
<td>2.081 ± 0.107</td>
<td>12.05</td>
<td>28.78</td>
</tr>
<tr>
<td>Color (gCL5.1)</td>
<td>&gt;3.20</td>
<td>5.51</td>
<td>1.510 ± 0.094</td>
<td>2.056 ± 0.137</td>
<td>11.62</td>
<td>28.78</td>
</tr>
<tr>
<td>Color (gCL8.2)</td>
<td>&gt;3.20</td>
<td>4.87</td>
<td>1.823 ± 0.098</td>
<td>1.459 ± 0.131</td>
<td>10.17</td>
<td>28.78</td>
</tr>
</tbody>
</table>

*Yellow shading indicating an increased effect on the trait

Abbreviations: BGR, lateral root volume (Vol. 1); root system maximum width (width), root system convex hull area (convex hull), crown root growing angle (angle), median number of roots (Num. roots), root system maximum depth (depth), crown root volume (Vol. 2); root pigment (color)
Table 6. Trait Correlation Scores. Strong positive correlations are colored in dark red and strong negative correlations are colored in dark green. The strongest negative correlations were between angle and %C and %C:N. The strongest positive correlations were between depth and width, and below-ground biomass and crown root volume.

<table>
<thead>
<tr>
<th>Trait Correlation Table (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>%C-N</td>
</tr>
<tr>
<td>%C</td>
</tr>
<tr>
<td>Num_roots</td>
</tr>
<tr>
<td>Vol_2</td>
</tr>
<tr>
<td>Depth</td>
</tr>
<tr>
<td>BGB</td>
</tr>
<tr>
<td>Vol_1</td>
</tr>
<tr>
<td>Width</td>
</tr>
<tr>
<td>Convex hull</td>
</tr>
<tr>
<td>Color</td>
</tr>
<tr>
<td>Angle</td>
</tr>
<tr>
<td>Height</td>
</tr>
<tr>
<td>Tiller_number</td>
</tr>
<tr>
<td>AGB</td>
</tr>
</tbody>
</table>

Abbreviations:
- %C-N: above-ground biomass (AGB), below-ground biomass (BGB), lateral root surface area (SA 1), lateral root volume (Vol_2), root system maximum width (width), root system convex hull area (Convex hull), crown root growing angle (angle), crown root surface area (SA 2), median number of roots (Num_roots), root system maximum depth (depth), root system perimeter (perimeter), crown root volume (Vol_1), root pigment (color), percentage of carbon in the rhizosphere (%C), percentage ratio of carbon to nitrogen in the rhizosphere (%C:N), above-ground biomass (AGB), number of tillers (Tiller_number), plant height (height)
Table 7. Exudation Regression Models and Statistical Significance. Statistical significance and coefficient of determinations from exudation regression models. RSA trait models that explained more phenotypic variation than genotype alone are highlighted in yellow. Angle and genotype explained more variation in %C and %C:N than genotype alone. Volume and height models did not add significance to %C or $C:N models.

<table>
<thead>
<tr>
<th>Exudation Regression Models</th>
<th>r2a</th>
<th>df</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>%C:N Model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%C:N ~Angle</td>
<td>0.0233</td>
<td>179</td>
<td>2.25E-02</td>
</tr>
<tr>
<td>%C:N ~Angle + Genotype</td>
<td>0.7207</td>
<td>104</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>%C:N ~ Genotype</td>
<td>0.7108</td>
<td>105</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>%C:N ~ Height</td>
<td>0.0314</td>
<td>179</td>
<td>9.67E-03</td>
</tr>
<tr>
<td>%C:N ~ Height + Genotype</td>
<td>0.7099</td>
<td>104</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>%C:N ~ Genotype</td>
<td>0.7108</td>
<td>105</td>
<td>&lt;2.2E-16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>%C Model</th>
<th>r2a</th>
<th>df</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>%C ~Angle</td>
<td>0.0286</td>
<td>179</td>
<td>1.29E-02</td>
</tr>
<tr>
<td>%C ~Angle + Genotype</td>
<td>0.2892</td>
<td>104</td>
<td>7.11E-04</td>
</tr>
<tr>
<td>%C ~ Genotype</td>
<td>0.2491</td>
<td>105</td>
<td>2.80E-03</td>
</tr>
<tr>
<td>%C ~ Vol. 2</td>
<td>0.0231</td>
<td>179</td>
<td>2.30E-02</td>
</tr>
<tr>
<td>%C ~ Vol. 2 + Genotype</td>
<td>0.2426</td>
<td>104</td>
<td>3.79E-03</td>
</tr>
<tr>
<td>%C ~ Genotype</td>
<td>0.2491</td>
<td>105</td>
<td>2.80E-03</td>
</tr>
</tbody>
</table>

*highlight indicates that RSA trait explained more phenotypic variation than genotype alone

Abbreviations:
- crown root growing angle (angle)
- crown root volume (Vol_2)
- percentage of carbon in the rhizosphere (%C)
- percentage ratio of carbon to nitrogen in the rhizosphere (%C:N)
- plant height (height)
Table 8. Above-ground Regression Models and Statistical Significance. Statistical significance and coefficient of determinations from above-ground trait regression models. Above-ground trait models that explained more phenotypic variation than genotype alone are highlighted in yellow. Height and genotype explained more phenotypic variation than genotype alone for crown and lateral root volume. However, height did not add significance to the crown root volume model. Above-ground biomass explained more phenotypic variation of below-ground biomass than genotype alone. The number of tillers did add significance to the crown root volume model.

<table>
<thead>
<tr>
<th>Model</th>
<th>r2a</th>
<th>df</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. 1 ~ Height</td>
<td>0.0519</td>
<td>1+642</td>
<td>3.03E-09</td>
</tr>
<tr>
<td>Vol. 1 ~ Height + Genotype</td>
<td>0.2927</td>
<td>168+475</td>
<td>8.52E-16</td>
</tr>
<tr>
<td>Vol. 1 ~ Genotype</td>
<td>0.2870</td>
<td>167+476</td>
<td>2.32E-15</td>
</tr>
<tr>
<td>Vol. 2 ~ Height</td>
<td>0.0651</td>
<td>1+642</td>
<td>2.95E-11</td>
</tr>
<tr>
<td>Vol. 2 ~ Height + Genotype</td>
<td>0.3543</td>
<td>168+475</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>Vol. 2 ~ Genotype</td>
<td>0.3425</td>
<td>167+476</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>Vol. 2 ~ Tillers</td>
<td>0.0036</td>
<td>1+642</td>
<td>6.90E-02</td>
</tr>
<tr>
<td>Vol. 2 ~ Tillers + Genotype</td>
<td>0.3424</td>
<td>168+475</td>
<td>&lt;2.22E-16</td>
</tr>
<tr>
<td>Vol. 2 ~ Genotype</td>
<td>0.3425</td>
<td>167+476</td>
<td>&lt;2.2E-16</td>
</tr>
<tr>
<td>BGB ~ AGB</td>
<td>0.1880</td>
<td>1+642</td>
<td>&lt;2.22E-16</td>
</tr>
<tr>
<td>BGB ~ AGB + Genotype</td>
<td>0.3420</td>
<td>168+475</td>
<td>&lt;2.22E-16</td>
</tr>
<tr>
<td>BGB ~ Genotype</td>
<td>0.3001</td>
<td>167+476</td>
<td>&lt;2.22E-16</td>
</tr>
</tbody>
</table>

*highlight indicates that above-ground trait explained more phenotypic variation than genotype alone

Abbreviations:
- below-ground biomass (BGB), lateral root volume (Vol. 1), crown root volume (Vol. 2), above-ground biomass (AGB), number of tillers (tillers), plant height (height)
The illustration on the left is showing the distinct cellular regions of the transition, elongation, and differentiation zones as well as the root cap. The cross-section illustration on the right is showing the cellular distinction of phloem, xylem, and Casparian strip regions. (De Smet, et al., 2015)
Figure 2. Experimental Greenhouse Design

Each of the 24 trays held 36 pots further grouped into two large rectangular plots.
The imaging stage with a black felt background, a Nikon d1150 camera positioned at a height of 52.5 cm, and a white overhead sheet to reduce lighting variation.
Figure 4. RILs Imaged in Three Distinct Orientations

Three separate orientations of replicate RILs were imaged; named Image A, B, and C. Root system perimeter, convex hull area, maximum depth, maximum width, and crown root growing angle measurements were taken using Image A and B. The median number of roots, crown and lateral root volume and surface area, and color measurements were taken using Image C.
Figure 5. Rhizosphere Collection Methodology

Graphic depicting the methodology and root surface of which the rhizosphere was collected. To minimize residual soil contamination, the rhizosphere was collected up to one inch below the soil plug line, indicated by the dashed green line.
Figure 6. Variation in the Crown Root Growing Angle

Images reflect the population’s crown root growing angle variation. The angle was measured as the widest point between the left crown root and the right crown root.
Figure 7. Variation in Red Pigmentation of Roots and the Respective Ranking System

Images reflect the population's pigment variation and the manually measured ranking system. Roots were ranked from 0 to 4, with 0 being indicative of no pigment and 4 being indicative of pigmentation on the entire root system.
A graph of the RIL population’s genetic bins across *Sorghum*’s 10 autoossomes. Individual RILs are on the y-axis and genetic markers (bins) are on the x-axis. Vertical lines represent *Sorghum*’s 10 autosomes. Bins colored red depict *S. propinquum* alleles, bins colored blue depict *S. bicolor* alleles, and bins colored white depict bins that were removed, i.e., heterozygosity, double crossovers.
Figure 9. Variation in RILs’ Root Morphology

Images illustrating the phenotypic variation in RIL’s root morphology. Observed variations include root system width and depth, number of crown roots, and amount of lateral branching.
A comparison of raw images and skeletonized output images from two RIL replicates. In the output images, crown roots are colored by blue pixels (dimension 2) and lateral roots are colored by red pixels (dimension 1).
Figure 11. A Map of the Resulting 11 QTL and Respective Mapping Positions (cM)

A graph of the resulting 11 novel root QTL. The mapping positions (cM) are on the y-axis and 10 autosomes on the x-axis. Colored bars represent the 95% confidence interval of each respective QTL with the peak marked by a black line. The figure legend contains QTL names and confidence intervals in base pairs, respectively. Traits that had a multiple QTL model are mapped in identical colors; root pigment (color) has purple bars and the number of roots (Num. roots) has orange bars. Traits that had a single QTL model are mapped by distinct colors; below-ground biomass (BGB) has a green bar, lateral root volume (Vol. 1) has a blue bar, maximum root system depth and width has a burgundy bar, crown root growing angle (angle) has a pink bar, crown root volume (vol. 2) has a magenta bar, and root system convex hull area (convex area) has a gold bar.
Supplementary Tables

Supplemental Table 1. Candidate genes for Respective QTL. Candidate genes for each of the 11 resulting QTL are highlighted in yellow. Columns contain Sorghum gene name, Sorghum gene annotation, and gene orthology with Arabidopsis and Oryza.