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Convergent evolution of root system architecture in two independently evolved lineages of weedy rice

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Summary

- Root system architecture (RSA) is a critical aspect of plant growth and competitive ability. Here we used two independently evolved strains of weedy rice, a de-domesticated form of rice, to study the evolution of weed-associated RSA traits and the extent to which they evolve through shared or different genetic mechanisms.
- We characterised 98 two-dimensional and three-dimensional RSA traits in 671 plants representing parents and descendants of two recombinant inbred line populations derived from two weed × crop crosses. A random forest machine learning model was used to assess the degree to which root traits can predict genotype and the most diagnostic traits for doing so. We used quantitative trait locus (QTL) mapping to compare genetic architecture between the weed strains.
- The two weeds were distinguishable from the crop in similar and predictable ways, suggesting independent evolution of a 'weedy' RSA phenotype. Notably, comparative QTL mapping revealed little evidence for shared underlying genetic mechanisms.
- Our findings suggest that despite the double bottlenecks of domestication and de-domestication, weedy rice nonetheless shows genetic flexibility in the repeated evolution of weedy RSA traits. Whereas the root growth of cultivated rice may facilitate interactions among neighbouring plants, the weedy rice phenotype may minimise below-ground contact as a competitive strategy.

Introduction

An active and important question in evolutionary biology is the extent to which the genetic basis of adaptation is flexible or constrained by phylogeny (Orr, 2005; Protas *et al.*, 2006; Weller *et al.*, 2012; Ng & Smith, 2016). One of the most common ways this question has been examined is through comparisons of separate populations or species that have independently evolved the same phenotype under similar environmental conditions and selective pressures. Evidence for this type of repeated phenotypic evolution is abundant across all kingdoms of life (for example Fong *et al.*, 2005; Losos, 2011; Pichersky & Lewinsohn, 2011), but what is less well understood is the extent to which it results from selection acting on the same genes and/or developmental pathways (referred to herein as parallel evolution or parallelism) (Weller *et al.*, 2012) or different genetic and developmental processes (referred to herein as convergent evolution or convergence) (Larson, 2014; Ng & Smith, 2016). In general, parallelism is predicted to be most likely for lineages that are phylogenetically closely related and hence genetically similar (Losos, 2011), as well as for lineages that are constrained by low genetic diversity (limiting the pool of potentially advantageous alleles) (Orr, 2005), and traits that are controlled by genes with highly specialised functions (Pfenning *et al.*, 2014).

In plants, repeated evolution has been described for a diverse range of traits, including photoperiod response (reviewed in Lenser & Theißen, 2013), abiotic stress adaptation (Lyu *et al.*, 2018), chemical defence metabolites (Takos *et al.*, 2011), floral pigmentation and morphology (Ng & Smith, 2016), mating system (Fishman *et al.*, 2015), and other agronomic traits in crop species (Sang, 2009). Many of these studies have found evidence for evolutionary convergence. However, as most such studies have compared species across different families or higher taxonomic groups, their findings may be inherently biased towards observations of convergence over parallelism. Far fewer studies in plants have examined instances of repeated evolution within species. In addition, virtually all of these studies have focused on the above-ground half of the plant phenotype. As a result, little to nothing is known about the extent to which adaptive changes in root growth and development may be evolutionarily constrained to a greater or lesser extent than above-ground growth.

Weedy rice (*Oryza sativa* f. *spontanea*) offers an attractive system for overcoming these gaps in our understanding of repeated phenotypic evolution in plants. This agricultural weed is a feral (de-domesticated) descendant of the genomic model crop species rice (*O. sativa*). Weedy rice has evolved multiple times independently from different domesticated rice varieties around the world (Federici *et al.*, 2001; Cao *et al.*, 2006; Londo & Schaal,

2007; Grimm *et al.*, 2013; Wedger & Olsen, 2018). The process of weedy rice evolution is associated with the repeated emergence of suites of adaptations that distinguish the weed from its domesticated ancestor and allow it to aggressively outcompete rice in the field. Weedy rice adaptations include highly shattering seeds (Qi *et al.*, 2015), strong seed dormancy (Gross *et al.*, 2010), herbicide resistance (Singh *et al.*, 2017b), and the ability to outcompete cultivated rice for light and soil nutrients (Burgos *et al.*, 2006; Xu *et al.*, 2018). Infestations of as few as eight weedy rice plants/m^2 can reduce rice yields by almost two-thirds (Xu *et al.*, 2018). The weed's competitive ability has been proposed to be directly related to the pattern of root growth (Burgos *et al.*, 2006), although this hypothesis has not been directly tested. As independently derived, conspecific relatives of domesticated rice, weedy rice strains are highly amenable for directly comparing the genetic basis of repeated weediness evolution.

The genetic and genomic differences that distinguish different weedy rice varieties are very well characterised (Londo & Schaal, 2007; Vigueira *et al.*, 2013a; Li *et al.*, 2017), and this information has provided a basis for recent studies that are elucidating mechanisms of repeated evolution of weedy rice (Qi *et al.*, 2015; Li *et al.*, 2017). In the USA, two genetically distinct weed strains predominate in the major rice growing region of the southern Mississippi valley. These two strains are distinguishable based on grain characteristics and are referred to as black-hull awned (BHA) and straw-hull awnless (SH) weedy rice. BHA weeds are feral descendants of cultivated *aus* rice varieties, while SH forms are descended from cultivated *indica* rice varieties. As neither *aus* nor *indica* varieties of rice were ever commercially cultivated in the USA, the BHA and SH weed strains are presumed to have originated in southern Asia, where *aus* and *indica* varieties are traditionally grown. Later introductions into the USA are likely to have occurred through weed-contaminated seed grain imports (Londo & Schaal, 2007). In the 150-yr history of BHA and SH weed presence in the USA, minimal amounts of hybridisation have been detected between the weed strains due to high selfing rates (Singh *et al.*, 2017a).

Several factors support the prediction that parallelism rather than convergence might be expected to underlie the emergence of weediness traits in the BHA and SH weeds. First, like most annual crop species, *O. sativa* underwent a genome-wide loss of genetic diversity during the process of domestication. This domestication bottleneck would have left a more limited pool of genetic diversity as a starting point for weed evolution compared with evolution in a wild species. In addition, outcrossing rates are very low in both cultivated and weedy rice (typically < 1%) (Cao *et al.*, 2006; Gealy *et al.*, 2009), which would further limit opportunities to enhance the genetic diversity of evolving weed strains. Consistent with these factors, genetic diversity in both BHA and SH strains is exceedingly low compared with their direct domesticated ancestors and to wild rice (Reagon *et al.*, 2010; Li *et al.*, 2017). Taken together with the close phylogenetic relationships among all weedy and cultivated rice populations, parallelism would therefore seem to be the most likely mechanism by which weedy traits would emerge. Interestingly, however, this is not the primary mechanism that has been observed in weedy rice studies to date (Mispan *et al.*, 2013; Thurber *et al.*, 2013; Qi *et al.*, 2015). These studies, all of which have examined above-ground traits, have revealed different genetic architectures for several weediness traits in the two United States weed strains.

Below-ground root growth and spatial organisation of root systems can be described in terms of root system architecture (RSA) (Topp *et al.*, 2013). Despite its critical role in determining efficiency of soil nutrient and water uptake, as well as neighbour-to-neighbour communication and levels of plant competition, RSA is far less characterised than above-ground aspects of plant growth (Casper & Jackson, 1997; Topp *et al.*, 2016). To the extent that the genetics of RSA have been examined, this has mostly been at the level of QTL mapping, in which many loci have been identified in crop varieties (Uga *et al.*, 2011; Topp *et al.*, 2013). Only two RSA genes have been cloned and functionally characterised, both in rice: *DEEPER ROOTING 1* (*Dro1*) (Uga *et al.*, 2013) and *Phosphorus-Starvation Tolerance 1* (*PSTOL1*) (Gamuyao *et al.*, 2012). To our knowledge, no study has investigated RSA or its genetic basis in weedy rice.

In this study we used comparative QTL mapping in two advanced-generation recombinant inbred line (RIL) mapping populations, derived from a BHA × *indica* cross and a SH × *indica* cross with the same *indica* parent, to examine the genetic basis of weedy rice RSA and the extent to which it has evolved through parallelism or convergence. We investigated three questions. First, are there RSA differences between the BHA, SH, and *indica* parents? Second, if so, are any of those differences shared by both weed ecotypes in a pattern suggesting repeated phenotypic evolution? Third, to the extent that there are shared weed-specific RSA traits, does their genetic architecture indicate that these are controlled by similar or different underlying genetic mechanisms?

Materials and Methods

Plant materials

Seeds for all accessions from two weed × crop RIL mapping populations were obtained from the USDA-ARS Dale Bumpers National Rice Research Center (Stuttgart, AR, USA), where they were advanced to the F_8 generation through single seed descent. The mapping populations were initiated in 2007–2009 at the University of Massachusetts – Amherst, USA by crossing the Taiwanese *indica* rice variety Dee Geo Woo Gen (DGWG; PI 653419) with each of two United States weedy rice ecotypes (Thurber *et al.*, 2013). The crop genotype used in our study is best known as the original source of the *sd1* semidwarfism allele that gained fame with the improved rice cultivars of the Green Revolution (Spielmeyer *et al.*, 2002). The first cross (source of the B mapping population below) was produced by crossing DGWG with a black-hull awned accession (MS-1996-6; GSOR 303535). The second cross (source of the S population below) was produced by crossing DGWG with a SH accession (AR-2000-1135-01; GSOR 303286). Seeds for 224 and 175 RILs from the B and S populations were obtained through the USDA-GRIN germplasm collection (https://www.ars-grin.gov).

Phenotyping

On a weekly basis over a 2-yr period in 2016–2017, replicates of the parental lines and RIL accessions were grown and phenotyped for below-ground root architecture using a modified root imaging protocol (Topp *et al.*, 2013). Two replicates per parent genotype were grown each week to serve as controls. Seeds were de-hulled and surface-sterilised with a 10-min bath of 35% hydrogen peroxide followed by three washes with distilled and deionised water. Sterilisation prevented fungal growth which would inhibit efficient imaging as described below. Sterilised seeds were placed on Petri dishes with Yoshida's nutrient solution containing 0.5% Gelzan gellan gum. Seeds were then placed in a dark incubation chamber at 29°C for 3 d to stimulate germination. Up to two healthy seeds per genotype were chosen for transplanting based on germination success, lack of microbial contamination and, when applicable, maximal distance from the nearest contaminated seedling. Germinated seeds were transplanted into glass 2 l ungraduated cylinders with 1 l sterilised Yoshida's nutrient solution containing 0.25% Gelzan gellan gum using flame-sterilised forceps and a sterile pipette (one seedling per cylinder). Transplanted seeds were assigned a unique identifier and left at room temperature and ambient light for 12 h to overcome transplanting shock. Plants were then moved into a growth chamber set for long day photoperiod (16-h days at 28°C and 600 μmol of light : 8-h nights at 24°C and 0 μmol of light) and left to grow for 10 d. On d 13 after germination, plants were removed from the growth chamber and imaged using a custom rig as described below (see also Supporting Information Fig. S1).

To facilitate imaging, cylinders with plants were placed individually on a turntable in a glass box filled with water (to correct for light diffraction) and backlit with a uniform green light panel. The cylinders were then rotated 360° on the turntable, and images were taken every 5° by a computer-controlled camera, resulting in 72 sequential images per plant. Plants that had become contaminated by microbial growth during the 10-d growth period were not imaged; these represented *c.* 10% of all seeds planted. Plants that failed to continue growing after transplanting were also not imaged; these represented *c.* 2% of all seeds planted. Wet shoot and root weights were taken immediately after imaging, and dry shoot and root weights were taken after sufficient drying time. Up to 40 plants were imaged per wk.

Images were analysed using a modified GiaRoots pipeline (Galkovskyi *et al.*, 2012; Topp *et al.*, 2013) which includes scaling, cropping, and thresholding the images to convert the greyscale image to a set of binary images. These binary images were then analysed by GiaRoots2D to measure two-dimensional (2D) traits. A three-dimensional (3D) reconstruction of the root was produced using the RootworkPerspective software. The 3D reconstruction was then analysed by GiaRoots3D to measure 3D RSA traits. The reconstruction was then further analysed by DynamicRoots, which can more finely measure traits from distinct root classes (for example primary, first-order lateral, second-order lateral). In total, we obtained phenotypic measurements for 98 RSA traits, many of which were strongly correlated (Fig. S2).

Phenotypic analysis and QTL mapping

To test for significant phenotypic differences between the crop and weed parental genotypes (DGWG, BHA, and SH), their phenotypic values were compared using a single factor analysis of variance (ANOVA) followed by a post-hoc Tukey honest significant difference (HSD) test in R. Equal variance and normality assumptions were tested in R using Levene's test and the Shapiro–Wilk test, respectively. When assumptions were violated, results from ANOVA were validated in R using Welch's one-way test and the Kruskal–Wallis rank-sum test. Traits that failed to show significant differences in the ANOVA, Welch's, or Kruskal–Wallis tests were considered not significantly different. Traits were binned into six patterns of significance based on a Tukey HSD significance threshold of $\alpha = 0.05$. The six bins were as follows for pairwise differences between the BHA parent (b), the *indica* crop parent (c) and the SH parent (s): $b = c = s$ (i.e. no significant differences between any lines); $b = c \neq s$ (SH different from other two parents); $b = s \neq c$ (DGWG different from other two parents); $c = s \neq b$ (BHA different from the other two parents); $b \neq c \neq s$ (all parents different from each other); and a catch-all bin for any other patterns (for example $b = c = s \neq b$ and other nontransitive relationships that reflected differences in confidence interval widths). The $c = s \neq b$ bin corresponds to a pattern predicted based on phylogenetic relationships alone, as *indica* rice is the putative direct ancestor of SH weedy rice, whereas BHA is less closely related to these genotypes. The $b = s \neq c$ bin would be consistent with repeated phenotypic evolution of shared root phenotypes in the two independently evolved weed strains that distinguish them from the crop. This analysis allowed us to begin describing the suite of traits that together characterise the below-ground weedy rice phenotype.

The parental phenotypes were further analysed using the R/randomForest machine learning package in R. This analysis was performed to determine first if the parental genotypes were reliably distinguishable from each other (as opposed to the ANOVAs above, which assessed whether the weeds were distinguishable from the crop) and, second, if so, which traits were the most diagnostic in differentiating the parental genotypes. The random forest model built 3000 trees and was trained on two-thirds of the data. The resulting model was applied to the remaining one-third of the data to assess predictive success.

Using modified linkage maps from previously published B and S mapping populations (Qi *et al.*, 2015; D.M. Goad, unpublished), QTL mapping of root phenotypes was performed in R/qtl using the *scanone* function and the Haley–Knott method for a balance in speed and performance (Haley & Knott, 1992; Broman *et al.*, 2003). Physical positions were determined relative to the Msu v.7.0 rice genome (http://rice.plantbiology.msu.edu). LOD thresholds were calculated on a trait-by-trait basis using 10 000 permutations. LOD confidence intervals represent a drop of 1.5 LOD on either side of the maximum value. Mapping was performed using 11 853 and 4733 single nucleotide polymorphism (SNP) markers from the F_5 generations for the B and S populations, respectively. These markers were obtained in an earlier generation of the RILs (F_5) and published in an earlier study

(Qi *et al.*, 2015). QTL positions were visualised using the R/QTLTOOLS package in R (Delaneau *et al.*, 2017).

Results

We imaged 671 rice plants for 98 2D and 3D RSA traits. The phenotyped plants included 30 replicates of the BHA parent, 29 replicates of the crop parent, 33 replicates of the SH parent, 237 plants from the B mapping population (BHA × DGWG RILs), and 342 plants from the S mapping population (SH × DGWG RILs). In the B population, 84 RILs were phenotyped twice and 23 RILs were phenotyped three times, yielding 107 RILs with two or more replicates. In the S population, 63 RILs were phenotyped twice, and 72 were phenotyped three times; this yielded 135 RILs represented by two or more replicates. Only RILs that were phenotyped at least twice were used in further analysis. It should be noted that the limited number of RILs analysed in each population could potentially bias our results towards the identification of a few large-effect QTL, leading to an underestimate of the total number of small -effect loci.

Parental line assessment

For the 98 RSA traits where we tested for differences between the three parental lines, 62 of them showed no significant differences (corresponding to a pattern of $b = c = s$, where b is black-hull awned parent, c is crop parent, and s is an SH parent). Among those with significant differences, eight of the traits fitted the pattern that would be predicted if phylogenetic history were the primary determinant of phenotypic differences (with the closely related SH and DGWG genotypes not significantly different from each other, but both significantly different from the evolutionarily diverged BHA genotype; i.e. $c = s \neq b$). The opposite pattern was observed for only one trait (no significant difference between the BHA and crop genotype, but SH significantly different from those accessions; $b = c \neq s$). Notably, 20 traits showed significant phenotypic differences in the pattern that would be predicted if the weedy rice strains had independently evolved shared root morphologies (no significant differences between the SH and BHA parents but significant difference between the weeds and DGWG; $b = s \neq c$). We refer to these as 'weed-specific RSA traits' below.

Weed-specific RSA traits Because many of the root traits are highly correlated (for example mean root depth and median root depth; Fig. S2), we condensed the 20 putative weed-specific RSA traits into eight summary descriptor traits: root width−depth ratio, average root width, maximum number of roots, width of the root system, specific root length, mean root depth, mean root tortuosity (i.e. degree to which roots are curved), and mean root−soil angle (i.e. degree to which roots grow horizontally or vertically). From these descriptor traits, the crop (DGWG) root system can be summarised as being different from the weeds in the following ways: it is wider and higher in the soil, with individual roots that are thinner, longer, more abundant, more curved, and at a lower angle to the soil (Fig. 1).

To assess whether the parental lines could be reliably differentiated using root traits, a random forest machine learning approach was undertaken in R using the R/RANDOMFOREST package. While random forest machine learning is usually used to predict unknowns, we used it here to reduce the dimensions of our data (Ramírez *et al.*, 2010). We found that the three parents were correctly identified *c.* 60% of the time when each data type was analysed separately (GIAROOTS2D, GIAROOTS3D and DYNAMICROOTS) (Table S1). By comparison, assignment to the correct parental genotype by chance alone would be expected 33.3% of the time. When all three data types were combined, the strains were correctly identified *c.* 70% of the time. Therefore, the analysis of root traits approximately doubles the probability of correct assignment compared to random chance alone.

The R/RANDOMFOREST package also generates a rank order of diagnostic traits. These traits can be considered the most important for distinguishing root phenotypes of the crop vs weed parents, although they should not be interpreted as necessarily related to the biology of weediness. For the 2D dataset, the most diagnostic traits were *Solidity (2D)* (density of the root system), and *Maximum Width (2D)*. For the 3D datasets, the most diagnostic traits are *Width−Depth Ratio*, and *Median Lateral Root−Soil Angle* for GIAROOTS3D and DYNAMICROOTS traits, respectively (Fig. 2). Although the results presented in Fig. 2 represent a typical run, highly correlated traits shifted in relative importance between individual runs. Regardless of the exact trait at the top of the list, the biological interpretation is robust between runs. All three datasets placed the most importance on traits related to width and exploration.

QTL analysis

Out of 98 root phenotypes that were evaluated in the F_8 generation of the two weed × crop mapping populations, we identified 65 significant QTLs distributed across 43 root traits (Table S2, see also Table S3). In the S population (SH × *indica*), 36 QTLs were identified, with 22 traits having one QTL apiece, and six traits mapping to more than one genomic location (up to three QTLs). In the B (BHA × *indica*) population, 29 QTLs were identified, with 19 traits mapping to one QTL apiece, and five traits mapping to two QTLs. Of the 43 traits with significant QTLs, 10 traits mapped to both populations; these traits fall into four broad trait categories (Table 1). We describe these shared mapped traits below.

Root depth Both *Depth (2D)* and *Major Ellipse Axis (2D)* are measures of rooting depth. *Depth (2D)* is the straight-line distance between the soil line and the tip of the deepest root at 90° from the soil line. *Major Ellipse Axis (2D)* is the distance between the two major vertices of the smallest possible ellipse encompassing the entire root system. If the root mass is symmetrically distributed along the depth axis, this measurement is very similar to *Depth (2D)*. If not, it captures differences in root mass distribution. In the B population, both traits map to the same position in the middle of chromosome 4 (Table 1; Fig. 3a) while, in the S population, both traits map to the same position in the middle of chromosome 8 (Table 1; Fig. 3b). Both weed parents are on

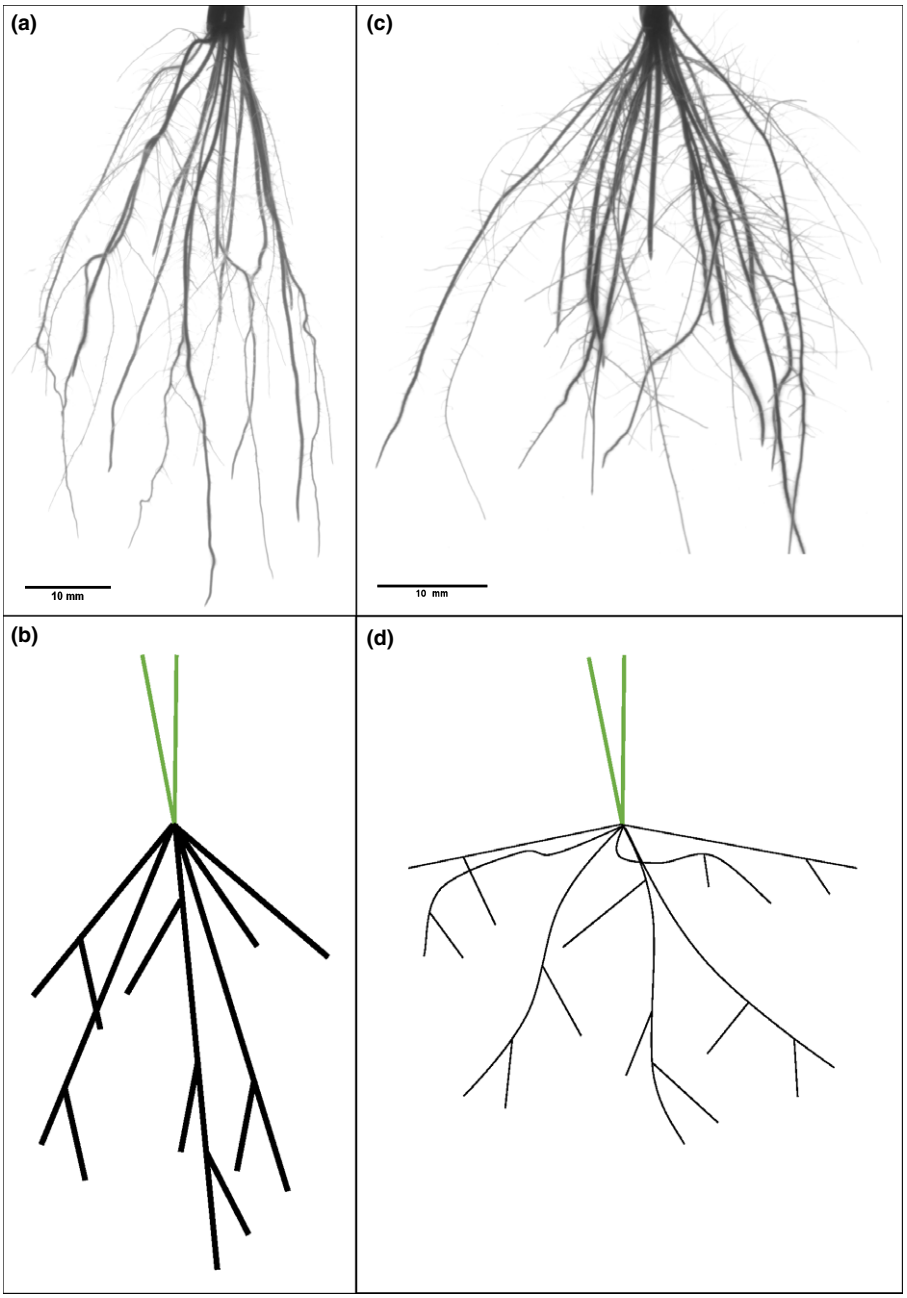

Fig. 1 Characteristic differences in root system architecture between weedy and cultivated rice. Panels show digitised images (a, c) and schematic drawings (b, d) of typical weed (a, b) and crop (c, d) roots. Individual crop roots are thinner, longer, more curved, and more abundant, while the root system as a whole is higher in the soil, wider, and has shallower root–soil angles than the weed.

average deeper than the crop. Interestingly, however, all the significant QTLs for root depth have increased effects conferred by the crop allele, ranging from a 10.8–16.5% effect and explaining 8.4–14.9% of the variation. This pattern suggests that there may be many small effect loci in the weeds that are undetected by this study and that collectively cause the weeds to grow deeper roots than the crop parent.

Root system width *Minor Ellipse Axis (2D)* and *Maximum Network Width (3D)* are both measures of the width of the root system. *Minor Ellipse Axis (2D)* is the distance between the two minor vertices of the smallest possible ellipse encompassing the entire root system. If the root mass is symmetrically distributed along the depth axis, this measurement is very similar to the *Maximum Network Width (2D)*. If not, it provides an alternative measure of differences in root mass distribution distinct from *Major Ellipse Axis (2D)*. *Maximum Network Width (3D)* is the widest span of the root system in a plane parallel to the soil line. In the B population, two width-associated QTLs were identified, including a *Minor Ellipse Axis (2D)* QTL at the top of chromosome 6 and a *Max Network Width (3D)* QTL at the top of chromosome 9 (Table 1; Fig. 3a). In the S population, we also identified two QTLs. The first QTL was mapped with both trait measures to the middle of chromosome 4, while the second mapped with *Maximum Network Width (3D)* at the top of chromosome 5 (Table 1; Fig. 3b). The crop parent has a wider root system than both weed parents. For three of the five significant QTLs, the crop alleles conferred increased width, ranging from

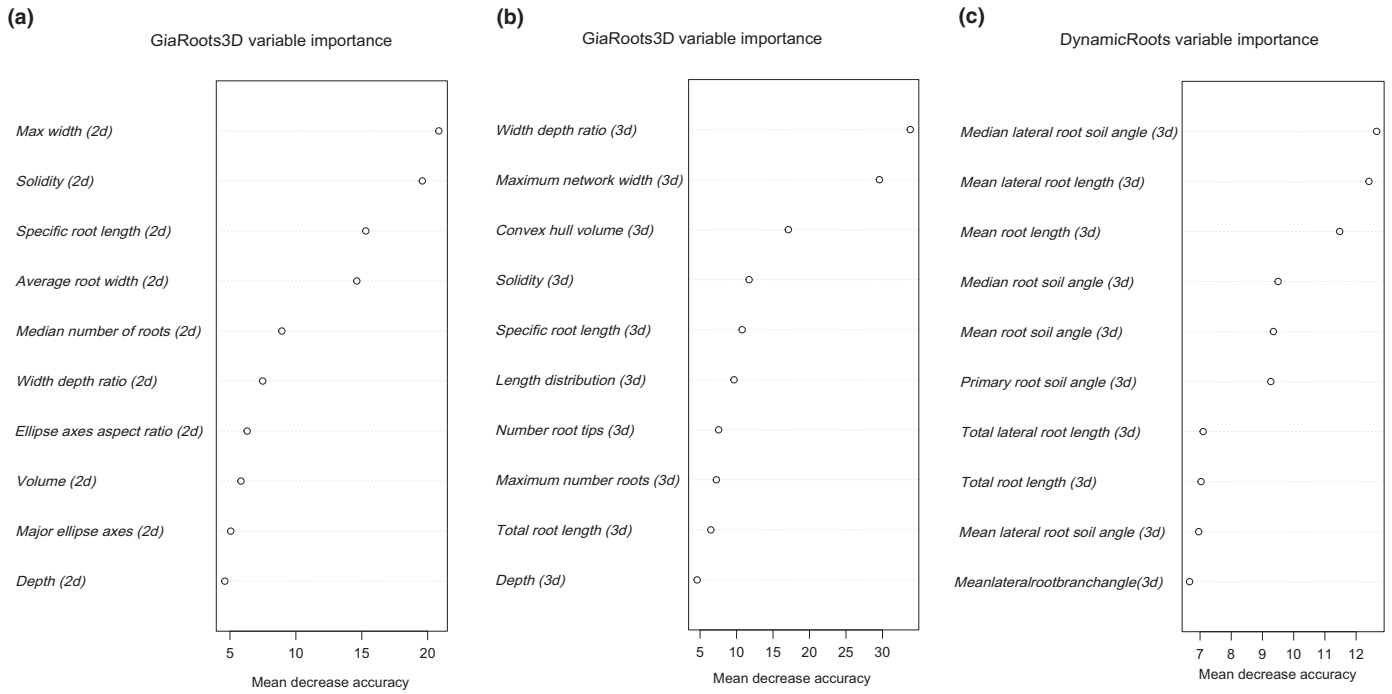

Fig. 2 Diagnostic importance of rice root phenotypic variables from random forest machine learning model. GiaRoots2D (a), GiaRoots3D (b), and DynamicRoots (c) datasets put highest diagnostic importance on exploration, system width, and root–soil angle traits, respectively. 'Mean Decrease Accuracy' is a measure of how many extra observations would be misclassified if the trait in question were removed. Highly correlated traits will shift in importance between runs, but these changes in rank order do not change the biological interpretation.

10.6–15.2% increased effects and explaining 7.4–13.3% of the phenotypic variation. For the other two QTLs, the weed alleles confer increased width, ranging from a 10–16.6% increase and explaining 6.6–13.4% of the phenotypic variation.

Exploratory space *Perimeter (2D)*, *Network Convex Area (2D)*, *Convex Hull Volume (3D)*, and *Solidity (3D)* are all measures to describe the volume of soil media explored by a root system. These measures approximate the extent to which the roots reach into their surroundings. *Perimeter (2D)* is calculated as the number of root pixels connected to a background pixel, an estimate of absorptive surface of the root system. *Network Convex Area (2D)* is calculated by drawing the smallest convex polygon around the root system and calculating the area inside the polygon. *Convex Hull Volume (3D)* is calculated in much the same way, but in three dimensions. *Solidity (3D)* can be thought of as the density of the root system and is calculated by dividing *Total Root Volume (3D)* by *Convex Hull Volume (3D)*. A larger solidity would be denser and therefore less exploratory. The latter three traits are correlated (Fig. S2).

For all four exploratory space measures, one QTL was identified in the B population at the top of chromosome 6, and one QTL was identified in the S population in the middle of chromosome 4 (Table 1; Fig. 3b). For *Convex Hull Volume (3D)* and *Network Convex Area (2D)*, another B population QTL was identified in the middle of chromosome 4, but statistically only the *Convex Hull Volume (3D)* QTLs overlap between the S and B population. Interestingly, Convex Hull Volume is the only trait of the 10 RSA traits considered in both mapping populations that maps to overlapping genomic regions in the two populations (Table 1; Fig. 3a). Therefore, most RSA QTLs for weedy rice are not shared between the BHA and SH ecotypes.

There was high variability in these exploratory space traits, but in general the crop parent had higher exploration than either weed ecotype. Effect directions are similarly variable, with the same QTL increasing exploration in both the BHA weed and crop depending on the particular exploratory space measure calculated. This variability in effect directions is likely to be due to the allometric relationships between the traits that can create nonlinear relationships as a function of dimensionality. Five of the other six QTLs had increased effects in the crop, conferring a 12.6–40% change in phenotype and explaining 8.7–14.3% of the phenotypic variation.

Root–soil angle *Mean Root–Soil Angle (3D)* and *Mean Lateral Root–Soil Angle (3D)* describe the angle of roots relative to the soil surface. A larger angle would result in a deeper, narrower root system. Both root–soil angle traits map to the top of chromosome 12 in the B population (Table 1; Fig. 3a), whereas they map to the bottom of chromosome 2 in the S population (Table 1; Fig. 3b). The crop parent had a lower root–soil angle than the weeds. For both significant QTLs, the weed parent alleles confer increased effects, leading to a 9.8–23.4% phenotypic change and explaining 9–13.7% of the phenotypic variation.

Discussion

Despite its critical importance for traits such as nutrient uptake and competition for soil resources, RSA remains one of the least

Table 1 QTLs for identified phenotypes that map in each of two mapping populations of cultivated × weedy rice.

Trait group	Phenotype	Mapping population	QTL‡	Chromosome	Genomic position (cM)	Physical position§	LOD drop 1.5 confidence interval	PVE* (R^2)	$\alpha = 0.05$ LOD threshold	LOD	Increased effect	Effect size per allele†	RIL average	% Effect per allele
Root depth	Depth2D	B	*qRDP4B-a*	4	72.1	23 609 792	21 631 772–27 286 914	14.9	3.3	3.9	Crop	−5.8	76	−7.63
		S	*qRDP8S-a*	8	51.6	10 219 719	5757 773–21 026 154	8.4	3	3.1	Crop	−3.8	70.4	−5.40
	majorElipseAxis2D	B	*qRDP4B-b*	4	72.1	23 609 792	21 631 772–27 286 914	14.9	3.2	3.9	Crop	−5.7	68.9	−8.27
		S	*qRDP8S-b*	8	47.6	8939 999	5757 773–17 076 446	9.9	3.2	3.6	Crop	−3.7	60.3	−6.14
Root system width	MinorEllipseAxis2D	B	*qRSW6B-a*	6	9.6	2472 895	2176 780–2867 607	13.4	3	3.5	Weed-B	2.8	33.7	8.31
		S	*qRSW4S-a*	4	101	29 817 550	21 631 772–30 808 550	9.6	3.1	3.5	Crop	−1.8	34.4	−5.23
	MaxNetworkWidth3D	B	*qRSW9B-a*	9	13.3	8199 257	7936 052–10 105 116	13.3	3.3	3.4	Crop	−2.6	34.2	−7.60
		S	*qRSW4S-b*	4	90.7	28 026 225	21 746 628–33 128 017	7.4	3.1	3	Crop	−1.8	33.8	−5.33
		S	*qRSW5S-a*	5	30.1	4184 550	3090 444–5478 629	6.6	3.1	2.7	Weed-S	1.7	33.8	5.03
Exploratory	Perimeter2D	B	*qREX6B-a*	6	9.6	2472 895	2176 780–2867 607	13	3.2	3.3	Weed-B	394.2	2897.8	13.60
		S	*qREX4S-a*	4	101	29 817 550	23 747 123–33 386 030	8.7	3.1	3.1	Crop	−195.5	3081.6	−6.34
	Solidity3D	B	*qREX6B-b*	6	9.6	2472 895	2176 780–25 947 874	14.2	3.2	3.7	Crop	−0.001	0.005	−20
		S	*qREX4S-b*	4	57	21 892 817	19 858 548–33 386 030	7	2.4	2.5	Weed-S	0.001	0.007	14.29
	ConvexHullVolume3D	B	***qREX4B-a***	4	84.4	27 210 802	24 867 620–27 286 914	14.3	3.2	4.4	Crop	−10 835	57 968.3	−18.69
		B	*qREX6B-c*	6	9.6	2472 895	2176 780–2867 607	11.2	3.2	3.6	Weed-B	9873	57 968.3	17.03
		S	***qREX4S-c***	4	90.7	28 026 225	21 844 394–30 808 550	10.8	3.2	4	Crop	−7949	53 084	−14.97
	NetworkConvexArea2D	B	*qREX4B-b*	4	84.5	27 247 058	21 631 772–27 286 914	13.9	3.2	4.3	Crop	−399	2870.6	−13.90
		B	*qREX6B-d*	6	9.6	2472 895	2176 780–2867 607	12.1	3.2	3.8	Weed-B	381.6	2870.6	13.29
		S	*qREX4S-d*	4	101	29 817 550	22 176 514–33 631 677	9.14	3.1	3.3	Crop	−230.7	2658.5	−8.68
Root–soil angle	MeanLateralRootSoilAngle3D	B	*qRSA12B-a*	12	10	1659 127	148 244–2023 121	13.7	3.2	3.5	Weed-B	3.8	32.5	11.69
		S	*qRSA2S-a*	2	170	34 784 617	32 697 234–35 053 040	9	3	3.3	Weed-S	1.1	22.6	4.87
	MeanRootSoilAngle3D	B	*qRSA12B-b*	12	10	1659 127	148 244–2023 121	13.6	3.3	3.4	Weed-B	3.9	32.8	11.89
		S	*qRSA2S-b*	2	167	3430 7545	32 697 234–35 053 040	9	3	3.3	Weed-S	1.1	22.8	4.82

*PVE is per cent of the phenotypic variation explained by the allelic variation at the QTL.
†A positive value in effect size represents a positive change in the weed, while a negative value represents a positive change in the crop.
‡QTLs in bold are the only QTL to map to the same position in both mapping populations.
§Physical positions were determined relative to the MSU v.7.0 assembly http://rice.plantbiology.msu.edu

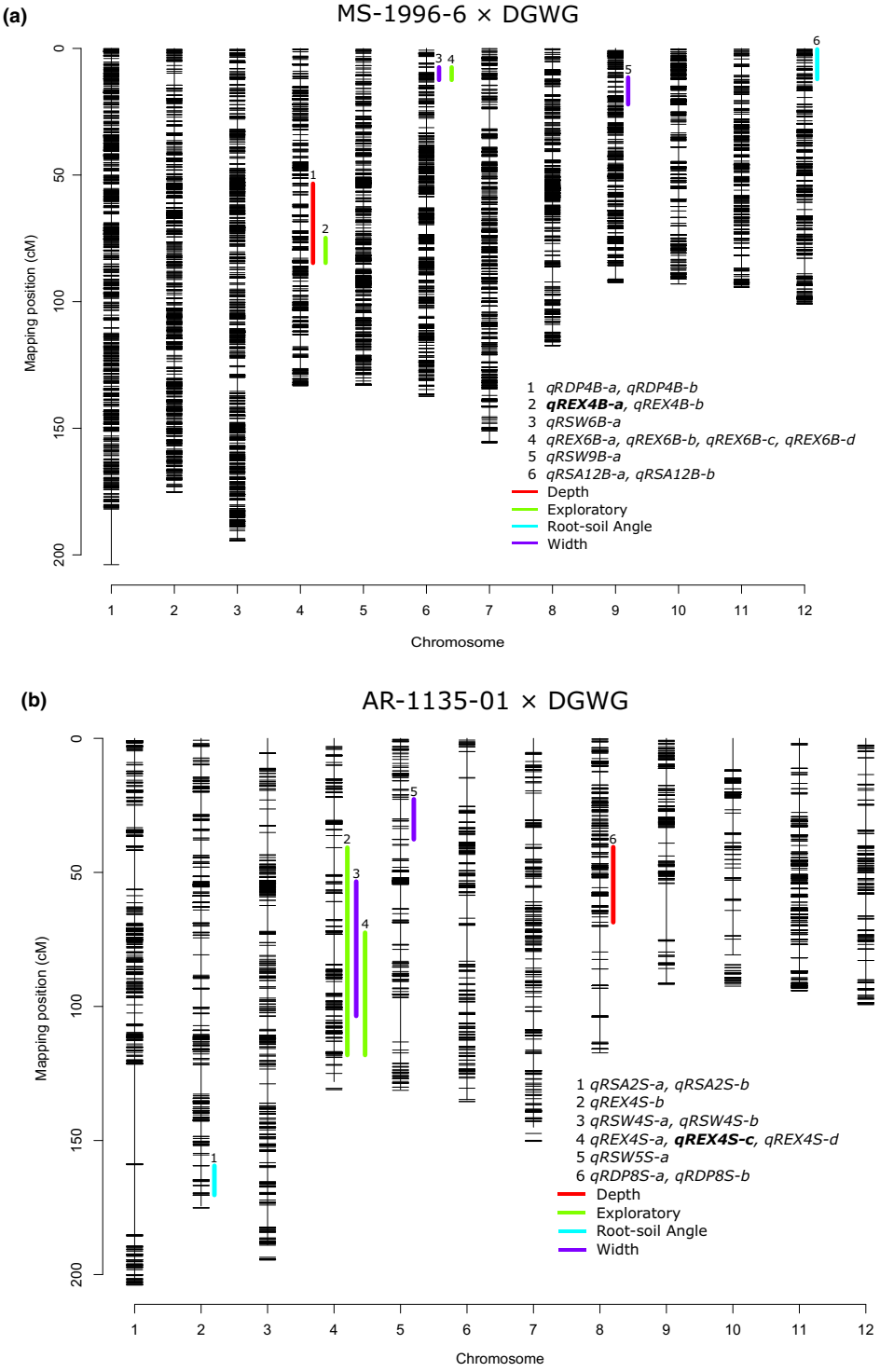

Fig. 3 Rice genome linkage maps of the MS-1996-6 × DGWG 'B' population (a) and AR-2000-1135-01 × DGWG 'S' population (b) with QTL from four broad trait groups highlighted. Each vertical black line represents a rice chromosome, while horizontal hash marks indicate one SNP. Coloured vertical lines represent the confidence intervals (LOD drop 1.5) of mapped traits. Only one trait (*Convex Hull Volume 3D*, an exploratory trait shown in bold font) maps to the same location in both populations.

well characterised aspects of plant growth morphology. Here we have used an integrated root imaging platform to precisely characterise RSA traits in a cultivated rice genotype and in two independently evolved ecotypes of weedy rice, feral descendants of the crop that aggressively outcompete it in the field. We have used this system to examine the extent to which weed-associated RSA traits have evolved in a pattern consistent with repeated phenotypic evolution, and whether comparative QTL mapping suggests that parallelism or convergence is more likely to have played a role in this process. We found clear evidence for repeated phenotypic evolution below ground, with the SH and BHA weedy rice parents independently evolving a shared suite of RSA traits (Fig. 1). Interestingly, despite the close phylogenetic relationship of the two weed ecotypes, we found very little evidence that this had occurred through shared genetic mechanisms. Of the 10 weed-specific RSA traits with significant QTLs in both mapping populations (Table 1), only a single trait (*Convex Hull Volume (3D)*) mapped to overlapping genomic positions in both sets of RILs (Fig. 3a,b). Below we discuss these results in the context of RSA variation, repeated phenotypic evolution in plants, and potential implications for combatting weedy rice in crop fields.

Repeated phenotypic evolution

Our analyses revealed clear evidence of weed-specific RSA traits. Compared with the crop genotype, the two major United States weedy rice ecotypes were characterised by root systems that were deeper, thinner, straighter, and less spread out, with fewer individual roots that were thicker and steeper relative to the soil line (Fig. 1). These patterns suggested independent evolution of shared RSA traits in these weedy rice lineages. As both weed ecotypes were being compared to the same crop variety, DGWG, one potential contributor to these patterns could be the occurrence of root traits that are unique to the crop accession. If this were the case, the traits that we interpreted as independently evolved in the weeds would in fact be DGWG-specific traits. Our QTL mapping results did not support this possibility, however, as we did not find shared QTL in the two mapping populations. Therefore, the determining genetic factors cannot be attributed to the shared crop parent. Nonetheless, our understanding of RSA trait evolution in weedy rice would clearly benefit from follow-up studies with expanded sampling of multiple weed and crop genotypes at multiple life stages to assess the generalisability of our results.

In this study we used a clear Gelzan-based growth medium combined with a shadow imaging technique (Fig. S1). With this imaging technique, any amount of microbial growth in the medium would cast a shadow on the camera and alter our measurements. Therefore, all of our RSA traits are based on growth in sterile media. There is no doubt that microbial communities are important for root growth (Rolli *et al.*, 2015; Saleem *et al.*, 2016). Indeed, anecdotally, we observed that plants heavily contaminated by microbial growth (and therefore not imaged for this study) had visibly different growth patterns. It is an unfortunate constraint of this root imaging technique that microbial growth cannot be considered. Follow-up studies using the 2D mature root-crown imaging software DIRT (Bucksch *et al.*, 2014), or advanced imaging techniques using X-ray computed tomography (X-ray CT) could potentially provide additional insights into the impact of microbial communities on RSA traits.

Above-ground traits have been extensively described for the domestication syndrome in crop species (Morrell *et al.*, 2011; Vigueira *et al.*, 2013b; Li & Olsen, 2016), as well as for the agricultural weed syndrome in their weedy relatives (Zhu *et al.*, 2012; Subudhi *et al.*, 2014; Qi *et al.*, 2015). By contrast, very little information is known about what constitutes domestication and weediness traits for RSA. It is therefore difficult to assess whether the repeated phenotypic evolution we observed for RSA traits in weedy rice is typical of other agricultural species. When considered in comparison with above-ground phenotypes in weedy rice, the independent evolution of RSA traits is consistent with the extensive phenotypic convergence observed in previous studies (Zhu *et al.*, 2012; Qi *et al.*, 2015). At the genetic level, the repeated detection of different underlying QTL in comparative studies of weedy rice ecotypes suggested multiple instances of independent evolution, including for emergence date, shattering, and pericarp colour (Qi *et al.*, 2015). In this respect, our RSA study parallels previous findings for above-ground traits in weedy rice.

Given the current lack of information on plant RSA traits, it is also difficult to assess the biological significance of the RSA differences we observed in cultivated and weedy rice. Here, we found that DGWG roots were more abundant and more exploratory (although not more massive) than the weedy counterparts (Fig. 1). At face value this finding seems counterintuitive for a crop phenotype, as a more compact root system could reduce neighbour-to-neighbour competition for soil nutrients. Indeed, for above-ground traits, much of the progress that plant breeders have achieved in increasing cereal crop yields has been through breeding for traits that minimise or reduce plant-to-plant interactions (thereby reducing competition for light and growing space) (Duvick, 2005). One possible explanation for this unexpected pattern is that the growth of cultivated rice could be enhanced by root-to-root interactions. Consistent with this hypothesis, a study that examined the root growth of cultivated rice when growing the same genotype or a different genotype found that homotypic pairings led to greater intermingling of roots than heterotypic pairings (Fang *et al.*, 2013). That finding has been further supported by a more recent study that suggested that below-ground kin recognition in cultivated rice plays an important role in root behaviour and therefore could explain the exploratory nature of the crop roots (Yang *et al.*, 2018). In maize, modern varieties have been found to have shallower root angles than their historical progenitors (York *et al.*, 2015); this was also found to be consistent with selection for increased root interactions in this crop. As our study was performed using individual plants grown alone in sterile gel media, field experiments should be undertaken to address the extent to which kin recognition may occur in crop fields and the role of the soil microbiome in mediating below-ground interactions. Expanded sampling of genotypes and plant growth stages could also be particularly insightful in this context.

Lack of parallelism

We found very little evidence for parallelism in this study, with only a single trait (*Convex Hull Volume (3D)*) mapping to overlapping genomic locations in both the S and B populations (Li *et al.*, 2017). This finding provides an interesting contrast with observations from studies of domestication traits in cereal crop species, which sometimes suggest a one gene–one trait pattern for domestication traits (reviewed in Sang, 2009). Our results in the weedy rice system showed that this pattern did not necessarily extend to direct descendants of crop species. This inference has been further borne out by genome scans in weedy rice, in which signatures of selection suggested little parallelism for above-ground trait QTLs or for genomic regions showing signatures of selection (Qi *et al.*, 2015; Li *et al.*, 2017). It should be noted that as the level of genetic resolution in the present study is on the scale of QTL intervals, the identity of the underlying causal genes remains unknown. Therefore, it is possible that different QTL for a given RSA trait corresponded to different genes within a single developmental pathway. If this is the case, then the prevalence of parallelism in RSA trait evolution may be greater than is apparent from our QTL data alone. Identification of candidate genes and confirmation of developmental pathways would be needed to definitively address this possibility.

Only two genes that directly control RSA have been cloned and functionally verified in plants, and neither of these genes appears to play a role in the RSA variation observed in the present study. *Dro1* occurs in the middle of chromosome 9 and encodes an auxin-sensitive gravitropic response protein which controls rice root–soil angle, with plants homozygous for the upland allele developing roots with a higher angle relative to the soil (Uga *et al.*, 2011, 2013). This effect results in a deeper root system that is more drought tolerant. Similar phenotypes were linked to overexpression of *Dro1* in *Arabidopsis thaliana* and plum (*Prunus domestica*) (Guseman *et al.*, 2017). In the present study, although two width-associated QTL were mapped to chromosome 9, neither QTL overlapped with the genomic region containing *Dro1*. Root–soil angle QTLs in this study were localised to chromosomes 3, 7, and 12.

The other gene, *PSTOL1*, is an enhancer of early root growth in the middle of chromosome 12, which enables rice to increase the intake of phosphorus in early growth stages (Gamuyao *et al.*, 2012). This gene was identified in the traditional *aus* rice variety Kasalath and was found to occur as a gene presence/absence polymorphism in other rice varieties. While it is known that DGWG lacks the *PSTOL1* gene, it is highly likely that our weedy rice parents both possess the gene as every United States weed genotyped to date carries it (Vigueira *et al.*, 2016). Given that we did not find any QTL mapping to this locus, despite the probable presence/absence polymorphism in the RILs, it seems likely that *PSTOL1* is not a contributing factor for phenotypic variation in this system. This finding is consistent with a previous study of *PSTOL1* variation in cultivated and weedy rice, which detected no observable phenotypes associated with this gene (Vigueira *et al.*, 2016).

Implications for agriculture

Previous studies have linked phosphorus starvation tolerance and drought tolerance to root architecture, suggesting that breeders can select for a more optimum RSA to take advantage of soil conditions (Uga *et al.*, 2011; Gamuyao *et al.*, 2012). In this study, we identified an early life-stage root depth QTL not associated with *Dro1* (Table S2). Although no test of drought tolerance was performed in this study, further experimentation would be relatively simple. If the prediction holds that plants with deep rooting-associated QTL are more drought tolerant, the weeds studied here could be a valuable resource in marker-assisted selection. In addition, our observations of differences in root system width and exploration between cultivated and weedy rice suggest that neighbour-to-neighbour root communication may be important to growth in cultivated rice (Fang *et al.*, 2013; Yang *et al.*, 2018). This study sheds light on potential QTLs of interest for further characterising this trait and its potential agronomic value.

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Author contributions

KMO planned and designed the research. MJW performed the experiments, analysed the data, and wrote the manuscript. CNT contributed to the design of the research and provided laboratory equipment and space.

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Supporting Information

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

Fig. S1 Schematic of the custom rig used to image the root system of rice plants growing in 2 l glass cylinders. Camera and turntable are controlled with the same computer, allowing the camera to take 72 images exactly 5° apart.

Fig. S2 Correlation matrix of 98 rice root system architecture traits measured in this study.

Table S1 Success of random forest machine learning model.

Table S2 All root QTLs identified in this study.

Table S3 Average phenotypic values for all rice genotypes phenotyped in this study.

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