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Received: 1 July 2021 | Accepted: 25 January 2022

DOI: 10.1111/pce.14272

ORIGINAL ARTICLE

Plant, Cell & Environment | WILEY

Aus rice root architecture variation contributing to grain yield under drought suggests a key role of nodal root diameter class

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Funding information
Ministry of Science and Technology, Bangladesh, Bangabandhu Fellowship on Science and ICT; Generation Challenge Programme, Grant/Award Number: G3008.06; Chinese Scholarship Council, Grant/Award Number: Hengygang Guiding Plan Project 2020jh022798

Abstract

The aus rice variety group originated in stress-prone regions and is a promising source for the development of new stress-tolerant rice cultivars. In this study, an aus panel (~220 genotypes) was evaluated in field trials under well-watered and drought conditions and in the greenhouse (basket, herbicide and lysimeter studies) to investigate relationships between grain yield and root architecture, and to identify component root traits behind the composite trait of deep root growth. In the field trials, high and stable grain yield was positively related to high and stable deep root growth ($r = 0.16$), which may indicate response to within-season soil moisture fluctuations (i.e., plasticity). When dissecting component traits related to deep root growth (including angle, elongation and branching), the number of nodal roots classified as 'large-diameter' was positively related to deep root growth ($r = 0.24$), and showed the highest number of colocated genome-wide association study (GWAS) peaks with grain yield under drought. The role of large-diameter nodal roots in deep root growth may be related to their branching potential. Two candidate loci that colocated for yield and root traits were identified that showed distinct haplotype distributions between contrasting yield/stability groups and could be good candidates to contribute to rice improvement.

KEYWORDS
GWAS colocations, rice, root angle, root length, root plasticity, yield stability

1 | INTRODUCTION

Having originated in the stress-prone regions of Bangladesh and Eastern India (Glaszmann, 1987), the aus subgroup of rice has been identified as a valuable source of stress tolerance genes. Examples include the submergence tolerance QTL/gene SUB1 (Xu et al., 2006), Pup1/PSTOL1 for phosphorus-deficiency tolerance (Gamuyao et al., 2012; Wissuwa et al., 1998, 2001), and drought-yield QTLs $qDTY_{2.2}$ and $qDTY_{4.1}$, which were identified from aus genotypes FR13A, Kasalath, and Aday Sel, respectively. Nagina-22 (N22), a

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heat-tolerant variety (Satake & Yoshida, 1978) and the source of major-effect drought-yield QTL *qDTY*$_{1.1}$ (Vikram et al., 2011), also belongs to the aus subgroup. However, despite their promising utility in improvement of rice stress tolerance breeding, relatively few studies (drought/leaf traits: Cal et al., 2019; salinity: Chen et al., 2020) have conducted large-scale phenotypic and genetic surveys of aus accessions for stress tolerance.

Drought stress is increasingly affecting crop productivity in both developed and developing countries (Lesk et al., 2016), pointing to the urgency of exploring valuable resources such as the aus variety group for improving rice productivity. Drought stress response and productivity in rice have been attributed to deep root growth both in upland and lowland conditions (Gowda et al., 2011), and to root architectures that respond to soil moisture levels (plasticity; Suralta et al., 2018). Deep root growth in lowland rice has previously been defined as below 15 or 30 cm, based on reports that up to 94% total rice root length can be found from 0 to 10 cm soil depths and that few roots are typically found below 30 cm (as reviewed by Gowda et al., 2011). In addition to environmental factors, genetic variation also affects deep root growth in response to drought: in terms of constitutively-expressed root traits, aus accessions showed some of the greatest values for vertical root distribution (Lafitte et al., 2001) as well as higher water uptake rates and root length densities at depth under drought stress in lysimeters compared to other rice subgroups (Gowda et al., 2012). Moreover, compared with genotypes in other subgroups, aus genotypes showed the least yield reduction across multiple seasons under drought (Henry et al., 2011). Therefore, given the promising physiological and genetic evidence for the aus subgroup based on the performance of individual genotypes, characterization of larger panels representing the aus subgroup could help pinpoint individual traits and genes to target for the development of new drought-tolerant rice cultivars.

Following the historic collection, curation and availability of traditional rice varieties to the public through Genebanks such as the International Rice Genebank Collection at the International Rice Research Institute (IRRI) (Jackson, 1997), efforts for large-scale phenotypic and genetic analysis of rice diversity panels began to surge upon the availability of sequence data. This includes the resequencing of 3000 rice genotypes (The 3000 Rice Genomes Project, 2014; D. R. Wang et al., 2018) and their alignment to the Nipponbare sequence that has been developed into a user-friendly database for genetic analysis of the 3000 sequenced genomes (https://snp-seek.irri.org/; Alexandrov et al., 2015; Mansueto et al., 2016, 2017). Along with the sequence availability, high-throughput phenotyping protocols were developed to generate phenotypic data on large rice diversity panels, including root measurements and field studies (e.g., Shashidhar et al., 2012). By targeting conditions such as those relevant to stress-prone regions where rice is produced, and by dissecting component traits related to deep root growth, characterizing stress-tolerance traits on rice diversity panels aimed to facilitate identification of potential donor genotypes for breeding, key stress tolerance traits, and related genetic regions.

In this study, the relationships between grain yield and root dry weight (RDW) in a panel of over 200 aus genotypes were investigated across field studies with different soil moisture treatments, and root component traits (length, angle and branching) of the aus panel were characterized in complementary greenhouse studies. Our aims were to identify composite root traits related to rice yield under drought and to identify the key component root traits behind the yield-related composite root trait of deep root growth based on phenotypic correlations and co-locations from genome-wide association study (GWAS) analyses. By exploring a large panel of aus genotypes across multiple seasons and growth conditions, we aimed to identify stable relationships between rice root architecture and yield, to refine phenotypic and genetic selection targets for rice drought breeding.

2 | MATERIALS AND METHODS

2.1 | Plant material and experiments conducted

An aus panel consisting of over 210 genotypes was selected from the International Rice Genebank Collection maintained by IRRI's T.T. Chang Genetic Resource Center based on the information from the Generation Challenge Program (GCP) Composite Collection genotyping (Generation Challenge Program, 2005), as well as additional entries known to be in the aus variety group based on molecular characterization of the high-density rice array (HDRA) panel (McCouch et al., 2016). Four field experiments and three greenhouse experiments (a lysimeter study, a basket study and a herbicide study; Table 1) were conducted using the aus panel (Table S1), followed by a fifth field experiment on selected aus genotypes. Leaf and water uptake results from the 2010 to 2012 field experiments and the lysimeter study were reported by Cal et al. (2019).

2.2 | Field experiments

Similar sets of 212, 239, 238 and 225 aus genotypes were grown in the dry seasons of 2010, 2011, 2012 and 2018, respectively, for the field experiments (Table S1). Based on the results of the first four field experiments, a set of 16 selected aus genotypes was grown in the wet season for the 2019 field experiment. The soil in the 2010–2012 and 2019 experimental fields is an Isohyperthermic Typic Hapludalf and the soil in the 2018 experimental field is an Aquandic Epiaquall (see Table S2 for soil nutrient and bulk density levels). The experimental designs consisted of alpha lattice with three replicates in each treatment for all field experiments except in 2019, which was in a randomized complete block design (RCBD). Well-watered and drought stress treatments were included in all field experiments. In 2018, to increase the range of environments under which genetic variation in grain yield was assessed, four treatments with different water, nitrogen and phosphorus application levels were grown: a well-watered treatment with nitrogen but without phosphorus added

TABLE 1 Summary of all experiments conducted in this study

Envt	Expt. name (field year, season and treatment)	Expt. design	Genotypes	Reps	Average temp. (°C)	Relative humidity (%)
Field	2010DS_WW	AL	212	3	26.8	87.1
	2010DS_RS	AL	212	3		
	2011DS_WW	AL	239	3	25.9	87.9
	2011DS_RS	AL	239	3		
	2012DS_WW	AL	238	3	24.5	85.2
	2012DS_RS early	AL	67	3		
	2012DS_RS med	AL	141	3		
	2012DS_RS late	AL	30	3		
	2018DS_WW	AL	225	3	27.5	82.6
	2018DS_RS	AL	225	3		
	2018DS_WW + N0P	AL	225	3		
	2018DS_WW0N + P	AL	225	3		
	2019WS_WW	RCBD	16	3	27.7	84.0
	2019WS_RS	RCBD	16	3		
Greenhouse	Basket study	RCBD	225	4	32.2	59.4
	Lysimeter study_WW	RCBD	209	4	29.3	69.2
	Lysimeter study_RS	RCBD	209	4		
	Herbicide study	RCBD	154	4	≥25.0	

Abbreviations: AL, alpha lattice; DS, dry season; N, nitrogen; P, phosphorus; RCBD, randomized complete block design; RS, reproductive stage drought stress; WS, wet season; WW, well-watered.

(+N), a well-watered treatment with neither phosphorus or nitrogen added (0N, 0P), and well-watered and drought stress treatments with nitrogen and phosphorus added (+N, +P); Table 1).

All field trials were conducted under lowland conditions with transplanting into puddled soil as described by Henry et al. (2011) and Cal et al. (2019) in an open field, except the 2019 drought stress treatment that was conducted in an automated rainout shelter. Each field experiment was established in a seedbed nursery and transplanted to the experimental field around 17 days after sowing (DAS) into three-row plots with 3-m row length, and spacing of 25 cm between rows and 20 cm within rows. Basal fertilizer was applied at a rate of 40–40–40 kg N–P–K ha^{-1} complete fertilizer before transplanting, and ammonium sulphate was applied as topdressing at 40 kg ha^{-1} 3–4 weeks after transplanting, except in the 2018 field experiment in which the +N well-watered experiment received 100 kg ha^{-1} basal and 50 kg ha^{-1} topdressing of ammonium sulphate, the well-watered 0N, 0P experiment received no fertilizer, and the well-watered and drought +N, +P experiments received 100 kg ha^{-1} basal and 50 kg ha^{-1} topdressing of ammonium sulphate plus 50 kg ha^{-1} ordinary superphosphate. The drought stress treatments were initiated at 54, 49, 59 and 44 DAS in 2010, 2011, 2018 and 2019, respectively, by stopping irrigation and opening drains at the edge of the field. In 2012, the genotypes were separated into three groups to target reproductive stage drought across genotypes with different phenologies. The drought stress in 2012 was initiated at 39 DAS in the early-flowering group (68 genotypes), 47 DAS in the medium-duration group (144 genotypes) and 54 DAS in the late flowering group (34 genotypes).

The drought treatments were rewatered 3–4 days before root sampling at 81 DAS in 2011, and 79, 87 and 99 DAS in the 2012 early, medium and late-flowering groups, respectively, and at 78 DAS in 2018 to soften the soil that had become hard and cracked during the drydown. In 2019, roots were sampled at three time points: (1) at 44 DAS (just before initiating the drought stress), (2) at 62 DAS (during drought stress and just before rewatering) and (3) at 70 DAS (7 days after rewatering). The drought treatments in all experiments were also rewatered one to two times during the reproductive stage to ensure a harvestable grain yield. The soil moisture in the drought stress treatments was affected by rainfall (except in 2019 due to the rainout shelter), as reflected by tensiometer readings (Soilmoisture Equipment Corp.) at a depth of 30 cm in each replicate (Table S3).

Root sampling was conducted by soil coring using 4-cm diameter steel tubes (fabricated at IRRI according to Henry et al., 2012b, in 2011–2012, and fabricated by Giddings Inc., in 2018–2019) to a depth of 30 cm, except in 2019 when soil cores were sampled to a depth of 60 cm. Roots were sampled in the drought treatments of all field trials except 2010 and in the well-watered treatments of 2012 and 2018 only. One soil core per plot was sampled in 2011, 2012 and 2018, and three subreplicate soil cores per plot were sampled in 2019. Soil cores were systematically sampled between the second and third hills in a row. After sampling, the soil cores were separated into 15-cm depth segments from which the roots were carefully washed according to Böhm (1979) and described in Henry et al. (2012b). In 2011, 2012 and 2018, RDW at 0–15 cm and 15–30 cm depths were recorded, and the deep root percentage was calculated by RDW at 15–30 cm/(RDW at 0–15 cm + RDW at 15–30 cm) × 100. In 2019, roots were scanned (Calibrated Color Optical Scanner STD4800, Epson) and analysed to determine root length density (RLD) (cm root cm^{-3} soil) using WinRHIZO Prov. 2013e, Regent Instruments (Regent Instruments). For the purpose of this study, roots at the 0–15 cm depth segment are considered 'shallow roots', and roots at the 15–30 cm depth segment and below are considered 'deep roots'. Root classes (Yamauchi et al., 1987) were assigned as <0.05 for S-type lateral roots, 0.05–0.2 mm diameter for L-type lateral roots and >0.2 mm diameter for nodal roots.

In 2019, shoot dry weight (one hill per plot) was determined at each root sampling date. The grain yield and straw biomass were determined in all field experiments at maturity by harvesting an area of 1.5 m^2 per plot and normalizing the grain weight to a 14% moisture content.

2.3 | Lysimeter study

The lysimeter study was carried out in an IRRI greenhouse as described by Cal et al. (2019) and Kijoji et al. (2012) in the wet season of 2011. Briefly, each lysimeter was composed of a polyvinyl chloride (PVC) cylinder with a diameter of 18 cm and a height of 95 cm into which a plastic liner was inserted and filled to 80 cm with upland soil and an additional 15 cm of paddy soil as the topsoil. The experimental design was RCBD with three replicates in the drought stress treatment and two replicates in the well-watered treatment. The seeds of 209 aus genotypes were germinated in Petri dishes, and three plants were transferred to each lysimeter, which were then thinned to one plant per lysimeter. Roots were harvested at 88 days after germination of the well-watered treatment and 89–91 days after germination (by replicate) in the drought treatment by pulling out the plastic liner and separating the soil into 20-cm depth segments, except for the deepest sample which was 60 cm and below. The root length, RDW, percent lateral roots (% laterals) and percent nodal roots (% nodal roots) were then determined using WinRhizo as described above on the roots sampled from a depth of 60 cm and below. Based on their proportion of the total root length in each soil segment sampled, lateral roots were expressed as follows:

$$\%\ \text{lateral roots} = \text{root length of lateral roots} / \text{total root length} \times 100,$$

$$\%\ \text{L-type lateral roots} = \text{root length of L-type lateral roots} / \text{total root length} \times 100.$$

2.4 | Basket study

The basket study was carried out in a greenhouse at IRRI according to Uga (2012) in the dry season of 2013. One plant was grown in each open stainless steel mesh basket, which had a top diameter of 7.5 cm, depth of 5.0 cm and mesh size of 2 mm, and a ring indicator that divided the basket into the upper and lower halves, representing an angle of 45°. The basket was filled with sieved and dried soil from the IRRI upland farm, placed in a PVC pipe with 7.5-cm diameter and 15-cm height, and then placed in a plastic tray. The seeds of 225 aus genotypes were germinated in Petri dishes and then transferred to the baskets in an RCBD experimental design with three replicates per genotype. Each replicate was grown on separate dates; Rep 1 from March to April, Rep 2 from April to May and Rep 3 from May to June. The setup was watered with tap water for the first week, half-strength Yoshida nutrient solution (Yoshida et al., 1976) for the second week, and full-strength Yoshida solution for the third week. At 25–27 days after planting, the plants were harvested to determine maximum root length, the number of large-diameter and small-diameter nodal roots emerging above the ring indicator (shallow roots) and the number of large-diameter class and small-diameter class nodal roots (based on a visual assessment) emerging below the ring indicator (deep roots). The percentage of steep-angled roots was calculated as:

$$\%\ \text{steep-angled roots} = \text{total number deep nodal roots} / \text{total number of nodal roots} \times 100.$$

2.5 | Herbicide study

A total of 154 aus genotypes were screened in the herbicide study at the Cruickshank greenhouse of the University of Aberdeen, Scotland, in 2013 according to the method described by Al-Shugeairy et al. (2014). Four boxes with 200 cm length, 81.5 cm width and 35 cm depth were positioned on a bench in an RCBD. The boxes were filled with topsoil, and filter paper impregnated with Diuron was placed at a depth of 25 cm. Full strength Yoshida's nutrient solution was applied to saturate the soil of the box. Theta probes were placed at the depth of 15 and 25 cm (at the herbicide layer) to measure the soil moisture throughout the experiment. Seeds were sown directly to the box at a depth of 1.5 cm using 5 × 5 cm spacing. Water was supplied to the seeds until germination, after which watering was stopped. The herbicide injury of the plants was recorded every other

day from the time symptoms first appeared at Day 20 after sowing until Day 47 after sowing, on a scale of 6 according to the leaf chlorosis and death: A score of 0 indicated no symptoms, 1 indicated noticeable leaf yellowing (5%–15% of leaf area affected), 2 indicated substantial leaf yellowing (15%–50% leaf area affected), 3 indicated substantial leaf yellowing (>50% leaf area affected) and noticeable leaf death (5%–15% of leaf area), 4 indicated substantial leaf death (15%–50% leaf area dead) and 5 indicated virtual to complete plant death (>50% leaf area). The herbicide injury score on Day 36 was used for this analysis, which is when the soil moisture had declined to about 5% at the 15 cm depth and 8% at the 25 cm depth (at the herbicide layer).

2.6 | Statistical analysis

Data analyses were conducted in R version 3.1.3 (R Core Team, 2020). The three maturity groups grown in 2012 were pooled to calculate lsmeans and for subsequent analyses. The function used for analysis include *lmer* (lme4 package v. 1.1.27.1; Bates et al., 2015) for lsmeans, *rcorr* (Hmisc package v. 4.5.0; https://hbiostat.org/R/Hmisc/, https://github.com/harrelfe/Hmisc/) for correlation, *AMMI* (agricolae package v. 1.3.5; De Mendiburu, 2009) for the additive main effects and multiplicative interactions (AMMI) models analysis, and *lavaan* v. 0.6.9 (Rosseel, 2012) for path analysis.

AMMI analysis and correlations were conducted on all treatments and trials together and also on all drought treatments together, but not on the well-watered treatments separately since root samples were taken in only two seasons and the analysis requires three seasons of data. The AMMI model used was:

$$P_{ij} = \mu + \tau_i + \delta_j + \sum_{k=1}^{t} \lambda_k \alpha_{ij} \gamma_{ij} \varepsilon_{ij}.$$

P_{ij} is grain yield or RDW, μ is the grand mean; τ_i is the genotypic effect; δ_j is the environmental effect; the constant λ_k is the singular value of the kth bilinear (multiplicative) component, which is ordered $\lambda_1 \geq \lambda_2 \geq \ldots \geq \lambda_t$; α_{ik} are elements of the kth left singular vector of the true interaction and represent genotypic sensitivity to hypothetical environmental factors represented by the kth right singular vector with elements γ_{jk}; ε_{ij} is the average of the corresponding random error, and t is $i \times j$ (the total number of genotypes × the number of environments). The terms α_{ik} and γ_{jk} satisfy the constraints:

$$\sum_{i=1}^{g} \alpha_{ik} \alpha_{ik'} = \sum_{j=1}^{s} \gamma_{jk} \gamma_{jk'} = 0 \quad \text{for} \quad k \neq k' \quad \text{and} \quad \sum_i \alpha_{ik}^2 = \sum_j \gamma_{jk}^2 = 1,$$

where g is the number of genotypes and s is the number of environments.

The stability index (SI) and AMMI stability value (ASV) were determined by index.AMMI (model). The ASV was calculated as described by Purchase et al. (2000):

$$\text{ASV} = \sqrt{\left[\frac{\text{IPCA1}_{SQ}}{\text{IPCA2}_{SQ}}(\text{IPCA1}_{score})\right]^2 + (\text{IPCA2}_{score})^2},$$

where IPCA1_{SQ} and IPCA2_{SQ} are the sum of squares of the two principal component analysis scores (IPCA1_{score} and IPCA2_{score}). SI was calculated as the sum of rASV and rmeans, where rASV is the rank of ASV and rmeans is the rank of mean rice yield across all environments.

Mean values for each genotype were scaled (value/maximum genotypic value for the trait) before running the path analysis using $\text{RDW}_{15-30\,cm}$ from 2011, 2012 and 2018 field trials, root length >60 cm from the lysimeter study, % large diameter roots and root length from the basket study and the herbicide score on Day 36 from the herbicide study.

2.7 | Association study

Genome-wide association studies were performed to identify single-nucleotide polymorphism (SNP) markers associated with grain yield, RDW at depths of 0–15 cm and 15–30 cm and deep root percentage in the field study in 2011, 2012 and 2018. In addition, root traits recorded in the lysimeter study, basket study and herbicide study were also subjected to GWAS to identify the associated markers. The genotype data was extracted from the Rice Reference Panel (RICE-RP; W. Wang et al., 2018) that includes an imputed version of the HDRA data set (McCouch, et al., 2016), from which 157 of the aus genotypes grown in the field studies, 140 genotypes in the lysimeter and basket study and 97 genotypes in the herbicide study were available. The SNP data was filtered by PLINK 1.90 (Chang et al., 2015) based on the call rate (>80%) and minor allele frequency (>0.05), and the linkage disequilibrium (LD) pruned data set was generated by the 'indep-pairwise' command with parameters: window size = 2 kb, $r^2 = 0.85$. The ped and map files generated by PLINK were then converted to the hapmap files via Tassel 5.0 (Bradbury et al., 2007). Finally, the GWAS was performed using the Genome Association and Prediction Integrated Tool (GAPIT) package (Lipka et al., 2012) using the efficient mixed-model association expedited (EMMAX) model (Kang et al., 2010) in R version 3.1.3.

To determine GWAS peak colocations, we divided the reference genome into windows of size =1 kb and assigned the lowest p value among the SNPs in the window as the window p value. The colocation analysis was done using narrow (1 kb) windows to minimize the chance of finding false positives. For a given pair of traits, the windows with p values for both traits below the threshold 1e-3 were counted as colocations. The R packages used in colocation analysis include readr v. 2.0.1 (https://github.com/tidyverse/readr), ggplot2 v. 3.3.5 (Wickham, 2016), dplyr v. 1.0.7 (https://github.com/tidyverse/dplyr) and reshape2 v. 1.4.4 (Wickham, 2007).

To estimate the expected number of colocations and the empirical p values for the observed numbers of colocations, we estimated the probabilities of having a peak in each trait as the observed proportion Prob(peak) = *N*_peaks/*N*_windows, and the probability of

colocation as the product of probabilities of peaks for each of the two traits. The number of colocations between GY and a root trait T was then modelled as a binomial random variable with parameters *prob* = (*N*_peaks(GY)/*N*_windows) × (N_peaks(T)/*N*_windows) and *size* = N_windows = 276973 (the number of 1-kb windows with SNPs in our data set). The 95% quantile for the number of colocations and the *p* value for the observed number of colocations were computed using R functions *qbinom* and *pbinom* from package stats v. 4.1.1 (R Core Team, 2020).

Of the 438 loci that colocated between grain yield and root traits, we selected 50 loci that showed colocations four or more times (i.e., in multiple seasons or for multiple root traits). A haplotype group for each sequenced accession in the 3k RGP or in the Top 25 (15 accessions) and Bottom 25 (18 accessions) for $GY_{drought}$ from the AMMI analysis (Tables S5 and S6) was generated using the SNP-Seek database 'Genotype' function. The 'Haploview' function was set to 15 variety groups. Variety order and image tabs were downloaded. The 'variety order' file from the SNP-seek table was then loaded in R to get the kgroup (haplotype group) of each locus. Five loci showed significantly different distributions among haplotype groups between the Top 25 and Bottom 25 accessions for $GY_{drought}$ using a χ^2 test (chisq.test) in R v. 4.0.3 (R Core Team, 2020): Chr2_35194, Chr4_29223, Chr6_11688, Chr7_14998 and Chr11_19463 (the position indicated after underscore denotes the start of the locus in kb) of which two (Chr2_35194 and Chr6_11688) were most significant ($p < 0.01$).

Haplotype groupings were then visualized by the 'Haplotype view' tool in SNP-Seek. A smaller list of varieties was prepared using the accessions from the Top 25 and Bottom 25 accessions for $GY_{drought}$ for which sequence data were available. Using the GOBii Haplotype Visualization Tool (https://haplotool-docs.readthedocs.io), parameters such as the genotype file in PLINK binary format and the phenotype file (Top 25 and Bottom 25 for $GY_{drought}$) were inputted, the monomorphic sites were removed and a new haplotype plot was generated.

3 | RESULTS

To investigate genetic variation in the relationships between rice grain yield and root architecture under drought stress, a panel of about 220 aus genotypes (Table S1) was evaluated in four field seasons under well-watered and drought conditions and in three greenhouse studies (a basket study, a herbicide study and a lysimeter study).

3.1 | Stable or plastic deep root growth was related to yield stability, depending on the group of genotypes considered

In each individual field season, in which drought reduced grain yield and RDW at the 0–15 cm soil depth (Figure S1A,B), no consistent direct correlations were observed between grain yield and RDW at 0–15 or 15–30 cm (Table S4). However, across trials (all well-watered and all drought treatments, 212-239 genotypes per season) from which an AMMI analysis was conducted (Figure 1a), correlation analyses (Figure 1b) revealed a significant relationship between the yield SI (SI_{yield}; lower values indicating high and stable yield) with high and stable RDW at the 15–30 cm depth ($SI_{RDW15-30\,cm}$; $R = 0.16$). This correlation between grain yield stability and deep root stability was also observed when drought trials were considered separately for the AMMI analyses (Figure S2; $R = 0.15$).

Genotypes with contrasting yield stability indices (SI_{yield}) were selected for further comparison of deep root growth between well-watered and drought conditions. Across all three AMMI analyses on grain yield (well-watered [Figure S3A], drought [Figure S3B] and combined well-watered and drought treatments [Figure 1a]), the SI_{yield} rankings consistently included four genotypes (Sreerampur Shaita, Chandarhat, DJ 24 and Aus 257) in the Top 25 (Table S5, H genotypes) and seven genotypes (AusJota, Basmati 1, Baran Boro, Kharsu 80, ARC 7229, Gerdeh and Molladigha) in the bottom 25 (Table S6, L genotypes). There was no difference in deep root percentage between H and L genotypes under well-watered treatments both in 2012 and 2018 (Figure 1c), but the average deep root percentage of H genotypes was numerically but not significantly ($p = 0.098$) higher than that of L genotypes under drought stress in 2011, 2012 and 2018 (Figure 1d). This difference in deep root growth between yield stability groups, which was only observed in the drought treatments and not in well-watered treatments, suggests a plastic response.

3.2 | The proportion of nodal roots classified as 'large diameter' was a key trait linking early-stage greenhouse studies with field studies

To further characterize variation in root architecture of the aus panel and to dissect the root component traits behind deep root growth, a series of greenhouse experiments was conducted at a range of developmental stages: a seedling stage basket study, an early vegetative stage herbicide-at-depth study, and a late vegetative stage lysimeter study (Figure S4). Path analysis indicated the following interconnectedness between root architecture in the field and the traits measured in greenhouse experiments: $RDW_{15-30\,cm}$ values in the 2011 and 2012 drought field trials were significantly positively related to root length below 60 cm in the lysimeter study, which was positively correlated to the percentage of nodal roots classified as large diameter in the basket study. In turn, the percentage of nodal roots classified as large diameter in the basket study was highly correlated with nodal root length in the basket study, which was positively related to the herbicide response score at day 36 in the herbicide study (Figure 2). Thus, the proportion of large-diameter nodal roots was a trait that appeared to link the detailed seedling and vegetative stage root characterization experiments in greenhouse conditions with those conducted at later stages and in field conditions.

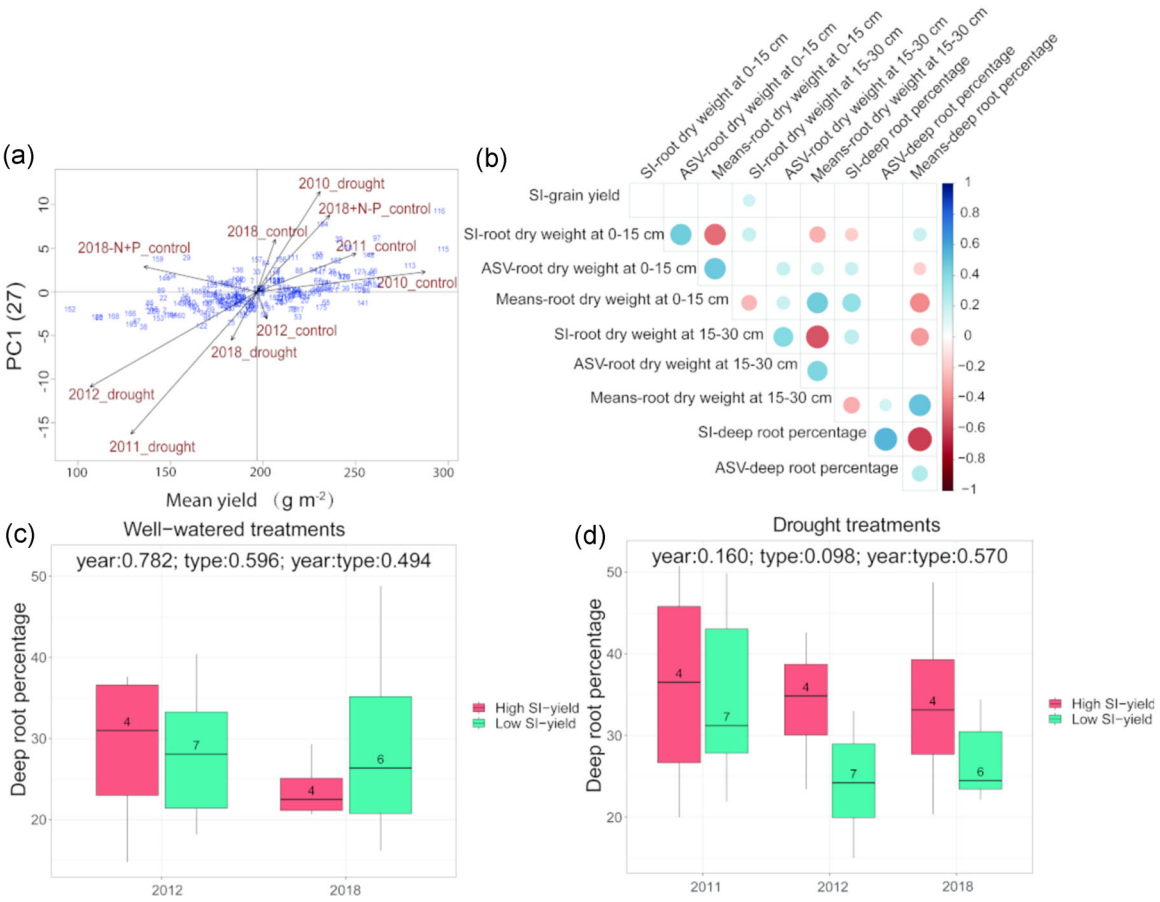

FIGURE 1 Grain yield and stability was related to deep root growth under drought across field trials. (a) Biplot of the AMMI analysis of grain yields in all field experiments under well-watered and drought treatments. (b) Corrplot of grain yield and root traits across both well-watered and drought conditions across all years measured from the AMMI analysis (grain yield: six well-watered experiments, four drought experiments; root traits: two well-watered experiments and three drought experiments). Deep root percentage was calculated by root dry weight in depth 15–30 cm/(root dry weight in depth 0–15 cm + root dry weight in depth 15–30 cm) × 100. Values shown represent the correlations across all years and environments. AMMI, additive main effects and multiplicative interaction; ASV, AMMI stability value; SI, stability index (the sum of genotype ranks for ASV and means). The dots inside the plot indicate significant correlations (blue, positive correlation, red, negative correlation; larger dots indicate stronger correlations) and a blank indicates that the traits were not significantly correlated. (c) Boxplot of the deep root percentage of the selected genotypes with contrasted grain yield stability in under well-watered (root samples not taken in 2011) and (d) drought conditions in 2011, 2012 and 2018. H: genotypes with high and stable grain yield (see Table S2), L: genotypes with low and variable grain yield (see Table S3). In (c,d) the *p* values from analysis of variance (ANOVA) analysis are presented at the top, and the number of genotypes (*n*) are shown on each bar

3.3 | The proportion of nodal roots classified as 'large diameter' showed the highest number of colocated GWAS peaks with grain yield under drought

In addition to phenotypic correlation, we took another approach to examining relationships between root traits and grain yield by GWAS (Table S7). For association analysis, a total of 905 912 SNPs remained after SNP filtering. Based on a cutoff calculated as $P = -Log_{10}(1/\text{total SNPs})$ (which was previously used in rice drought-yield and other studies; Ma et al., 2016), no significant SNP was detected for grain yield under drought in 2010 and 2012, two significant SNPs on chr10 were observed in 2011 and six significant SNPs were observed in 2018 (Table 2). Among the significant peaks for the three root traits measured in the field drought experiments, none were consistent among years (Table 2). For the lysimeter study and basket study, a total of 903 687 SNPs remained after SNP filtering and a cutoff of −LogP = 5.956 (calculated as $P = -Log_{10}(1/903\,687)$) was used to identify significant GWAS peaks. Of the five root traits from the lysimeter study used in GWAS, only one significant SNP was identified on chr11 for % lateral roots (Table 2). Fifteen of the basket study root traits were used in GWAS; of these, six significant SNPs were detected on chr5 and chr8 for RDW, four significant SNPs were identified for the number of small-diameter shallow nodal roots, and significant SNPs were also identified for the number of large-diameter deep nodal roots, the number of large-diameter shallow nodal roots, the ratio of large to small diameter nodal roots and the total number of shallow nodal roots (Table 2). No significant SNPs were detected from the herbicide study.

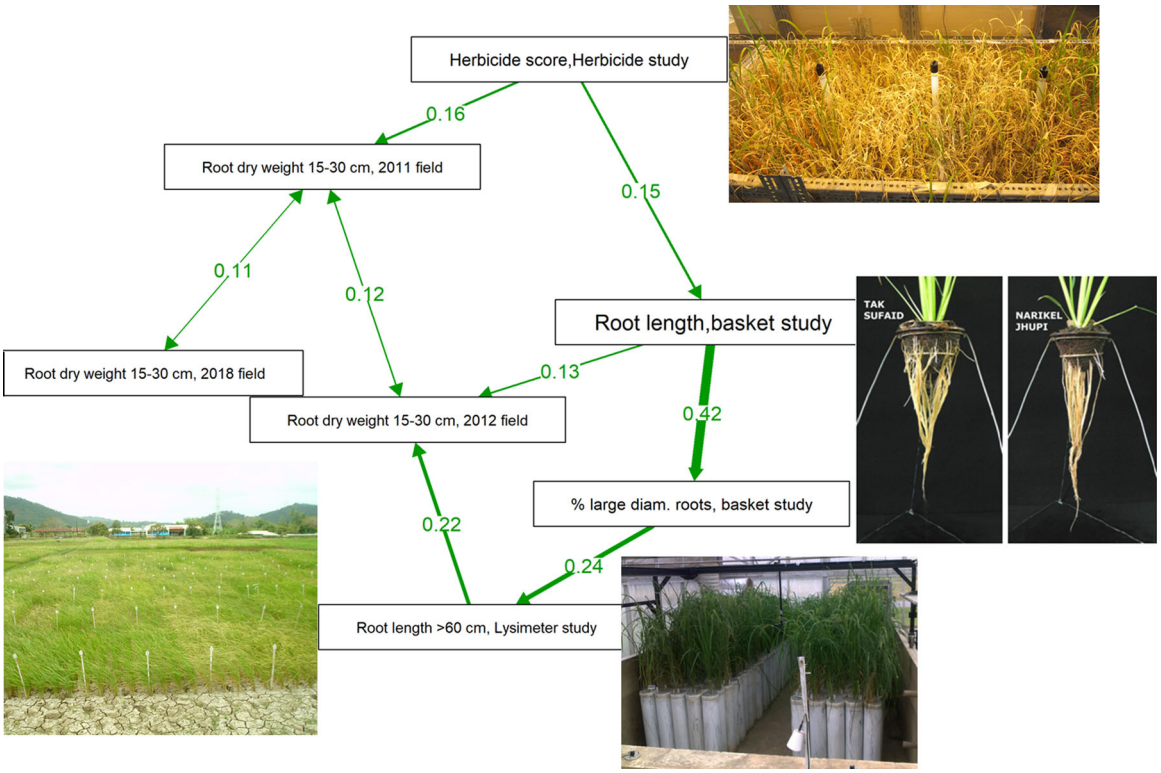

FIGURE 2 Path analysis relating traits measured in the aus panel in field experiments under drought, as well as those measured in the greenhouse lysimeter study, basket study and herbicide study. Values shown are correlation coefficients and the wide arrows represent stronger correlations [Color figure can be viewed at wileyonlinelibrary.com]

Very few colocations between root traits and grain yield in the field trials, or root traits in the greenhouse experiments and grain yield in the field trials, were identified with the same threshold used for genome-wide significance cutoffs (Table 2). Therefore, to investigate which root traits showed the highest degree of genetic correspondence with grain yield under drought, we examined SNP co-locations within a 1-kb window between grain yield under drought and root traits from all experiments using a reduced threshold of −LogP = 3 (to increase the power while still controlling the number of false positives). The root trait with the highest number of colocations with grain yield under drought (in 2011, 2012 and 2018) was the number of large-diameter nodal roots with shallow angles in the basket study (Table 3) and the next highest number of colocations was with the proportion of L-type lateral roots in deep soil from the lysimeter study (Table 3). These colocations point to roles for both nodal root diameter class and deep lateral root production in maintaining grain yield under drought.

3.4 | Aus genotypes with consistently highest yields and deep RDWs showed the greatest degrees of responses to rewatering after drought

We selected genotypes for further study based on their different combinations of yield and deep root growth across trials. Based on the grain yield under drought and $RDW_{15-30\,cm}$ under drought in 2011, 2012 and 2018 field trials, we divided the 239 aus genotypes into four groups: genotypes with high grain yield and high $RDW_{15-30\,cm}$ (HH), genotypes with high grain yield but low $RDW_{15-30\,cm}$ (HL), genotypes with low grain yield but high $RDW_{15-30\,cm}$ (LH) and genotypes with low grain yield and low $RDW_{15-30\,cm}$ (LL) (Figure S5A–C). Among the four groups, we found that there were 16 genotypes consistently located in the HH (red), HL (green), LH (blue) and LL (purple) group in the three years (Figure S5A–C) as well as based on SI_{yield} and means of $RDW_{15-30\,cm}$ across the three years (Figure 3a). The selected HH genotypes were Aus 257, Chikon Shoni, Moshur and Dhala Bhadoi; HL genotypes were DM 43, Chengri 2, Ikra and ARC 10955; LH genotypes were Fulkati, ARC 10352, T 26 and Jamri; and LL genotypes were Aus 41, Jhum Begunbichi, Sada Solay and Miriti.

To further examine the question about deep root growth being related to grain yield and stability in a stable or plastic manner, the 16 consistently differentiated genotypes were grown in a field experiment in 2019 in which root samples were measured before and after a 20-day drought stress treatment, as well as 7 days after rewatering following drought. In terms of the trends in RLD over time, no significant difference was observed among the four categories in the well-watered treatment (Figure S6). However, in the drought treatment, the HH genotypes responded most quickly to rewatering: their RLD at both the 15–30 cm depth (Figure 3b) and the 30–45 cm depth

TABLE 2 Significant SNPs for root traits measured in the field trials (root dry weight at 0–15 cm [$RDW_{0-15\,cm}$], root dry weight at 15–30 cm [$RDW_{15-30\,cm}$] and deep root percentage) taken from 188.5 cm^3 soil cores; and greenhouse experiments (lysimeter study, basket study and herbicide study) by GWAS, based on a cutoff of −Log10(1/total SNPs)

Exp.	Traits	Chr.	Position	logP
Field trial-2011	$GY_{drought}$	10	1494242	6.02
		10	1472412	6.00
	$RDW_{0-15\,cm}$	5	9972175	6.12
	$RDW_{15-30\,cm}$	5	28476801	5.97
	Deep root percentage	5	19010376	6.05
		5	19021823	6.00
		5	18952876	5.99
Field trial-2012	$RDW_{0-15\,cm}$	5	9972175	6.12
		4	5061626	6.56
		9	14189771	6.39
		7	21608421	6.20
		12	7825500	6.10
		4	4773858	6.07
		4	5012248	6.02
Field trial-2018	$GY_{drought}$	6	9683272	7.80
		6	10565735	6.96
		7	16611957	6.46
		7	17887592	6.21
		7	16599505	6.01
		6	8475519	6.01
Lysimeter study	% Lateral roots>60 cm	11	26499434	5.99
Basket study	# Large-diameter deep nodal roots	4	28868811	6.06
	# Large-diameter shallow nodal roots	11	5173390	6.98
	The ratio of large and small roots	2	10641230	6.64
	Root dry weight	5	5817822	6.69
		5	18176872	6.63
		5	5823790	6.15
		5	5841282	6.06
		5	5842488	6.05
		8	17328185	6.02
	# Small-diameter shallow nodal roots	1	35262284	8.65
		4	34290240	7.97
		5	27316837	6.33
		8	21940266	5.99
	Total #shallow nodal roots	11	5173390	6.52

Note: In the lysimeter study, lateral roots were defined as those with a diameter <0.2 mm and % lateral roots was the proportion of the total root length as lateral roots, sampled below a soil depth of 60 cm. In the basket study, seep-angled nodal roots represented those growing through the lower part of the basket mesh (46–90° angle) and shallow-angled nodal roots represented those growing through the upper part of the basket mesh (0–45° angle).

(Figure S7) increased sharply between the sampling point during the drought stress treatment and after rewatering. Such responses to rewatering indicate plastic responses in genotypes that were classified as stable deep rooting based on AMMI analysis from root data taken at one-time point in each trial.

In addition to their root growth patterns over time, the four-grain yield/$RDW_{15-30\,cm}$ groups were explored to help dissect the components behind their deep root growth. In the 2019 field study, the HH and LH genotypes (high $RDW_{15-30\,cm}$) showed relatively lower shoot dry weights compared with the HL and LL genotypes (low $RDW_{15-30\,cm}$) both in the well-watered treatment (Figure S6B) and at the end of the drought stress and following rewatering (Figure 3c). These results suggest that the higher $RDW_{15-30\,cm}$ and RLD in the HH and LH genotypes was not an allometric effect. We also applied the grain yield and $RDW_{15-30\,cm}$ groupings to the results from the greenhouse studies to examine how each group ranked in terms of root traits measured in the lysimeter, basket and herbicide studies. The most distinct separation was observed in the basket study where the percent deep-angled nodal roots of HL genotypes ranked lower than that of all HH genotypes (Figure 4), pointing to the importance of deep root angle as a component trait of these aus genotypes in the field.

3.5 | Two candidate loci that colocated for yield and root traits best distinguished the accessions with contrasting yield under drought

Of the loci (Chr2_35194 and Chr6_11688) that showed the most significantly different distribution among haplotype groups between the Top 25 and Bottom 25 accessions for $GY_{drought}$, those colocations were with grain yield under drought in 2010 and 2018 and the percentages of deep and shallow nodal roots, as well as with the number of large-diameter deep and total deep nodal roots for the locus at Chr2_35194. Based on their high proportion of Top 25 accessions for $GY_{drought}$ (Figure 5), haplotype groups 1, 2 and 10 at Chr2_35194 and haplotype groups 1, 4, 7, 9 and 15 at Chr6_11688 appear to be the most favourable.

TABLE 3 Colocations of grain yield under drought and root traits by GWAS across different trials and years, based on a cutoff of −logP = 3 and a window of 1 kb

Expt	Trait	# colocations with grain yield under drought			
		2010	2011	2012	2018
Field-2011	Root dry weight at 0–15 cm	6	9	2	6
	Root dry weight at 15–30 cm	14	5	10	14
	Deep root percentage	16	4	3	16
Field-2012	Root dry weight at 0–15 cm	25	8	6	25
	Root dry weight at 15–30 cm	4	5	5	4
	Deep root percentage	7	4	7	7
Field-2018	Root dry weight at 0–15 cm	7	4	6	7
	Root dry weight at 15–30 cm	8	7	3	8
	Deep root percentage	7	10	5	7
Lysimeter study	Root dry weight > 60 cm	8	5	3	8
	%Lateral roots > 60 cm	18	2	4	18
	%L-type lateral root length > 60 cm	17	7	4	17
	Root length > 60 cm	7	5	2	7
Basket study	% Large-diameter nodal roots	3	2	1	3
	Nodal root length	8	3	6	8
	# Large-diameter deep nodal roots	6	2	2	6
	# Large-diameter shallow nodal roots	16	44	19	16
	% Steep-angled nodal roots	9	9	4	9
	Ratio of large to small diameter nodal roots	3	2	4	3
	Root dry weight	5	6	8	5
	# Small-diameter deep nodal roots	1	4	1	1
	% Shallow-angled nodal roots	9	9	4	9
	% Small-diameter nodal roots	3	2	1	3
	# Small-diameter shallow nodal roots	10	5	4	10
	Total # large-diameter nodal roots	10	12	10	10
	Total # deep nodal roots	10	10	3	10
	Total # nodal roots	10	9	14	10
	Total # shallow nodal roots	5	14	9	5
	Total # small-diameter nodal roots	1	3	1	1
Herbicide study	Herbicide response score at Day 36	3	4	3	3

Note: Lateral roots were defined as those with a diameter <0.2 mm, and % lateral roots was the proportion of the total root length from the depth of lateral roots, sampled below a depth of 60 cm. In the basket study, steep-angled nodal roots represented those growing through the lower part of the basket mesh (46–90° angle) and shallow-angled nodal roots are those growing through the upper part of the basket mesh (0–45° angle)

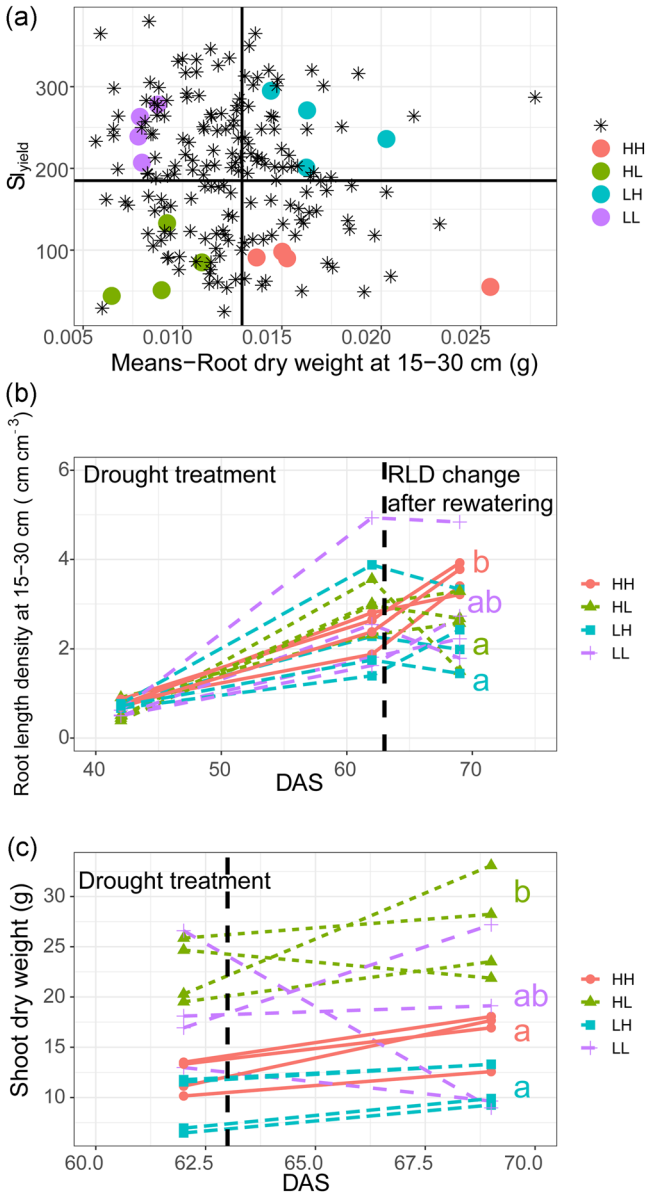

FIGURE 3 Selected contrasting aus genotypes and their responses to drought and rewatering. (a) The distribution of ~220 aus rice genotypes based on the grain yield stability (SI_{yield}; the sum of genotype ranks or ASV and means) and root dry weight at 15–30 cm (in a 188.5 cm^3 soil core). Each dot represents one genotype. Red symbols: genotypes with high grain yield and high $RDW_{15-30\,cm}$ (HH); green symbols: genotypes with high grain yield but low $RDW_{15-30\,cm}$ (HL); blue symbols: genotypes with low grain yield but high $RDW_{15-30\,cm}$ (LH); purple symbols: genotypes with low grain yield and low $RDW_{15-30\,cm}$ (LL). (b) The root length density at 15–30 cm of the 16 contrasting genotypes sampled before initiating the drought stress treatment (44 days after sowing [DAS]), 18 days after initiating drought (62 DAS) and 7 days after rewatering (70 DAS). (c) The shoot dry weight sampled at the same time as the last two root samples. The black dashed line represents the day of rewatering. The letters indicate significant differences at $p < 0.05$ among groups for the individual sampling date [Color figure can be viewed at wileyonlinelibrary.com]

Both Chr2_35194 and Chr6_11688 exhibit interesting structural variations. Of the 3 K RGP, there is a deletion in 139 lines from 3 K overlapping the 1 kb window at Chr2_35194 (del-164735 chr02:35106958.35583062), and a tandem duplication in eight lines. This deletion is large and also contains two OGRO trait genes 20 kb upstream, one of which is MIR (annotated as 'other stress resistance' [osa-MIR396c]). The alignments of Nipponbare with 9311, DJ123 and Kasalath have a breakpoint inside this region, which could indicate a deletion, while the alignment of Nipponbare and N22 shows a ~330 bp length insertion in N22. Furthermore, both loci showed a large degree of heterozygosity as indicated by the genotype heat maps across the 3k RGP (Figure S8).

4 | DISCUSSION

This study explored the aus rice variety group, with a genomic background that evolved in one of the most stress-prone rice-growing areas and whose traditional landraces have been subjected to natural and farmers' selection under those conditions. We aimed to identify potential drought tolerance donor material to improve the current breeding pool and to characterize how root growth was physiologically and genetically correlated with grain yield across field trials of varying soil moisture.

Compiling grain yield data across aus panel field trials, AMMI analysis enabled the identification of a small group of genotypes (H) with high and stable grain yield, which may be considered as drought-tolerant parents for use in breeding. Notably, three of the cultivars identified for high and stable yield in Table S5 (DM43, DJ 123 and DJ24) showed consistently higher yield under both continuously flooded and alternate wetting and drying conditions than five of the cultivars identified for low and unstable yield in Table S6 (ARC6578, ARC7229, Aswina, N22 and T1) that overlapped with the Bengal and Assam Aus Panel studied over several seasons in Bangladesh (Norton et al., 2018). Based on its increase in RDW at a depth of 15–30 cm ($RDW_{15-30\,cm}$) under drought compared to well-watered trials, the H group showed more plastic deep root growth than the group (L) with low and unstable grain yield, which showed similar $RDW_{15-30\,cm}$ values between the drought and well-watered trials. Further investigation across the entire aus panel, however, revealed a relationship between high and stable yield with stable deep root growth (i.e., genotypes with consistently greater deep RDW). This apparent contradiction in the contribution of plasticity may reflect the level of contrast in accessions being compared in the two analyses (H and L groups versus the entire set of ~220 aus genotypes), as well as the definition of plasticity considered in the context of root response to drought, which is often based on between-trial rather than within-trial responses.

In our previous report of the aus panel, we suggested that conclusions based on comparing small numbers of the most extreme genotypes/phenotypes may not show the same subtle variation across a large set of genotypes (Cal et al., 2019). That study referred

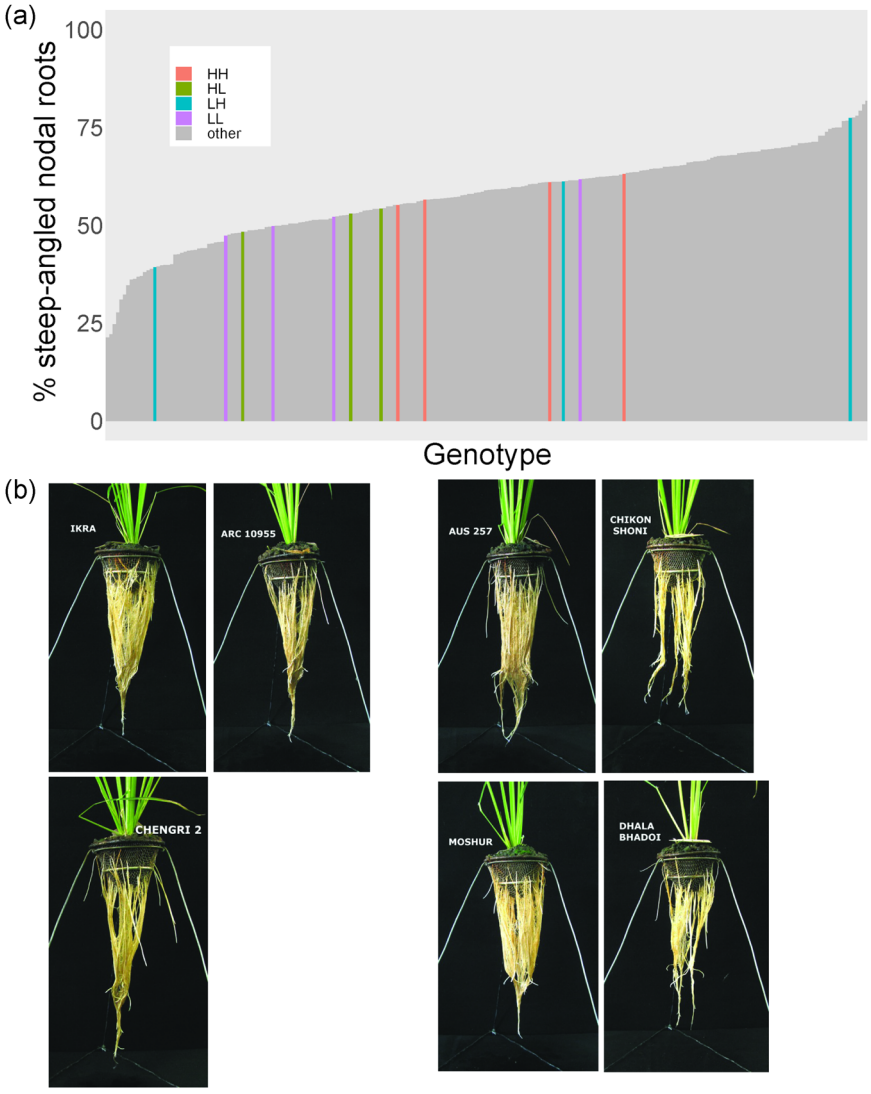

FIGURE 4 The selected contrasting genotypes in the greenhouse basket study. (a) Bar plot in order of steep root percentage (in terms of angle). Each bar represents one genotype and the colours indicate groups identified from the field studies: red bars indicate genotypes with high grain yield and high $RDW_{15-30\,cm}$ (HH), the green bars indicate genotypes with high grain yield but low $RDW_{15-30\,cm}$ (HL), the blue bars indicate genotypes with low grain yield but high $RDW_{15-30\,cm}$ (LH) and the purple bars indicate genotypes with low grain yield and low $RDW_{15-30\,cm}$ (LL). (b) Images of the selected HH and HL genotypes. HL genotype DM43 was not included in the basket study [Color figure can be viewed at wileyonlinelibrary.com]

to the lack of correlation between leaf rolling under drought and leaf water status parameters across the aus panel despite previous reports of leaf water and osmotic potential affecting leaf rolling in smaller sets of genotypes. In the present study, comparison of root phenotypes in the H (four genotypes) and L group (seven genotypes) is a similar contrast to using the entire aus panel and points to the value of examining phenotypic correlations using larger sets of genotypes in examining relationships among traits.

Previously, Sandhu et al. (2016) also characterized root architectural plasticity by comparing RDW in drought versus well-watered conditions in a given season and reported that rice genotypes with a higher degree of root architectural plasticity in response to drought showed high and stable yield in two mapping populations with aus donor parents. However, our 2019 field experiment, in which the HH genotypes showed the most rapid increase in RLD upon rewatering, revealed that genotypes we previously classified as showing stable deep root growth may in fact be quickly increasing their root growth at depth in response to rewatering: this within-treatment response can also be classified as a type of plasticity. Since such 'lifesaving irrigation' is common in rice drought field trials (Venuprasad et al., 2007), we should therefore reconsider our definition of root architectural plasticity to not only consider differences in root growth between treatments but also changes that occur within treatment in a given season. Indeed, previous greenhouse studies imposing fluctuating soil moisture levels reported genotypic differences in nodal and lateral root length, number, elongation rates and branching that was defined as root plasticity triggered by water deficit (Bañoc et al., 2000; Kano-Nakata et al., 2013; Niones et al., 2015; Owusu-Nketia et al., 2018).

Given that lateral root growth has often been highlighted as the key trait related to rice drought response and root architectural plasticity (Hazman & Brown, 2018; Henry et al., 2012; Suralta et al., 2018), a surprising result from the current study was that the nodal root number and diameter classification stood out in both the path analysis linking early-stage greenhouse studies with root growth in the field at later stages, as well in the GWAS analysis: root length in the lysimeter study was positively correlated with the percentage of 'large diameter' nodal roots in the basket study (Figure 2), and the

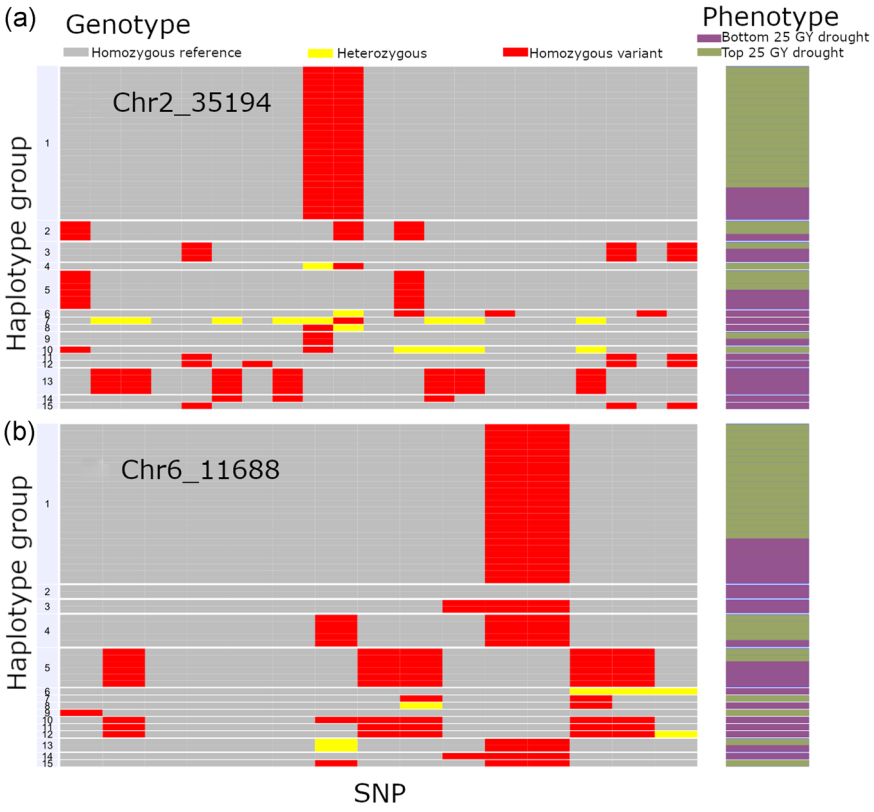

FIGURE 5 Haplotype groupings for the regions on (a) Chromosome 2 and (b) Chromosome 6 (the position indicated after underscore denotes the start of the locus in kb) that showed a high number of colocating GWAS peaks for $GY_{drought}$ and root traits (Table 3), and the most significantly different distribution among haplotype groups between the Top 25 and Bottom 25 accessions for $GY_{drought}$ (Tables S5 and S6). A total of 35 accessions are shown (those with available sequences), and the height of each row is proportional to the number of accessions in each haplotype group. The phenotype of each accession is indicated on the right [Color figure can be viewed at wileyonlinelibrary.com]

number of shallow nodal roots classified as 'large diameter' showed the highest number of colocated GWAS peaks with grain yield under drought (Table 3). Previously, large maximum diameter of nodal roots has been highlighted as a beneficial root trait in drought-prone environments in the context of hardpan penetration; large-diameter nodal roots were documented to better penetrate hard soil layers caused by drought and to promote deep root growth (Zhao et al., 2018; Zheng et al., 2000) and qRT9 controlling root thickness and contributing to root elongation in upland rice was identified (Li et al., 2015). Previous studies showed that greater maximum nodal root diameter contributed to better hard pan penetration (Clark et al., 2008; Samson et al., 2002), although the role of a hardpan in limiting nodal root elongation may vary across environments (Suralta et al., 2018). Compared to this emphasis on the maximum diameter of rice nodal roots, the distinction of nodal roots into different diameter classes and their distribution between those classes has received less attention despite having been documented decades ago (Matsuo & Hoshikawa, 1993).

Typical rice phytomer models indicate that smaller-diameter nodal roots emerge from the upper file of a phytomer and that larger-diameter nodal roots emerge from the lower file, but a number of nodal root types have been observed that may vary not only within a phytomer but also among successive nodes (Yamazki & Harada, 1982). More research is necessary to characterize rice nodal root types and their function. Although our study suggests a positive relationship between the proportion of the total number of nodal roots classified as 'large-diameter' and grain yield (via $RDW_{15-30\,cm}$ in the phenotypic correlations and directly by GWAS colocations), this is in contrast to recent results from simulation modelling of rice roots which concluded that a larger proportion of 'small-diameter' nodal roots was beneficial to plant growth and yield under low-nitrogen, rainfed direct-seeded conditions (Ajmera et al., In Press). Therefore, the function of various nodal root class distributions may differ depending on the soil conditions and should be further investigated.

Dissecting the component root traits that underly composite traits such as RDW at depth is more likely to reveal a greater degree of genetic variation than measuring the composite traits alone (Rangarajan & Lynch, 2021). As the trait that most consistently correlated with high and stable grain yield, we hypothesized that greater deep RDW of aus genotypes could be due to three main mechanisms involving deep root growth component traits (in addition to the evidence of plasticity mentioned above): nodal root elongation, nodal root angle and lateral root formation. Our analysis of consistent correlations between RDW in the field and root component traits, as well as genetic correlations identified through GWAS, provides evidence that all of these component traits were involved across the aus panel. A role of nodal root elongation was indicated by the significantly positive correlations between RDW at depth in the field trials and nodal root length in the lysimeter study (Figure 2). Furthermore, the path analysis identified a connection between nodal root elongation (as evidenced by herbicide response score and root length in the basket study) with deep RDW in the field (Figure 2). Previously, few direct correlations between early-stage root screens and later stage root results from field have been reported (Rich et al., 2020), however, path analysis may facilitate these linkages, albeit with low correlation coefficients, as reported by Catolos et al.

(2017) and in the present study. In terms of nodal root angle, a number of colocated GWAS peaks between grain yield and deep root angle were identified (Table 3). The role of lateral roots was also indicated by our GWAS results that identified a high number of co-locations of percent lateral roots in the lysimeter study with grain yield across years (Table 3). The trend of HH genotypes showing initially lower shoot dry weight than the HL group (Figure 3c) rules out an allometric effect behind the higher deep RDW of those genotypes. Taken together, deep root growth related to yield stability across different soil moisture levels was not simply affected by single root traits, but appeared to be the cumulative effect of nodal root length and angle, lateral root growth and root architectural plasticity across the aus panel. The contribution of each component trait to deep RDW likely varied among genotypes.

In rice, QTLs for the above-mentioned deep root growth component traits have been reported, including qRL6.1 for nodal root elongation (related to nitrogen supply; Obara et al., 2010), Dro1 controlling root angle (Uga et al., 2013), qSOR1, a homolog of Dro1 that modifies root angle (Uga et al., 2012) and *qLLRN-12* for lateral root plasticity (Niones et al., 2015). $qDTY_{3.2}$, a drought-yield quantitative trait locus, was also associated with deep root growth under drought via reduced shallow root growth (Grondin et al., 2018). In addition, overexpression of OsNAC5 enlarged root diameter and promoted drought tolerance and grain yield in rice (Jeong et al., 2013). Although we aimed to determine if some of these genes/QTLs were responsible for deep root growth in the aus panel through GWAS, we were unable to identify such association due to low levels of significance in our GWAS analysis, which is likely caused by the high degree of variability in our data common to field/drought/root studies. Therefore, we took an alternate approach in interpreting our GWAS data by considering all peaks including those below the significance cutoff to identify traits that showed the greatest number of colocations with grain yield under drought, rather than identifying significant peaks that colocated with previous studies.

Although the use of $-\log P = 3$ as a cutoff may increase the likelihood of detecting false positives, the requirement that a peak in one trait coincides with a peak in the other trait will remove most chance associations, since these will arise independently in the two traits (provided the traits are independent, such as those measured in this study from distinct root parameters and grain yield). With the observed number of peaks for each trait, one expects to see less than 11 colocations 95% of the time (see Section 2). Thus, our observation of more than 11 colocations between root traits and grain yield for a number of cases (Table 3) is highly likely to contain true genetic associations. This approach of identifying colocations of GWAS peaks with a relaxed threshold may be well-suited to root and drought response studies since many of the related traits are thought to be polygenic traits controlled by many quantitative trait loci with low heritability resulting from complex genetic regulation and interaction due to environmental cues, rather than single, major-effect loci.

Of both loci that showed a high number of colocating GWAS peaks for $GY_{drought}$ and root traits, and the most significantly different distribution among haplotype groups between the Top 25 and Bottom 25 accessions for $GY_{drought}$, it was interesting to note that both showed a considerable degree of heterozygosity (Figure S8). A higher than expected proportion of heterozygous SNP calls at a locus may be due to a 'hidden' homologous locus in the aus genome, which is missing from the reference genome. That is, the locus has one copy in Nipponbare but two copies in the aus genome, where one copy is diverged and another copy is the same as in Nipponbare. Both copies in the aus genome are mapped to the same locus in Nipponbare when calling SNPs, thus causing these heterozygous SNPs. Since these two loci showed multiple colocations between grain yield and root traits in our study (including nodal root diameter class), and they exhibit interesting structural variation and a high degree of heterozygosity, we recommend further investigation to evaluate their utility in breeding for improvement of drought tolerance in rice.

5 | CONCLUSIONS

From multiple angles, this study highlighted that scale of observation can drastically affect the interpretation of phenotypic plasticity. Regarding scale in terms of panel size, our comparison of small but contrasting sets of lines for yield suggested the importance of root plasticity in response to drought, but our analysis of the entire panel suggested a role of stable deep root growth across environments. Regarding scale in terms of temporal changes in root growth, the stable deep root growth observed from sampling at one time-point in a field trial immediately after rewatering likely missed genetic variation in plastic responses to rewatering that could be observed by sampling later. These results highlight the importance of strategically timing the sampling in field trials, to better capture genetic variation in response to drought.

Our study also highlighted the large degree of genetic variation in component root traits underlying deep root growth, from both the phenotypic and genomic associations. The relationships (direct or indirect) of a number of component traits—some unexpected, such as nodal root diameter class—with deep root growth suggest that root architecture can be even further dissected at both the phenotypic and genomic levels to better pinpoint traits for rice improvement. Given its adaptation to abiotic stresses such as drought, and the promising genotypes and loci identified in this study and elsewhere, the aus variety group of rice merits further exploration of stress tolerance traits, genes and potential drought donor genotypes for breeding.

ACKNOWLEDGEMENTS

We thank L. Holongbayan, N. Turingan, N. Driz, A. Los Añes, N. Sadiasa, A. Reyes, D. Morales, P. Zambrano, T. Batoto, E. Mico, L. Satioquia, M. Manalili and C.A. Centeno for technical support. We also acknowledge R. Serraj for initiating the aus field trials, M. Wissuwa and J.G. Monroe for contributions to the 2018 field trial, J. Xu and D. Pisano for advice on statistical analysis, Y. Uga for advice

on the basket study and I. Ajmera for helpful discussions about nodal roots. This study was supported by the Generation Challenge Program (GCP) project G3008.06, 'Targeting Drought-Avoidance Root Traits to Enhance Rice Productivity under Water-Limited Environments'. Q. Liao was supported by the Chinese Scholarship Council (CSC) and 'Hengygang Guiding Plan Project 2020jh022798' award. M. S. Islam was supported by a Bangabandhu Fellowship on Science and ICT from the Ministry of Science and Technology, Bangladesh.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data from this study will be made available on Dataverse upon publication.

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SUPPORTING INFORMATION

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How to cite this article: Liao, Q., Chebotarov, D., Islam, M.S., Quintana, M.R., Natividad, M.A., De Ocampo, M. et al. (2022) Aus rice root architecture variation contributing to grain yield under drought suggests a key role of nodal root diameter class. *Plant, Cell & Environment*, 45, 854–870. https://doi.org/10.1111/pce.14272