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RESEARCH PAPER

Establishment method affects rice root plasticity in response to drought and its relationship with grain yield stability

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Abstract

By responding to the variable soil environments in which they are grown, the roots of rice crops are likely to contribute to yield stability across a range of soil moistures, nutrient levels, and establishment methods. In this study, we explored different approaches to quantification of root plasticity and characterization of its relationship with yield stability. Using four different statistical approaches (plasticity index, slope, AMMI, and factor analytic) on a set of 17 genotypes including several recently-developed breeding lines targeted to dry direct-seeding, we identified only very few direct relationships between root plasticity and yield stability. However, genotypes identified as having combined yield stability and root plasticity showed higher grain yields across trials. Furthermore, root plasticity was expressed to a greater degree in puddled transplanted trials rather than under dry direct-seeding. Significant interactions between nitrogen and water resulted in contrasting relationships between nitrogen-use efficiency and biomass stability between puddled-transplanted and direct-seeded conditions. These results reflect the complex interaction between nitrogen, drought, and even different types of drought (as a result of the establishment method) on rice root growth, and suggest that although rice root plasticity may confer stable yield across a range of environments, it might be necessary to more narrowly define the targeted environments to which it will be most beneficial.

Keywords: AMMI, direct-seeding, factor analytic, *Oryza sativa*, puddled transplanted, rice, root plasticity, yield stability,

Introduction

Rice crops can be grown in a range of edaphic conditions in terms of seedling establishment method, water availability, and nutrient availability, suggesting that adaptability to that range of conditions may be conferred by plasticity in root traits. Many components of rice root architecture have been documented to show plastic responses to changes in soil moisture

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and nutrient levels, including lateral root growth (as reviewed by Suralta *et al.*, 2018), crown root angle (Kano-Nakata *et al.*, 2019), and nodal root elongation/root growth at depth (Obara *et al.*, 2010; Sandhu *et al.*, 2016). However, few studies have quantified the relationship between root architectural plasticity and productivity in rice.

The calculations and analyses used to quantify plasticity in plant traits have been a topic of recent discussion in both the ecological and agricultural research communities. Laitinen and Nikoloski (2019) have described relative and nominal approaches to quantifying plasticity in plants, and Arnold *et al.* (2019) have advocated random regression mixed models for plasticity studies. We have previously quantified rice root architectural plasticity using a plasticity index (Sandhu *et al.*, 2016) that compares the difference in root growth between two treatments as

$$\frac{x_{\text{stress}} - \bar{x}_{\text{control}}}{\bar{x}_{\text{control}}} \quad (1)$$

This index is useful for statistical comparisons of genotypic differences in plasticity as it allows for replication of values in the stress treatment; however, it presents certain limitations. One is that it compares only two conditions (e.g. drought versus well-watered), although more environments can be included in the analysis by taking the average of plasticity index values across environments. Another limitation is that in cases of extreme plasticity, for example at soil depths where roots are present in the drought-stress treatment but are absent in the well-watered control, there is no data-point that can be recorded since the denominator in the equation is 0. Hence, alternative approaches to quantifying rice root plasticity may facilitate inclusion of more results from contrasting conditions, and improve the characterization of genotypic variation.

Since our overall aim in investigating root architectural plasticity across environments is to improve the yield of rice under stress (i.e. to improve the yield stability across stress-prone and optimal conditions), we have considered using yield stability analyses to examine root architectural plasticity. In principle, plasticity is the opposite of stability (see Bradshaw, 2006), specifically when stability is defined as low among-environment variance (i.e. the Type I concept of stability; Lin *et al.*, 1986). Therefore, it should be appropriate to employ the same approaches to analysing yield stability as for root plasticity. Our previous comparison of root architectural plasticity and yield stability unexpectedly resulted in some negative relationships (Sandhu *et al.*, 2016), which could be attributable to either the soil depths considered or the fact that we used the plasticity index for root plasticity and the additive main effects and multiplicative interaction (AMMI) model for yield stability due to the limited number of environments available for the root plasticity analysis in that study. In the current study we therefore aimed to clarify the relationships between root plasticity and yield stability by including additional conditions and seasons, in order to generate root datasets that could be evaluated using yield stability approaches.

Yield stability is commonly evaluated by analysing yield values for the same set of genotypes across multiple environments, using genotype-by-environment (G×E) interactions (ANOVA), linear regression (Eberhart and Russell, 1966), and mixed models. AMMI is a model that combines ANOVA for the main effects of environment and genotype with principal components analysis of G×E interactions. The factor analytic (FA) model is similar to AMMI in that both assume that G×E can be explained by latent variables and both models can be used to examine patterns of G×E and stability across environments (Piepho, 1998; Meyer, 2009; Elias *et al.*, 2016). The FA model might be superior to the AMMI model for our experimental data because, firstly, it is flexible and allows both fixed and random factors to be accounted for, whereas the AMMI model assumes that experimental error is homogenous across environments, and secondly, it assumes that the varieties/breeding lines are random effects whereas the AMMI model assumes that they are fixed effects.

In this study, we grew a set of rice drought-breeding lines and released varieties across a range of soil moisture and nitrogen conditions, using both dry direct-seeding and puddled transplanting as establishment methods. We compared root architectural plasticity in terms of root-length density at depth with yield stability using a common method for the two parameters and comparing four different approaches of analysis, in order to add confidence to our conclusions regarding the relationship between yield stability and root plasticity. We hypothesized that genotypes with the most stable yield would be those with the greatest degree of root plasticity. After discovering that root plasticity was not necessarily correlated with greater yield stability, we explored the traits behind yield stability by characterizing the physiological differences between stable-yielding genotypes with more- or less-plastic root growth.

Materials and methods

Experimental design

In field trials, 17 experiments were conducted at the International Rice Research Institute (IRRI, Los Baños, Laguna, The Philippines, 14°10′11.81′′N, 121°15′39.22′′E) spanning from the 2016 dry season to the 2017 dry season (Table 1). Seventeen rice (*Oryza sativa*) genotypes (IR 115844-B-154, IR 115844-B-32, IR 115844-B-332, IR 115845-B-154, IR 115845-B-310, IR 115845-B-388, IR 92801-504-B, IR 92801-527-B, IR 94226-B-265, IR 94226-B-364, IR 94226-B-419, IR 95783-6-2-2-3, IR 97041-5-1-1-2, IR 98976-20-1-2-1, IR64, MTU1010, UPLRi7) were grown in each experiment, except for experiments 1–4 in which IR 92801-504-B, IR 92801-527-B, and IR 94226-B-265 were excluded, and experiments 7 and 8 in which UPLRi7 was excluded. Of these genotypes, IR92801-504-B, IR92801-527-B, IR 94226-B-265, and IR 94226-B-419 have previously been identified as having stable yield and plastic root architecture, such as root-length density and total root length (Sandhu *et al.*, 2016). IR64 and MTU1010 are high-yielding but drought-susceptible varieties, while UPLRi7 is a drought-tolerant variety. The remaining genotypes were

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Table 1. Summary of experiments

Experiment [a]	Environment code[b]	Date of sowing / transplanting	Measured traits[c]
1	2016DS_PTR_CN(S)	18 December 2015 / 5 January 2016	CT, NDVI, RLD, GY, Biomass, HI
2	2016DS_PTR_CN(W)		
3	2016DS_DSR_CN(S)	2 February 2016	CT, NDVI, N uptake, RLD, GY, Biomass, HI
4	2016DS_DSR_CN(W)		
5	2016WS_DSR_CN(S)	16 July 2016	CCI, LOP, NDVI, N uptake, RLD, GY, Biomass, HI
6	2016WS_DSR_CN(W)		
7	2016WS_PTR_CN(S)	1 July 2016 /19 July 2016	CT, LOP, NDVI, N uptake, RLD, GY, Biomass, HI
8	2016WS_PTR_CN(W)		
9	2017DS_PTR_LN(S)	23 December 2016 / 10 January 2017	CT, LOP, NDVI, CCI, N uptake, RLD, GY, Biomass, HI
10	2017DS_PTR_LN(W)		
11	2017DS_PTR_HN(S)	23 December 2016 / 10 January 2017	CT, LOP, NDVI, CCI, N uptake, RLD, GY, Biomass, HI
12	2017DS_PTR_HN(W)		
13	2017DS_DSR_LN(S)	24 January 2017	CT, LOP, NDVI, CCI, N uptake, RLD, GY, Biomass, HI
14	2017DS_DSR_LN(W)		
15	2017DS_DSR_HN(S)	24 January 2017	CT, LOP, NDVI, CCI, N uptake, RLD, GY, Biomass, HI
16	2017DS_DSR_HN(W)		
17	Lysimeter	17 October 2016 / 27 October 2016	CCI, RDW, RL below 60 cm, N uptake, WUE, Biomass

[a]Seventeen genotypes were grown in experiments 5–6 and 9–17: IR 115844-B-154, IR 115844-B-32, IR 115844-B-332, IR 115845-B-154, IR 115845-B-310, IR 115845-B-388, IR 92801-504-B, IR 92801-527-B, IR 94226-B-265, IR 94226-B-364, IR 94226-B-419, IR 95783-6-2-2-3, IR 97041-5-1-1-2, IR 98976-20-1-2-1, IR64, MTU1010, and UPLRi7. In experiments 1–4 IR 92801-504-B, IR 92801-527-B and IR 94226-B-265 were not included, and in experiments 7 and 8 UPLRi7 was not included.

[b] Environment codes show the year, the season (DS, dry season; WS, wet season); the establishment method (PTR, puddled transplanted rice; DSR, direct-seeded rice); the nitrogen treatment (CN, conventional N rate; LN, low N rate; HN, high N rate), and the water treatment (W, well-watered; S, drought stress at the reproductive stage). The N application rates were as follows: CN, 100 kg N ha^{-1}; LN, 60 kg N ha^{-1} for DSR and 75 kg N ha^{-1} for PTR; HN, 120 kg N ha^{-1} for DSR and 150 kg N ha^{-1} for PTR. The lysimeter study included PTR (W), PTR (S), DSR (W) and DSR (S).

[c] CT, canopy temperature, only measured in drought stress experiments; NDVI, normalized difference vegetation index; RLD, root-length density at different depth intervals; CCI, chlorophyll concentration index; GY, grain yield; HI, harvest index; RDW, root dry weight at different depth intervals; RL below 60 cm, root length below soil depth of 60 cm; WUE, water-use efficiency.

from multi-parent crosses as part of a direct-seeded rice breeding program (Supplementary Table S1; Sandhu *et al.*, 2019; Subedi *et al.*, 2019).

Experiments 1–16 were arranged in a randomized complete block design with 3–4 replications in puddled transplanted trials that represented rainfed lowland conditions, and in direct-seeded trials that represented upland conditions (see Supplementary Table S2 for soil characteristics). In the puddled transplanted rice (PTR) experiments, 17–18-d-old seedlings were transplanted into ploughed and leveled rainfed lowland fields in 3 or 4 rows (25 cm between rows, plant spacing of 20 cm, row length of 3 m) and with 3 or 4 seedlings per hill for each genotype. Experiments 1, 7, 9, and 11 were reproductive-stage drought stress trials that were maintained under well-watered conditions during the vegetative stage and drained at the panicle initiation stage. Correspondingly, experiments 2, 8, 10, and 12 were well-watered trials that were maintained under well-watered conditions until 7 d before harvest. In the 2016 wet season, experiment 7 was laid out in a field with an automatic rainout shelter. After draining at panicle initiation, this shelter closed and re-opened automatically when rainfall started and stopped. In the direct-seeded rice (DSR) experiments, non-germinated seeds were dry-sown in ploughed, leveled, and furrowed upland fields with 4 rows (25 cm between rows, plant spacing of 10 cm, row length of 3 m) and with 4 or 5 seeds per hill for each genotype. After sowing, seeds were covered with 2–3 cm of soil. Experiments 3, 5, 13, and 15 were reproductive-stage drought stress trials that were subjected to sprinkler irrigation three times a week until panicle initiation, after which no irrigation was applied, except in cases where the plants could not flower due to severe drought stress. Correspondingly, the well-watered DSR experiments 4, 6, 14, and 16 were sprinkler-irrigated three times a week until 7 d before harvest.

A conventional N application rate of 100 kg N ha^{-1} was split equally between a basal application and one at the panicle initiation stage in experiments 1–8. A low N rate treatment of 75 kg N ha^{-1} was applied in experiments 9 and 10 and a high N rate of 150 kg N ha^{-1} was applied in experiments 11 and 12, with both being split and applied at proportions of 20%, 50%, and 30% at 0, 7, and 25 d after transplanting, respectively. A low N rate treatment of 60 kg N ha^{-1} in experiments 13 and 14 and a high N rate of 120 kg N ha^{-1} in experiments 15 and 16, with both being split and applied at proportions of 20%, 25%, 25%, and 30% at 0, 24, 3,6 and 46 d after sowing, respectively. Corresponding to the N application rates, 50 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1} were applied in experiments 1–8, 75 kg P_2O_5 ha^{-1} and 75 kg K_2O ha^{-1} were applied in experiments 9–12, and 60 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1} were applied in experiments 13–16 as basal fertilizer. The sources of N, P_2O_5 and K_2O at the basal application were from complete fertilizer (14-14-14) and the N source for top-dressing was from ammonium sulfate in experiments 1–8. In experiments 9–16, the sources of N, P_2O_5 and K_2O were from ammonium sulfate, superphosphate, and potassium chloride, respectively. In order to monitor soil moisture, 3–4 tensiometers (at a depth of 30 cm) were installed within 3–4 selected plots when the soil was close to field capacity after draining in the stress experiments (experiments 1, 3, 5, 7, 9, 11, 13, and 15; Supplementary Fig. S1). Lower solar radiation and temperatures, and higher rainfall, were observed in the wet season experiments (5–8; Supplementary Fig. S2).

In addition to the field experiments, a greenhouse lysimeter study (experiment 17) was conducted during the 2016 wet season using a split-split plot design with crop establishment method as the main blocks, water treatments as the sub-blocks, and genotypes as sub-sub-blocks, with four replications. The crop establishment treatments were PTR/lowland and DSR/upland, and the water treatments were drought stress and well-watered. The same 17 genotypes grown in the field trials were

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also used in the lysimeter study. Four concrete tanks (1.35 m deep, 3.5 m wide, and 6.8 m long) within a greenhouse were used, with one replication in each tank and a total of 272 lysimeters in the experiment. The lysimeters were constructed from PVC cylinders (0.95 m long and 0.18 m diameter) with plastic liners filled with 23 kg of dry, sieved soil (bulk density 1.1 g cm^{-3}) from the IRRI upland farm, as described by Sandhu *et al.* (2016). Briefly, the soil was added and manually compressed until it reached to a depth of either 70 or 90 cm for the lowland and upland treatments, respectively. Wet paddy soil collected from a lowland field that had been cleaned of debris was then added on top of the upland soil for the PTR/lowland treatment, leaving a clearance of 5 cm at the top of the cylinder. Three holes were drilled at the bottom of cylinder, which were blocked with rubber plugs for the well-watered treatment and unplugged for the drought-stress treatment after panicle initiation. Basal fertilizers (0.375 g N pot^{-1}, 0.75 g P_2O_5 pot^{-1}, and 0.75 g K_2O pot^{-1}) and top-dressing (0.375 g N pot^{-1}) were applied at 1 d before sowing/transplanting for the DSR/PTR treatments, and at 30 d after sowing (DAS) or 20 d after transplanting (DAT), for the DSR and PTR treatments, respectively. The sources of N, P_2O_5 and K_2O were from ammonium sulfate, superphosphate, and potassium chloride, respectively. These fertilizer levels corresponded to the high-N treatment in the field trials. Seeds of each genotype were germinated in Petri dishes. After 3 d, three uniform seeds were sown onto the soil surface of the cylinders for the DSR/upland treatment. At the same time, germinated seeds for the PTR/lowland treatment were sown in seedling trays, and after 10 d three seedlings were then transplanted into the cylinders for the PTR/lowland treatment. One plant was thinned from each cylinder at 18 DAS/7 DAT and again at 25 DAS/14 DAT. For the DSR/upland treatment, watering was done by hand every day in the first week after sowing and then three times a week until 30 DAS. For the PTR/lowland treatment, 2–5 cm of standing water was maintained until 20 DAT. Drought stress was initiated at 30 DAS/20 DAT by removing the rubber plugs to drain the excess water from the cylinders. Plastic sheets were then sealed around the base of each plant to minimize direct water evaporation from the surface of the cylinders.

Sampling and measurements

In the field trials, canopy temperature from panicle initiation to maturity was recorded from three readings per plot with an infrared sensor (MI-210; Apogee Instruments, UT, USA) around midday in the drought-stress experiments. The normalized difference vegetation index (NDVI) from panicle initiation to maturity was measured around midday in the drought-stress experiments for DSR throughout all seasons and for PTR in both the dry and wet seasons in 2016 using a Greenseeker Hand-held Sensor (NTech Industries, CA, USA) that was carried through the field, or with a Crop Circle ACS 470 sensor (Holland Scientific, NE, USA) mounted ~1 m above the soil on a rack that rolled along the tracks of the rainout shelter (experiment 7). Shoots from one hill per plot of experiments 3–8 and four hills per plot of experiments 9–16 were sampled from inside rows at 64–82 DAS for the DSR treatment and 58–78 DAT for the PTR treatments. The shoots were dried in an oven at 70 °C for 3 d to determine dry weight, and were then ground to a fine powder to determine N concentration using a Skalar SAN^{++} Continuous Flow Analyzer (Skalar Inc., Breda, The Netherlands). Shoot N uptake was calculated by multiplying shoot dry weight by the corresponding N concentration. The N-use efficiency for grain production (NUE_g, kg kg^{-1}) was calculated as grain weight divided by N uptake, and N-use efficiency for biomass (NUE_b, kg kg^{-1}) was calculated as total biomass at harvest (the sum of straw and grain dry weight) divided by N uptake.

Soil samples for root measurements were obtained in all experiments using a corer of 4 cm diameter (Giddings Inc., USA) to a depth of 60 cm at 89–94 d after sowing or 72–77 d after transplanting. Three sub-replicate core samples were collected from the middle of the inter-row spaces in each plot and were divided into increments of 15 cm depth. The root samples from each increment were washed carefully by repeatedly mixing the soil with tap water in a container and pouring the suspension into a 1-mm diameter plastic sieve to separate the roots from the soil and debris. The roots were then scanned using an Epson Expression 10000XL, and analysed for root-length density (RLD) using WinRhizo Pro (v.2007d, Regent Instruments Inc., Canada).

Total biomass and grain yield were determined from a harvested area of 1.5 m^2. Grain yield was adjusted to a moisture content of 0.14 g H_2O g^{-1} FW, and biomass was determined as the sum of straw and grain dry weights. The harvest index was calculated as the grain dry weight divided by total biomass. Chlorophyll concentration index was measured on six youngest fully-expanded leaves on plants in each plot using a CCM-200 Plus (Apogee Instruments Inc.) at 5 d intervals from panicle initiation to flowering, with three readings per leaf (lower, middle, and upper segments). Three flag-leaves per plot were sampled at 7–10 d after flowering for measurement of leaf osmotic potential using a Vapro 5600 (Wescor Inc.).

In the lysimeter study, plant water uptake was monitored by weighing the cylinders at 39, 46, 53, and 60 DAS or at 29, 36, 43, and 50 DAT. A mechanical hoist system was used to lift and place the cylinders on a balance (KERN SCE-3.0, Kern and Sohn) that was connected to a laptop computer (Kijoji *et al.*, 2013). For the well-watered treatment, the cylinder weight at 39 DAS or 29 DAT was recorded and used as a target, with water subsequently being added to maintain this level of soil moisture. At the time of weighing of each cylinder, the shoots were imaged and apparent leaf area was estimated in ImageJ (Abràmoff, 2004) based on the number of green pixels in reference to a 100-cm^2 size standard within each image. Normalized water uptake rates were calculated as the difference in weights between two consecutive weeks, divided by the leaf area measured in the second week. The shoots of the plants in each lysimeter were harvested by cutting at the base at 61 DAS or 51 DAT, and they were then dried in oven at 70 °C for 3 d to determine the above-ground biomass. Water-use efficiency was calculated as the shoot dry weight divided by the cumulative water uptake. The soil columns were then separated into four depth increments (0–20 cm, 20–40 cm, 40–60 cm, and >60 cm) and stored in a cold room at 4 °C until washing, which was carried out as described above within 2 months. Roots from below 60 cm were stored in 70% ethanol, and subsequently scanned and analysed for total root length as described above. Roots from all the depths were dried in an oven at 70 °C for 3 d to determine their weight. Shoots were ground to a fine powder to determine N concentration, as described above, and shoot uptake was calculated by multiplying the dry weight by the corresponding N concentration.

Approaches for calculating root plasticity and grain yield stability

We evaluated four different approaches for investigating the relationship between root plasticity and yield stability, as follows.

(I) Plasticity index. Root plasticity (in terms of RLD) and grain yield stability were calculated on each replicate from the drought-stress treatment and the mean values from the corresponding well-watered treatment (Sandhu *et al.*, 2016) within each establishment method and N treatment.

$$\text{Root plasticity or grain yield stability} = \frac{x_{\text{drought stress}} - \bar{x}_{\text{well-watered}}}{\bar{x}_{\text{well-watered}}} \quad (2)$$

The plasticity/stability index values were then averaged across the establishment method treatments, the nitrogen treatments, and the seasons to obtain a single index value for each genotype. Outliers were identified as the mean±2SD for all observed values and were excluded from the analysis. This approach was chosen because we had previously used it to quantify genotypic differences in rice root architectural plasticity.

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(II) Slope. Across all environments (year, season, establishment method, water and nitrogen treatment combination), the coefficient of root plasticity or grain yield stability was derived as the dimensionless slope of the linear regression between the traits (i.e. RLD or grain yield) of an individual genotype in a particular environment and the mean of the trait for all genotypes in that environment, as reported previously by Sadras *et al.* (2009). This approach was chosen because it has previously been used to calculate plasticity values, and because it allows results from a greater number of trials to be considered together compared to Approach (I). The slope approach was conducted by Finlay–Wilkinson analysis (Finlay and Wilkinson, 1963) using the *statgenSTA* and *statgenGxE* packages in R v.3.4.1 (www.r-project.org).

(III) AMMI. An additive main-effects and multiplicative interaction (AMMI) analysis was used to determine root plasticity and grain yield stability of the genotypes across all environments (Annicchiarico, 1997; Zhang *et al.*, 1998), using Plant Breeding Tools (PB Tools) v.1.3 (http://bbi.irri.org). The AMMI model used was:

$$P_{ij} = \mu + \ \tau_i + \delta_j + \sum_{k=1}^{t} \lambda_k \alpha_{ij} \gamma_{ij} \ \varepsilon_{\ ij} \tag{3}$$

P_{ij} is each root/yield parameter, μ is the grand mean for the trait; 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

$$\sum_{i=1}^{g} \alpha_{ik} \alpha_{ik'} = \sum_{j=1}^{s} \gamma_{jk} \gamma_{jk'} = 0 \tag{4}$$

$$\sum_{i} \alpha_{ik}^2 = \sum_{j} \gamma_{jk}^2 = 1 \tag{5}$$

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

We used the Euclidean distance (D), namely the significant interaction principal component (IPC) axes to the origin, as the measure of root plasticity and grain yield stability from the AMMI analysis, calculated as follows:

$$D = \sqrt{\sum_{\gamma=1}^{n} \gamma_{is}^2} \ (i = 1, \ 2, \ 3, \ldots, n) \tag{6}$$

where n is the number of significant IPCs, and γ_{is} is the score of genotype i in IPC s. In this study, we therefore extracted the significant PC1 and PC2 in terms of root traits or grain yield based on AMMI analysis to calculate D for each genotype. The AMMI approach was chosen since it is commonly used to evaluate yield stability.

(IV) Factor analytic. A factor analytic (FA) model (Cullis *et al.*, 2010; Smith *et al.*, 2015) was used to determine root plasticity and grain yield stability of the genotypes across all environments. The FA analysis was conducted in R v.3.4.1 using the *asreml* (Gilmour *et al.*, 2009), *AAfun4* (https://github.com/yzhlinscau/AAfun), and *naniar* (https://github.com/njtierney/naniar) packages.

We fitted the FA model in *asreml-r* according to:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \tag{7}$$

where $\mathbf{y}$ is a vector of phenotypes, $\mathbf{X}$ is the design matrix for the fixed effects of the mean and the environment effect, $\mathbf{b}$ is a vector of fixed effects, $\mathbf{Z}$ is the design matrix for the random line effect, $\mathbf{u}$ is a vector of line effects, and $\mathbf{e}$ is the residual error assumed independently and identically distributed. The variance of u was var($\mathbf{u}$) = $\mathbf{G} \otimes \mathbf{I}$, where $\mathbf{G}$ is the between-environment genetic variance matrix with the number of rows and columns equal to the number of environments, and $\mathbf{I}$ is an identity matrix with the number of rows and columns equal to the number of lines. For $\mathbf{G}$ we assumed a FA covariance structure of order 1.

The FA model used was:

$$P_{ij} = \mu + \ \text{Genotype}_j + \ \text{Expt}_i + \ \text{Expt(Genotype)}_{ij} + \varepsilon_{ij} \tag{8}$$

where P_{ij} is each root/yield parameter in the ith experiment (Expt) and the jth genotype, μ is the general mean, Genotype_j is the fixed main effect of the jth genotype, Expt_i is the random main effect of the ith Expt, $\text{Expt(Genotype)}_{ij}$ is the random interaction effect of the ith Genotype and the jth Expt, and ε_{ij} is the average error associated with the response of the jth genotype in the ith Expt.

The model fit of FA order 1 and order 2 models were evaluated based on the Akaike information criterion, and the FA order 1 model was selected. The absolute loading value of factor 1 was used as the root plasticity and grain yield stability index. This approach was chosen because FA is a random effects version of AMMI (see the Introduction).

For Approaches I, III, and IV, values close to zero indicated that a genotype showed a high degree of stability, while larger values indicated a greater degree of plasticity. For Approach II, a grain yield stability index (slope) value of 1 indicated an average degree of stability or plasticity across environments, slope >1 indicated a below-average degree of stability and an above-average degree of plasticity, and slope <1 indicated an above-average degree of stability and a below-average degree of plasticity.

We conducted ANOVA separately for each establishment method treatment (DSR and PTR) in R using the *aov* script in *agricolae* (http://tarwi.lamolina.edu.pe/~fmendiburu) with genotype and environment as factor or co-factor in the field trials, in which environment was a co-factor and represented each year, season, nitrogen treatment, and water treatment combination, as follows:

$$P_{ijk} = \ \text{Expt}_i + \ \text{Rep}_{k(i)} + \ \text{Genotype}_j \ + \ \text{Expt} \ \times \ \text{Genotype}_{ij} + \varepsilon_{ij} \tag{9}$$

where P_{ijk} is each parameter measured in the ith Expt and jth genotype, Expt_i is the random main effect of the ith experiment, $\text{Rep}_{k(i)}$ represents each replicate in each experiment, Genotype_j is the fixed main effect of the jth Genotype, Expt × Genotype_{ij} is the interaction effect of the ith Expt and the jth Genotype, and ε_{ij} is the average error associated with the response of the jth genotype in the ith Expt.

Results

By growing the same set of genotypes across a range of repeated establishment, water, and nitrogen treatments, we explored the genetic variation in grain yield and root growth, the environments in which root architectural plasticity was related to grain yield stability, and the physiological characteristics of genotypes with high yield stability and a relatively high degree of root architectural plasticity.

Factors affecting grain yield and root traits of genotypes across environments

In order to compare genotypic differences in grain yield stability with root architectural plasticity across the different treatments, we focused on grain yield, biomass, root-length density at soil depths of 15–30 cm ($RLD_{15–30}$) and 30–45 cm ($RLD_{30–45}$), and NUE with respect to grain yield and biomass as key traits, all of which varied greatly across the different environments (Supplementary

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Fig. S3). Grain yield was generally lower in the DSR experiments (0.01–181.50 g m^{-2} under drought and 139.10–262.80 g m^{-2} under well-watered conditions) compared to the PTR experiments (26.81–213.10 g m^{-2} under drought and 372.65–488.69 g m^{-2} under well-watered conditions; Supplementary Table S3). Compared with well-watered conditions, drought stress resulted in a mean decline in biomass across genotypes of 31% and 41% in the DSR and PTR experiments, respectively. $RLD_{15–30}$ and $RLD_{30–45}$ showed no consistent variation between drought stress and well-watered conditions in the DSR experiments, but it tended to increase under drought stress in the PTR experiments (Supplementary Table S3).

Genotypes IR 95783-6-2-2-3, UPLRi7, IR 97041-5-1-1-2, IR 115844-B-332, and IR 115845-B-154 showed the highest grain yield across the DSR experiments, and IR 98976-20-1-2-1, IR 95783-6-2-2-3, IR 115844-B-332, and IR 97041-5-1-1-2 showed the highest grain yield across the PTR experiments, but these genotypes did not show the same trends in biomass and root-related traits (Supplementary Table S4).

Environment (defined as year, season, water, and nitrogen treatments) had significant effects on grain yield, biomass, and root-length density at both depths, while genotype had significant effects on grain yield, biomass, and $RLD_{15–30}$ (Table 2). A significant interaction between genotype and environment (G×E) was observed for grain yield, biomass, RLD at both depths in the PTR experiments, but only for grain yield in the DSR experiments. However, the sum-of-squares values for G×E were consistently greater than those for G when all trials were separated by establishment method (Table 2). Significant effects of nitrogen (N), water treatment (W), genotype (G), and an interaction between nitrogen and water were observed for grain yield, biomass, and root traits in both the DSR and PTR experiments in 2017 dry season, but the G×N interaction was not significant for any trait in either DSR or PTR, and G×W was only significant for grain yield in PTR (Supplementary Table 2), and the sum-of-squares values for G×N and G×W were generally smaller than those for G.

In the lysimeter study, water treatment significantly affected biomass and root length below 60 cm in both the DSR and PTR treatments, and root dry weight between 40–60 cm depth ($RDW_{40–60}$) in DSR and between 20–60 cm ($RDW_{20–40}$ and $RDW_{40–60}$) in the PTR treatment (Supplementary Table S5). Effects of genotype on biomass and $RDW_{20–40}$ and $RDW_{40–60}$ in the DSR treatment and on $RDW_{0–20}$ in the PTR treatment were also observed, and there were no G×W interactions for biomass or root-related traits.

Grain yield stability, root plasticity, and their relationships

In the PTR experiments, rice grain yield stability showed positive linear relationships with mean grain yield when calculated using plasticity index (Approach I) and slope (Approach II), but not when it was calculated using AMMI (Approach

Table 2. Sum of squares as determined by ANOVA for grain yield, biomass, and root-length density (RLD) at depths of 15–30 and 30-45 cm in direct-seeded rice (DSR) and puddled transplanted rice (PTR) grown in the field.

Establishment method and season[a]	Source of variation	Grain yield	Biomass	$RLD_{15–30}$	$RLD_{30–45}$
DSR[b]	Genotype (G)	142 200***	633 928**	4.325**	0.546^{NS}
	Environment (E)	3 792 702***	9 733 992***	12.568***	3.785***
	G×E	292 692**	$1\ 895\ 521^{NS}$	15.595^{NS}	2.926^{NS}
PTR[c]	Genotype (G)	572 572***	592 177***	8.05*	1.094^{NS}
	Environment (E)	9 986 244***	22 430 010***	70.2***	16.56***
	G×E	582 199***	1 585 868***	35.37*	7.866*
2017DS_DSR	Genotype (G)	$52\ 110^{NS}$	327 446*	6.073**	0.3298^{NS}
	Nitrogen (N)	9665*	768 039***	0.392^{NS}	0.7558***
	Water (W)	1 188 090***	3 356 946***	2.947***	0.7056***
	G×N	$20\ 716^{NS}$	$187\ 438^{NS}$	1.767^{NS}	0.166^{NS}
	G×W	$52\ 093^{NS}$	$217\ 236^{NS}$	1.485^{NS}	0.3154^{NS}
	N×W	9352*	171 420***	4.491***	0.0005^{NS}
	N×W×G	$20\ 767^{NS}$	$154\ 097^{NS}$	2.866^{NS}	0.2387^{NS}
2017DS_PTR	Genotype (G)	404 208***	39 5117***	2.177^{NS}	0.562^{NS}
	Nitrogen (N)	161 296***	1 925 387***	1.471**	0^{NS}
	Water (W)	4 404 021***	9 406 956***	13.288***	0.119^{NS}
	G×N	$20\ 012^{NS}$	$46\ 079^{NS}$	2.187^{NS}	0.282^{NS}
	G×W	116 964***	$152\ 182^{NS}$	0.779^{NS}	0.514^{NS}
	N×W	182 402***	164 241***	0.002^{NS}	2.115***
	N×W×G	$21\ 709^{NS}$	$112\ 035^{NS}$	1.268^{NS}	0.394^{NS}

[a]DS, dry season.
[b]Includes all the DSR field trials.
[c]Includes all the PTR field trials.
*P<0.05; **P<0.01; ***P<0.001; NS, not significant.

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III) and factor analytic (FA, Approach IV; Fig. 1). The plasticity of $RLD_{15–30}$ under PTR showed positive linear relationships with mean $RLD_{15–30}$ when calculated using the slope and FA approaches, and the plasticity of $RLD_{30–45}$ under PTR showed positive linear relationships with mean $RLD_{30–45}$ when calculated using the slope approach (Fig. 2).

To better understand the relationships between grain yield stability and root architectural plasticity across treatments (establishment method, water, and nitrogen), as well as how our statistical approaches affected the interpretation of those relationships, we explored correlations between grain yield stability and plasticity of the four root traits. For each correlation analysis, we compared grain yield stability and root plasticity values that were both determined using the same approach. In general, this resulted in very few significant linear correlations being found, including when all trials were grouped together and when the PTR and DSR trials were separated (Supplementary Table S6). Significant linear relationships between grain yield stability and root plasticity for RLD at both depths were only observed when using plasticity index, and this was when the PTR experiments were considered separately from the DSR trials (Fig. 3). The direction of the relationship in the PTR experiments using the plasticity index approach indicated that genotypes with greater yield stability corresponded to those with a greater degree of root architectural plasticity. There were no tight relationships between grain yield stability and root plasticity across the DSR experiments, or using the slope, AMMI, and FA approaches either with or without separating the DSR and PTR experiments (Supplementary Table S6).

We were also interested to check whether the relationship we previously observed between yield stability and root architectural plasticity in an Aus 276 × MTU1010 population (Sandhu *et al.*, 2016), in which AMMI was used for the former

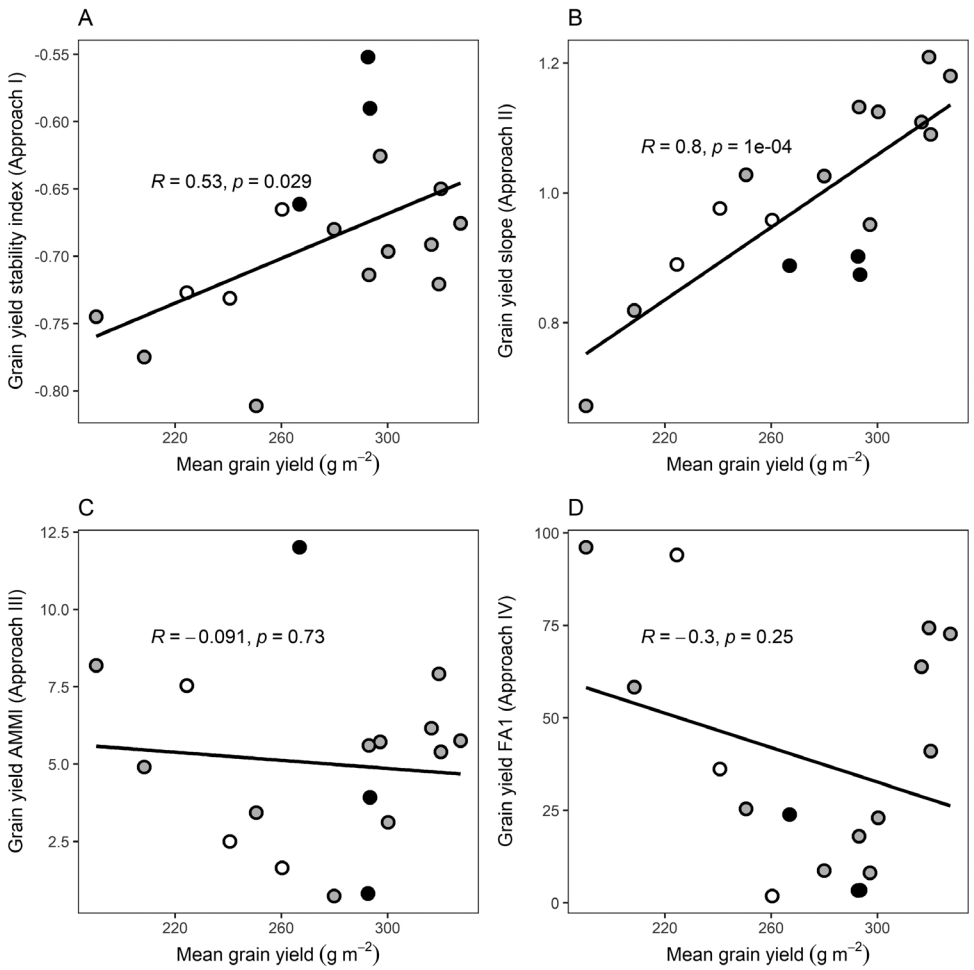

Fig. 1. Linear regressions between grain yield and grain yield stability across all the puddled transplanted rice field trials based on four different statistical approaches. (A) Plasticity index, (B) slope, (C) additive main effects and multiplicative interaction (AMMI; distance from the origin of PC1 versus PC2), and (D) factor analytic (FA; absolute loading values of factor 1). For each approach, a grain yield stability index closer to zero indicates a genotype with more stable yield. Open circles represent genotypes with stable grain yield but less-plastic root growth, and black circles represent genotypes with stable grain yield and more-plastic root growth (see Supplementary Table S8). The other genotypes are represented by grey-filled circles.

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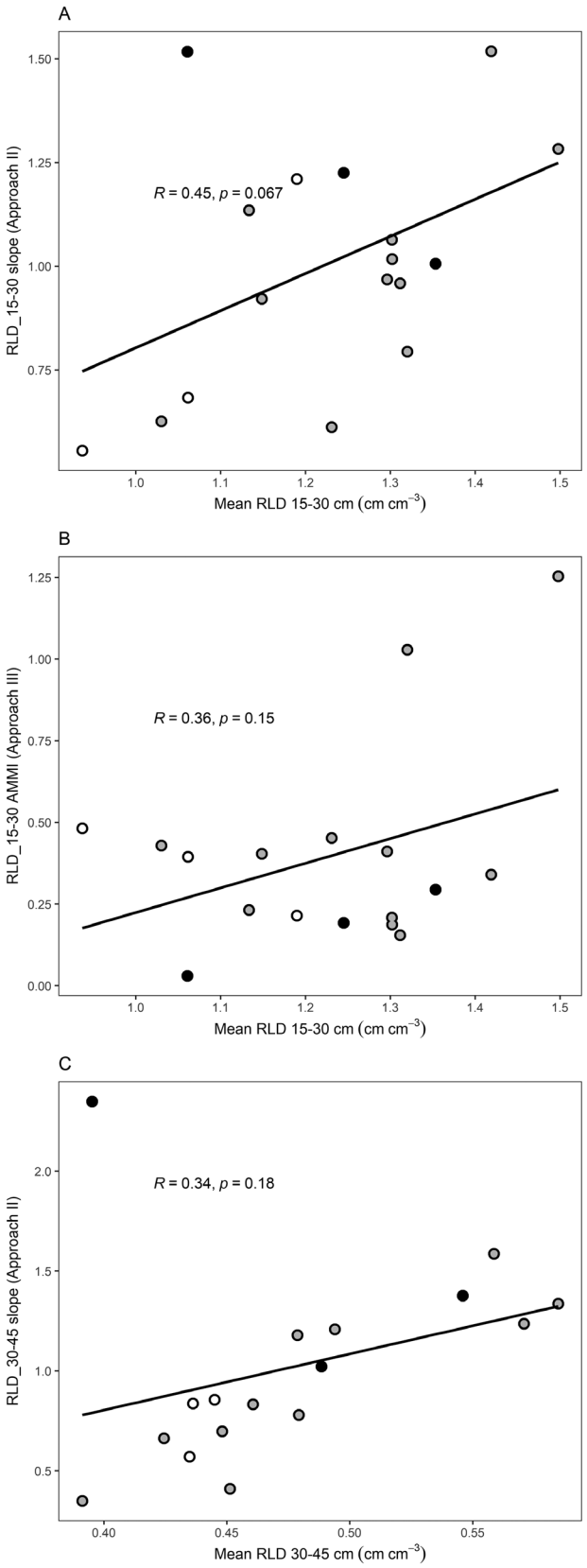

Fig. 2. Significant correlations between root-length density (RLD) and root architectural plasticity across all puddled transplanted rice field trials using different statistical approaches. (A) At a soil depth of 15–30 cm using the slope approach, (B) at a soil depth of 15–30 cm using an additive main effects and multiplicative interaction (AMMI) approach (distance from the origin of PC1 versus PC2), and (C) at a soil depth of 30–45 cm using the slope approach. Open circles represent genotypes with stable grain yield but less-plastic root growth, and black circles represent genotypes with stable grain yield and more-plastic root growth (see Supplementary Table S8). The other genotypes are represented by grey-filled circles.

and plasticity index was used for the latter, could be observed in the genotypes grown in the present study. Similar to the previous results, the best relationship observed with yield stability in the current set of genotypes was with root plasticity values from our lysimeter study at shallow depths (0-20 cm; Supplementary Fig. S4; Supplementary Table S7); however, we did not observe significant correlations between grain yield stability obtained with AMMI and plasticity index using data from the field.

In contrast to grain yield, no relationships between biomass and biomass stability index were observed using any of the four approaches (Supplementary Fig. S5). However, there were significant linear correlations between biomass stability and the plasticity of $RLD_{30–45}$ in the PTR experiments based on plasticity index and slope (Supplementary Table S6). Biomass stability calculated using the plasticity index was well-correlated with NUE, both in terms of NUE_g (grain yield/N uptake) and NUE_b (biomass/N uptake), but the relationships were opposite in the PTR and DSR experiments: genotypes with more stable biomass showed higher NUE in the PTR trials, and lower NUE in DSR trials (Supplementary Fig. S6). Biomass stability was not correlated with N concentration. Using the plasticity index approach, NUE_g was significantly affected by root plasticity at 15–30 cm depth across all experiments and NUE_b was affected under DSR (NUE_b), whilst NUE_b was significantly affected by root plasticity at 30–45 cm depth in the PTR experiments using the slope approach.

Comparisons of stable-yielding genotypes with contrasting degrees of root plasticity

We next identified the eight genotypes within each approach that had the highest degree of grain yield stability (Supplementary Table S8) and separated them into two groups based on their root plasticity response. Genotypes IR 115845-B-154, IR 115845-B-388, and UPLRi7 were selected as being commonly identified according to at least two approaches and showed relatively high yield stability as well as more plastic root growth for multiple root traits, whilst genotypes IR 94226-B-364, IR 94226-B-419, and IR64 showed higher and more stable yield but less plastic root growth.

In terms of the agronomic traits measured at harvest, the selected yield-stable/root-plastic genotypes (IR 115845-B-154, IR 115845-B-388, and UPLRi7) tended to stand out from the yield-stable/less-root-plastic genotypes (IR 94226-B-364, IR 94226-B-419, and IR64) across most environments. The yield-stable/root-plastic genotypes showed higher grain yield (>1.27-fold) than the genotypes with stable yield but

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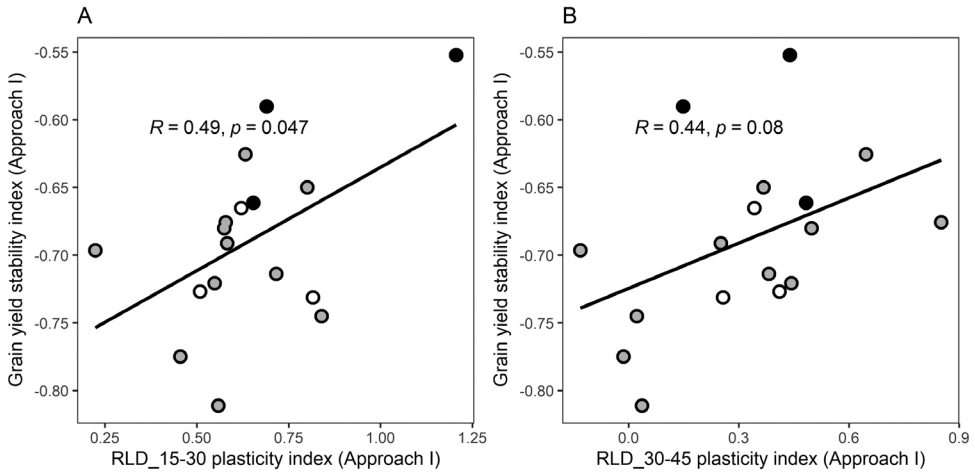

Fig. 3. Grain yield stability and root plasticity of each genotype based on the plasticity index approach across all puddled transplanted rice field trials. Grain yield stability values closer to zero indicate more stable yield, and higher root plasticity values indicate increased root growth in response to stress. Open circles represent genotypes with stable grain yield but less-plastic root growth, and black circles represent genotypes with stable grain yield and more-plastic root growth (see Supplementary Table S8). The other genotypes are represented by grey-filled circles.

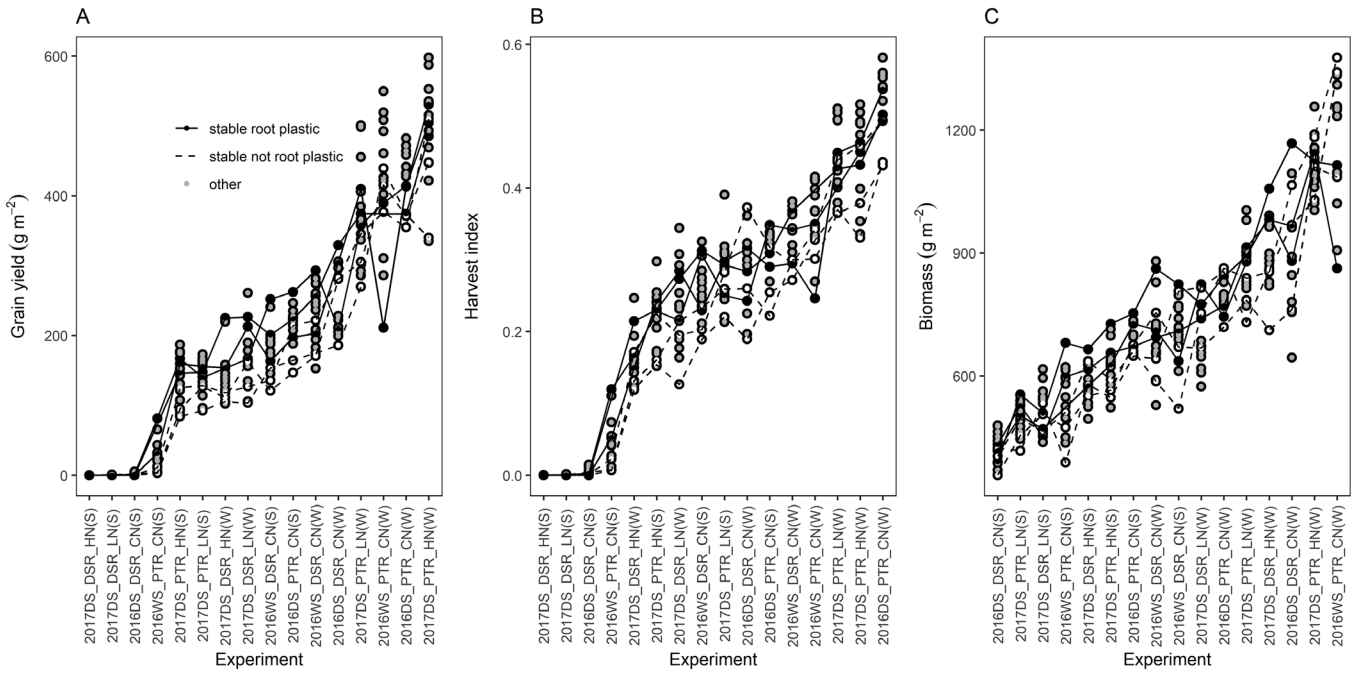

Fig. 4. Comparison of agronomic traits between rice genotypes with stable yield and contrasting root plasticity. (A) Grain yield, (B) harvest index, and (C) biomass of each genotype across 16 different environments in terms of establishment method, water availability, and nitrogen supply (see Table 1). The genotypes with stable yield and more-plastic root growth are IR 115845-B-154, IR 115845-B-388, and UPLRi7, while the genotypes with stable yield but less-plastic root growth are IR 94226-B-364, IR 94226-B-419, and IR64. Comparisons between the genotypes are shown in Supplementary Table S4.

less-plastic root growth across most environments, and this trend was most apparent in trials with yields up to 300 g m^{-2} (Fig. 4A). Higher harvest index (>1.10-fold; Fig. 4B) and biomass at harvest (except in the two severely stressed trials under DSR; Fig. 4C) were also observed for the yield-stable/root-plastic genotypes. Among the physiological traits measured, the yield-stable/root-plastic genotypes stood out in certain treatments/trials only: compared to the yield-stable/less-root-plastic genotypes they showed consistently higher NUE_g (Fig. 5A) and lower leaf osmotic potential (Fig. 5B, Supplementary Table S9) under DSR but not under PTR (Supplementary Fig. S7A, B). The yield stable/root-plastic genotypes did not show improved NUE_b in the field (Supplementary Table S4) or in the greenhouse lysimeter experiment (Supplementary Table S10). In the latter, the genotypes with stable yield but less-plastic root growth showed the lowest water uptake rates

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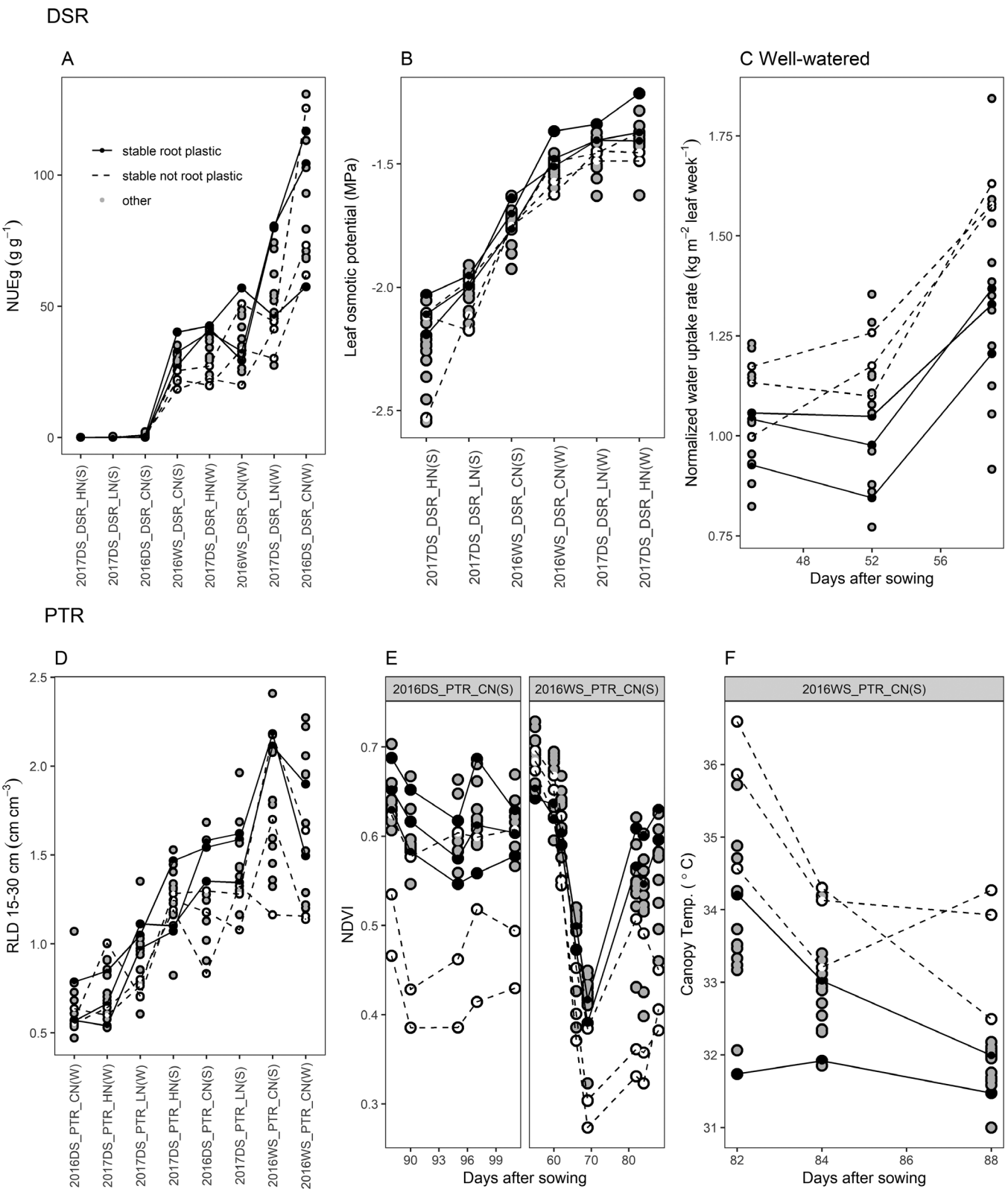

Fig. 5. Comparison of physiological traits between rice genotypes with stable yield and contrasting root plasticity. (A–C) Under direct-seeded rice (DSR). (A) Nitrogen-use efficiency for grain production (NUE_g) and (B) leaf osmotic potential in the dry direct-seeded rice (DSR) field trials across different environments in terms of establishment method, water availability, and nitrogen supply (see Table 1). (C) Normalized water uptake rate in the well-watered treatment in the lysimeter study. (D–F) Under puddled transplanted rice (PTR). (D) Root length density (RLD) at 15–30 cm depth, (E) normalized difference

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in the DSR well-watered treatment (Fig. 5C). In the PTR treatment, the genotypes with stable yield and more-plastic root growth showed consistently higher values for $RLD_{15–30}$ (Fig. 5D) and NDVI (Fig. 5E, Supplementary Table S9), but these differences between the genotype groups were not observed for DSR (Supplementary Figs S7C, S8). The canopy temperatures of genotypes with stable yield and more-plastic root growth were lowest only in the trial that had the lowest average canopy temperatures (experiment 7; Fig. 5E), and were otherwise similar to those of other genotypes (Supplementary Fig. S9, Supplementary Table S9). In the lysimeter study, all the genotypes showed similar values for water uptake and leaf area, except for in the well-watered DSR treatment where water uptake tended to be higher in the genotypes with less-plastic root growth (Supplementary Fig. S10). No differences in chlorophyll concentration index could be distinguished among the groups of genotypes in either the field trials or the lysimeter study (Supplementary Fig. S11), and the same was the case for WUE in the lysimeter study (Supplementary Fig. S12).

Linear regression of the combined data for DSR and PTR indicated the importance of shallow root growth for nitrogen uptake, which had a significantly positive linear relationship with $RLD_{0–15}$ in the well-watered and drought-stress treatments in the field trials, but in the lysimeter study nitrogen uptake was only related to $RDW_{20–40}$ in the drought-stress treatment (Supplementary Fig. S13).

Discussion

Rice cultivation environments even within a single region are becoming increasingly variable due to climate change, and hence an understanding of the physiological mechanisms that confer yield stability could contribute to the development of new varieties that growers can depend on regardless of the climate conditions in a given season. One of the conditions most prone to such variability across seasons is soil moisture, and therefore we focused our current study on assessing root plasticity as a mode of conferring yield stability within a set of rice genotypes that included breeding lines developed to target adaptability to multiple cultivation systems. Given the significant degree of phenotypic variation present among the genotypes in this study, the general lack of correlation between grain yield stability and root architectural plasticity—across various combinations of establishment methods and using four different statistical approaches—was unexpected. We hypothesize that this lack of direct correlation reflects the positive effect that root plasticity had on overall growth of the best-performing genotypes in this study as well as on yield stability. This trend was illustrated by the positive relationship between grain yield and yield stability (Fig. 1), as well as between root-length density (RLD) and RLD plasticity (Fig. 2), and by the fact that the stable-yielding/root-plastic genotypes showed overall higher grain yield and biomass across the experiments (Fig. 4). Hence, further research aimed at developing new indices for root architectural plasticity that incorporate measures of biomass or grain yield could help to clarify the relationships between yield stability and root plasticity and to pinpoint the planting environments, soil depths, and root traits within which those relationships are most apparent.

Across environments, it was clear that root architectural plasticity in the DSR trials was much less prevalent than in the PTR trials, and this corresponded with the only positive relationships between yield stability and root plasticity being observed when the PTR trials were analysed separately from the DSR trials (Fig. 3). The low levels of root plasticity observed in the DSR trials were probably due to the rate of soil drying in the drought treatments, which tended to occur very quickly in the DSR trials compared to the PTR trials (Supplementary Fig. S1). Such relatively rapid soil drying might be faster than the ability of the plant to respond to stress through root elongation and branching, which is typically observed when rice crops experience drought under puddled (lowland) conditions (Kamoshita *et al.*, 2004; Henry *et al.*, 2011). These effects of the soil-drying dynamics on root growth under dry direct-seeding also correspond with the observation that upland-adapted, drought-tolerant varieties tend to exhibit deeper root growth than lowland-adapted varieties regardless of the soil moisture conditions (Chang *et al.*, 1972, Yoshida and Hasegawa, 1982). However, the higher NUE and lower leaf osmotic potential of the selected yield-stable/root-plastic lines under DSR (Fig. 5) suggests that the roots conferred some functional advantages in this treatment in our study.

The ability of rice roots to respond to changes in soil moisture across the PTR trials was also reflected in the increased level of G×E interactions in these trials (Table 2). The lack of a relationship between yield stability and root plasticity in this study was therefore probably not due to a lack of G×E crossover among the genotypes. Furthermore, it was notable that the only relationships observed between yield stability and root plasticity were found by using the plasticity index approach (Fig. 3), rather than multivariate analyses. This may have been due to the relatively narrower scope we used for plasticity index, since we only compared water treatments within each establishment method and nitrogen treatment, in contrast to the other approaches that integrated all the treatments.

Aside from grain yield, the interactions between biomass and NUE showed some informative trends in relation to root plasticity. In the progressive drought conditions imposed in this study, higher N treatments appeared to exacerbate drought

vegetation index (NDVI), and (F) canopy temperature in experiment 7. The genotypes with stable yield and more-plastic root growth are IR 115845-B-154, IR 115845-B-388, and UPLRi7, while the genotypes with stable yield but less-plastic root growth are IR 94226-B-364, IR 94226-B-419, and IR64. Comparisons between the genotypes are shown in Supplementary Tables S4, S9.

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stress through the resulting high biomass, which presumably depleted soil moisture levels in the DSR drought treatments to the detriment of grain yield. These results differ from those of previous nutrient management studies that have reported improved productivity in drought-prone, rainfed rice with fertilizer application (Haefele *et al.*, 2013; Banayo *et al.*, 2018), but this might be due to the types of drought under which the plants were grown: we generally applied fertilizer before initiating severe, progressive drought whereas the previous studies were on-farm trials with fluctuating and less extreme soil moisture levels, and the fertilizer was strategically applied following rainfall. However, root hydraulic and stomatal leaf responses to growth under different N levels might also explain some of the variation observed across different soil moisture levels in our current study, since high N has been reported to act as a signal to increase water uptake and transpiration (as reviewed by Araus *et al.*, 2020) and might also result in depletion of soil moisture.

The effect of soil moisture interacted with the effects of low N on root growth (Table 2, Supplementary Table S3), with drought increasing root growth at depth only under the high-N PTR treatment. This interaction helps explain the opposite relationships that were observed between NUE and biomass stability in PTR versus DSR (Supplementary Fig. S6). Root plasticity was only correlated with NUE in the PTR trials, which in turn showed a positive correlation with biomass stability, whilst in the DSR trials it was low N rather than drought that increased root growth at depth, and that increase in growth in response to low N (and lower NUE) conferred biomass stability. The interactions between N and water have complex effects on plants and have been the focus of recent gene expression studies (e.g. Swift *et al.*, 2019); our current study indicates that the type of drought (i.e. under DSR or PTR conditions) also affects those interactions.

Our study was novel in that it included some of the most recently-developed rice breeding lines targeted for dry direct-seeding conditions (Sandhu *et al.*, 2019; Subedi *et al.*, 2019). It was interesting that the lines IR 94226-B-364 and IR 94226-B-419 that have previously been identified for their high degree of root plasticity were classified as yield-stable but less root-plastic in our study. This change in relative classification probably reflects the ongoing progress of breeding. For example, lines IR 115845-B-154 and IR 115845-B-388 were among the most recently-developed in this study, generated from multi-parent crosses (Supplementary Table S1) and screened under dry direct-seeding. Although these lines were not specifically selected for root plasticity, their diverse parentage and selection for yield under dry direct-seeding appears to have resulted in selection for a high degree of root plasticity. Thus, the older breeding lines appear less plastic in comparison.

In conclusion, the lack of a direct correlation between yield stability and root plasticity that we found highlights the fact that more research is necessary to characterize the environments in which root architectural plasticity is most beneficial, as recently discussed by Schneider and Lynch (2020). Our results showing increased root architectural plasticity in puddled-transplanted environments compared to dry direct-seeding environments further suggest that it might be necessary to more narrowly define the targeted environments in which root architectural plasticity should be selected. However, our observation that the most stable-yielding, root-plastic lines were also higher yielding in a certain set of environments provides strong support for the potential of root plasticity as a target for breeding.

Supplementary data

The following supplementary data are available at *JXB* online.

Table S1. Parentage of the breeding lines used in this study.

Table S2. Soil characteristics for the field trials and lysimeter study.

Table S3. Grain yield, biomass, harvest index, nitrogen-use efficiencies, and root-length densities in each field trial.

Table S4. Grain yield, biomass, harvest index, nitrogen-use efficiencies, and root-length densities of each genotype in the field trials.

Table S5. ANOVA for biomass and root-related traits in the lysimeter study.

Table S6. Correlations between grain yield, biomass, and nitrogen-use efficiencies with root architectural plasticity as determined using the different statistical approaches.

Table S7. Lysimeter root plasticity index based on root dry weight versus grain yield stability as determined by AMMI.

Table S8. Yield stability and root plasticity for the eight genotypes with the most stable grain yield as determined by each statistical approach.

Table S9. Normalized-difference vegetation index, leaf osmotic potential, and canopy temperature of the genotypes with the field environments grouped together.

Table S10. Biomass and biomass nitrogen-use efficiency of each genotype in the lysimeter trial.

Fig. S1. Soil water potential at a depth of 30 cm in the field stress experiments.

Fig. S2. Solar radiation, maximum and minimum temperature, and rainfall data for the field experiments.

Fig. S3. The linear regressions that were used to derive the stability/plasticity index according to the slope approach.

Fig. S4. AMMI grain yield stability (field) versus root plasticity index at the most shallow depth sampled (0–20 cm; lysimeter study).

Fig. S5. Linear regression between biomass and biomass stability index based on the four statistical approaches.

Fig. S6. Correlations of nitrogen-use efficiencies with biomass stability.

Fig. S7. Nitrogen-use efficiency for grain production and leaf osmotic potential under puddled transplanted rice, and root length density at 15–30 cm depth under direct-seeding.

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Fig. S8. Normalized difference vegetation index measured in direct-seeded field trial.

Fig. S9. Average canopy temperature in each field trial.

Fig. S10. Normalized water uptake rates and apparent leaf area in the lysimeter study.

Fig. S11. Chlorophyll concentration index for the field trials and the lysimeter study.

Fig. S12. Water-use efficiency in the lysimeter study.

Fig. S13. Linear regression of nitrogen uptake with root-length density in the field trials and the lysimeter study.

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Author contributions

AH and JER were responsible for conceptualization; XX and MRQ were responsible for data curation; XX, MRQ, JER, and AH were responsible for formal analysis; XX was responsible for investigation; NS, SS, AH, and YZ were responsible for resources; XX and AH were responsible for writing – original draft; XX, JER, and AH were responsible for writing – review and editing.

Data availability

The data that support the findings of this study are openly available in Dataverse at https://dataverse.harvard.edu/dataverse/RiceResearch

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