Original research article

Genotypic variation in rice root system distribution and activity in response to short-term soil drought

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Abstract: In this study, two experiments were conducted to evaluate the genotypic variation of rice root system distribution and root activity in response to short-term drought conditions. Seven rice genotypes were used, of which one (Rexmont) showed the greatest reduction in shoot biomass under drought, and two (Swarna and KDML105) showed the least reduction in shoot biomass under drought in both experiments. In a phytotron experiment (Experiment 1) in which root hydraulic conductivity (Lp_r) of 21-day-old rice plants was evaluated in well-watered (control) and dry down (drought) conditions, the Lp_r of Swarna, KDML105, and IRAT109 were significantly lower under drought compared to the control. In a field experiment (Experiment 2) conducted in the 2013 wet season at IRRI, stomatal conductance, bleeding rate, and root surface area density (RSAD) at 0-15, 15-30, 30-45, and 45-60 cm soil depths were measured in an irrigated (control) and rainfed (drought) treatments. Swarna, KDML105, and FR13A showed significant reductions in RSAD at 0-30 cm depth under drought in the field compared to the control, while Rexmont and IRAT109 showed no significant changes. In addition, Rexmont and Swarna both maintained higher bleeding rates than the other genotypes. Based on the root hydraulic and architectural traits of contrasting genotypes, we conclude that the bleeding rate did not explain the genotypic variations in the maintenance of shoot biomass, and that reducing shallow root growth and Lp_r in response to drought conferred the best ability to maintain shoot biomass under short-term drought conditions.

Keywords: rice (*Oryza sativa* L.), root hydraulic conductivity, drought, rainfed lowland, wet season, pressure chamber

Abbreviations: DAS, days after sowing; L_r, root hydraulic conductance; Lp_r, root hydraulic conductivity; LWP, leaf water potential; NRL, nodal root length; NRN, nodal root number; RSA, root surface area; RSAD, root surface area density; SDW, shoot dry weight; SWP, soil water potential; WS, wet season

Introduction

Rice is an important staple food crop, especially in Asia, but approximately 40% of the world's rice-growing area is rainfed with an unstable water supply (Maclean et al. 2013). The average rice yield in rainfed fields ranges from 1 to 2.3 t ha^{-1}, which is significantly lower than in irrigated rice fields (around 5 t ha^{-1}; Maclean et al. 2013). One of the main reasons for the lower yield in rainfed rice fields is reduced soil moisture. Furthermore, it is also becoming increasingly difficult to secure water for agricultural use in irrigated paddy fields. Therefore, the improvement of drought tolerance is one of the best strategies for increasing rice yield on a global scale. Roots play an important role in water absorption, and water absorption can be improved by improving root system functions.

Since crop growth and yield are closely related to water uptake, improvement of traits related to water uptake can be an effective approach to increase productivity and conserve water under drought

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stress. For example, the discovery of related genes has facilitated the breeding of new genotypes with deep rooting (Uga et al. 2011). The characteristics of root system distribution changes in response to the environment (termed 'plasticity') are important characteristics related to rice growth in rainfed conditions exposed to fluctuating soil moistures (Kano et al. 2011; Niones et al. 2015; Suralta et al. 2015, 2018). On the other hand, root physiological traits, such as root hydraulic conductivity (Lp_r), are also important for drought tolerance (Henry et al. 2012, 2016). Although deep rooting, root plasticity, and root physiological activity are independent traits, overall water uptake may be determined by their interactions.

In this study, we focused on the role of root physiological activity in rice growth under short-term drought stress representing rainfed conditions. Lp_r is thought to be an indicator of passive water uptake by transpiration (Steudle et al. 1987). Henry (2016) reported that at different vapor pressure deficit levels in rice, drought tolerant genotypes exhibited lower Lp_r under drought, suggesting that these root properties may be effective in saving water under drought conditions, as in other crop species (Richards and Passioura 1989, Zaman-Allah et al. 2011, Schoppach et al. 2013). Although it is difficult to evaluate Lp_r in the field, measurement of Lp_r in soil-grown rice plants has been done in pots using pressure chambers (Matsuo et al. 2009, Henry et al. 2012 and 2016, Grondin et al. 2016, Henry et al. 2019, Watanabe et al. 2020). Another measure of root activity is bleeding rate (the amount of sap exuded from the root system through cut stems), for which a simple field measurement method has been established (Morita and Abe 2002). Bleeding rate is an indicator of active water absorption based on root pressure, and since active water absorption is a metabolic process using energy, bleeding rate likely reflects root activity (Morita and Abe 1999). Bleeding rate has been used as an indicator of root activity to assess water absorption in multiple crops (Niu et al. 2020, Kumar et al. 2021, Zhang et al. 2022), especially during periods of no or low transpiration, such as at night or on cloudy days (Morita and Abe 2002). In rice, a significant negative correlation between the reduction rate of bleeding rate and the percentage of ripened grains was observed (Kusutani et al. 2000). Morita and Abe (2002) reported that the bleeding rate in rice decreased rapidly after heading, because no more new roots emerged while the senescence of the existing roots commenced at that time point. Ansari et al. (2004) reported that the stem bleeding rate during panicle formation stage was significantly related to the sink size due to its significant association with the number of spikelets in rice plant. Therefore, both Lp_r and bleeding rate may be useful indicators of plant function during drought stress.

Few studies have simultaneously evaluated and discussed the physiological activity of root systems and the distribution of root systems under drought conditions in the field (Matsuo and Mochizuki 2009, Matsuo et al. 2009, Henry et al. 2012, Grondin et al. 2016, Henry et al. 2016). In addition, studies on drought tolerance are often conducted during the dry season, when drought is more severe. It has been reported that the degree of plasticity of root system development is affected by the soil drying intensity, and phenotyping of root system development under relatively mild drying conditions such as wet season or short-term dry-down conditions is also important (Pantuwan et al. 2002, Kano et al. 2011, Kano-Nakata et al. 2011, Tran et al. 2014, Kameoka et al. 2016). The objective of this study was therefore to evaluate genotypic differences in root system distribution and root system activity, which may play an important role in rice water uptake under the short-term drought conditions characteristic of rainfed rice fields.

Materials and Methods

Plant materials

Seven rice genotypes were used in this study representing a range of previously observed responses to rainfed conditions: Nipponbare, IRAT109, Rexmont, LTH, FR13A, KDML105, and Swarna. KDML105 is one of the most widely-grown jasmine rice varieties in the rainfed lowland systems of Thailand (Jongdee et al. 2006). Although KDML105 lacks high yield potential, it is considered moderately drought tolerant (Jearakongman et al. 1995) and can show greater ability for root plasticity under drought or fluctuating soil moisture conditions (Bañoc et al. 2000a, 2020b, Kameoka et al. 2015, 2016). Rexmont is a long grain rice cultivar grown in the southern U.S (Bollich et al., 1990, Worthington and Horton 2013) with less root phenotypic plasticity under drought conditions (Kameoka et al. 2015, 2016). Swarna is widely consumed, especially in India, and is often reported to be sensitive to reproductive stage drought (Venuprasad et al. 2009). In a previous study, IRAT109 and Rexmont exhibited plasticity in deep root development and KDML105 in shallow root development under soil conditions where the soil surface layer was dry and the deep soil layer was wet (Kameoka et al. 2015). LTH and FR13A exhibited plasticity in root system development in response to mild soil drought stress in a simulated 20 cm deep paddy field assuming the presence of a hardpan layer in previous trials (Kameoka et al. 2016). Nipponbare

was used as a standard genotype in the evaluation of root system development (International Rice Genome Sequencing Project and Sasaki 2005).

Phytotron experiment (Experiment 1)

A phytotron experiment was conducted in 2012 at the International Rice Research Institute (IRRI) in Los Baños, Laguna, Philippines. The seven rice genotypes were planted in Experiment 1, although LTH was not evaluated due to stunting and failure to measure Lp_r. The light intensity and temperature in the phytotron mimicked the outside environment. The temperature was set at 29°C and 21°C from 6:30 to 18:30 and from 18:30 to 6:30, respectively, and within the phytotron, we used a combination of three 1000 W light sources turned on and off to simulate changes in the light environment under field conditions: from 6:30 to 8:00, one light source was turned on; from 8:00 to 11:00, two light sources were turned on; from 11:00 to 15:00, all three light sources were turned on; from 15:00 to 17:00, two light sources were turned on; from 17:00 to 18:30, one light source was on; and from 18:30 to 6:30 the next morning, all light sources were off, and the relative humidity setting was maintained at 70%. A second phytotron (phytotron 2) was also set up with the above settings (phytotron 1) staggered by 5 hours to maximize the number of Lp_r measurements that could be conducted within a day while eliminating the influence of diurnal variation.

Soil (pH 5.76, 0.26% N (Kjeldahl method), 33.6 mg kg^{-1} P (Olsen method), 1.4 meq 100 g^{-1} exchangeable K) collected from IRRI test plots was placed in 5 cm diameter, 50 cm high mylar resin cylindrical tubes with a bulk density of 1.1 g cm^{-3}. The soil was filled to a height of 40 cm from the bottom and no fertilizer was applied. Plants were grown in both well-watered (control) and dry-down (drought) conditions for 21 days (September-October 2012) with four replicates per genotype in both treatments. After seeding (3 seeds per cylinder), field capacity levels were maintained and then the seedlings were thinned to one plant per cylinder 10 days after seeding. After thinning, the control treatment was maintained at field capacity, while the watering was stopped in the drought treatment.

At 11 days after the start of drought treatment, shoots were cut at the base and oven-dried at 70°C for 3 days before recording the SDW. Immediately after shoot sampling, leaf water potential (LWP) was measured on one leaf per plant in a pressure chamber (3000HGBL Plant Water Status Console, Soilmoisture Equipment Corp. CA, USA) using compressed N_2. After sampling, the soil-filled cylinders with each plant were immersed in water for approximately 40 minutes. Subsequently, small tillers other than the main stem were cut near the base, while the main stem was cut at a height of approximately 5 cm above the soil surface. LWP measurements were started immediately after cutting the main stem.

A 1.6 l pressure chamber (3000HGBL Plant Water Status Console, Soil Moisture Equipment Corp., CA, USA) was used to evaluate root hydraulic conductance (L_r) in the root system and operating procedures followed those of Henry et al. (2012). The cut-off base of the main stem was sealed and pressurized at 0.2 MPa, 0.35 MPa, and 0.50 MPa to the root system and water-containing soil, and the sap output from the main stem was collected for 10 min at each pressure. The slope of the equation relating volume collected per unit time to pressure was calculated as the L_r (m^3 s^{-1} MPa^{-1}). Maximum root depth was recorded by unrolling the tube to expose the inner soil and measuring the depth at which the deepest root was located. Roots were carefully washed out with water. Nodal roots were cut at the base and then the number of nodal roots was measured manually. Root surface area (RSA) was calculated using WinRhizo v. 2005 (Regent Instruments, Quebec, Canada). L_r was divided by the RSA to calculate Lp_r (m^3 m^{-2} s^{-1} MPa^{-1}). A diameter of 0.2 mm was specified as the threshold for distinguishing nodal and lateral roots (Henry et al. 2011; Kato and Okami 2011).

Field Experiment (Experiment 2)

The field study was conducted during the 2013 wet season ("2013 WS"; June-November 2013) at the IRRI experimental farm. The study included an irrigated control and rainfed (drought) treatments.

The seedlings of all seven rice genotypes were transplanted to the experimental plots three weeks after sowing, and the soil was kept saturated but not flooded for two weeks after transplanting to prevent pest infestation. At 2 weeks after transplanting, both treatments were irrigated to a standing water level of approximately 10 cm. This water level was maintained by irrigation until one week prior to harvesting in the irrigated control fields, while the drought treatment fields were drained at 4 weeks after transplanting and were not irrigated thereafter. Tensiometers were placed at a depth of 30 cm soil depth at three locations within the drought fields as soon as the soil had consolidated after draining.

All plots were hand-weeded two to three times during each season. Basal fertilizer was incorporated into the soil at a rate of 40-40-40 kg N-P_2O_5-K_2O ha^{-1} before transplanting. Topdressing of $(NH_4)_2SO_4$ at a rate of 40 kg N ha^{-1} was conducted at about 3

weeks after transplanting. Mollusk pests were controlled during each season with niclosamide (0.25 L ha^{-1}) and saponin (20 kg ha^{-1}).

Bleeding rate measurements were carried out 5 times for each genotype, according to the protocol described by Morita and Abe (2002) and Henry et al. (2012). Shoots were cut at a height of around 15 cm from the soil surface, and the sap coming from the root zone was collected by covering the cut stems with a cotton towel inside a polyethylene bag sealed at the base with a rubber band. After 4 h, the previously weighed towel, polyethylene bag, and rubber band were collected and immediately weighed again to quantify the exuded sap from the intact root system. Shoots were sampled after each sap collection, oven-dried, and weighed to determine shoot biomass. The exuded sap was calculated as grams of sap exuded per hour, and values were normalized by shoot mass of the plant from which sap was collected.

To characterize root distribution, soil cores were taken at depths of 0-15 cm, 15-30 cm, 30-45 cm, and 45-60 cm using a 4 cm diameter core sampler with two sub replicate core samples taken per plot. The core sampler was fabricated at the International Rice Research Institute (Los Baños, Philippines). Roots were washed out of the soil immediately after sampling or stored at −4°C until washed out (within 3 weeks). All samples were stored in 50% ethanol until scanning. Root samples were scanned at 400 dpi (Epson V700, California, USA). Scanned images were measured for root surface area using WinRhizo v. 2007d (Régent Instruments, Québec, Canada).

At maturity, a 0.5 m^2 area from each plot was harvested to determine straw biomass and grain yield (normalized to a 14% moisture content). Nipponbare was not included in the yield evaluation because it was not harvested due to poor growth.

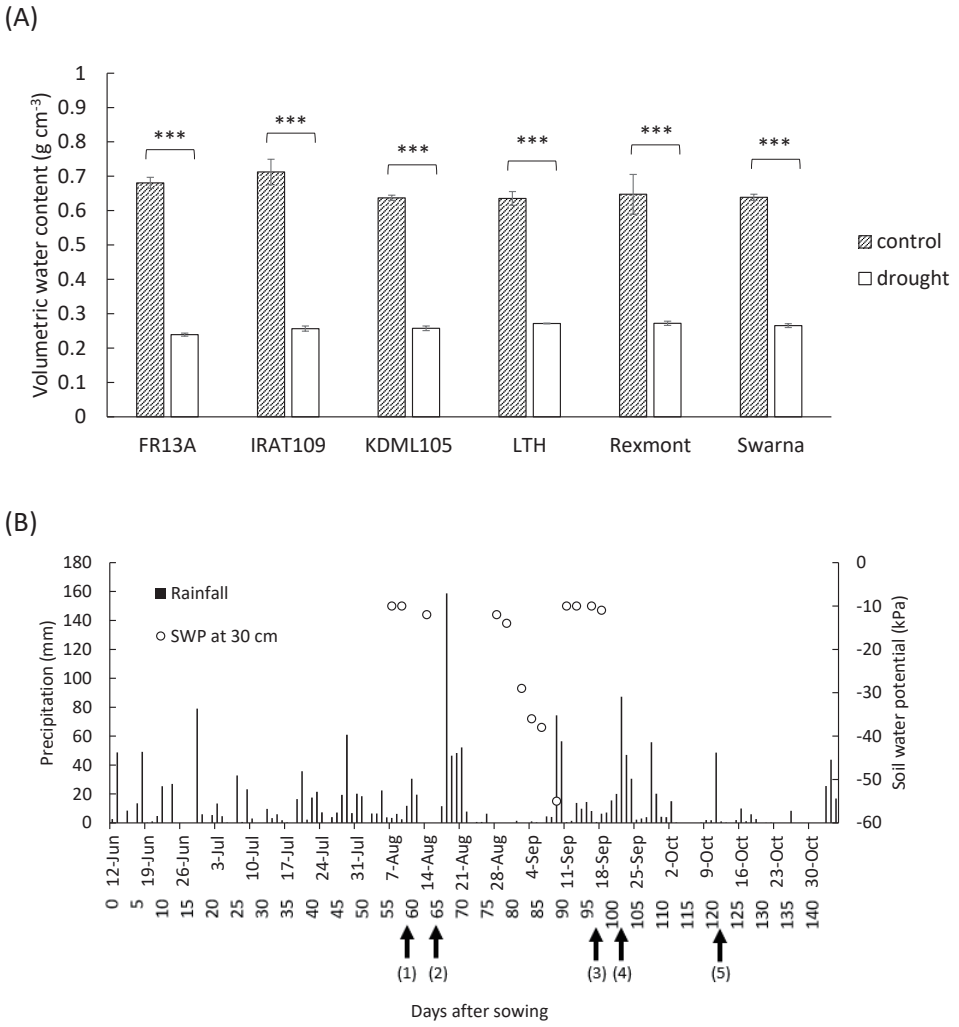

Fig. 1. Soil moisture conditions in (A) after the dry-down in Experiment 1 (phytotron experiment), and (B) Experiment 2 (field experiment). Arrows indicate the flowering date of each genotype: LTH (1), IRAT109 and Rexmont (2), KDML105 (3), Swarna (4), FR13A (5). Bars indicate the standard error. Significance levels among treatments are ***$P < 0.001$.

Data analysis

SDW reduction was calculated as (SDW control – SDW drought)/SDW control x 100. This was calculated using the SDW data on the final bleeding rate measurement (90 days after sowing (DAS); the timepoint with the most negative soil water potential (SWP) in Experiment 2), and at the time of Lp_r measurement in Experiment 2. Genotype and treatment effects for all traits measured were analyzed in R v. 4.1.3 (R Development Core Team, 2008) using analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) for mean comparison. Correlation analysis was performed by determining Pearson's correlation coefficient, using Microsoft Office Excel, 2019.

Results

Environmental conditions

In Experiment 1, the soil in the cylinders dried down to an average of 0.26 g water cm^{-3} soil over the 11-day drought treatment period (Fig. 1A), at which time the SDW, LWP, Lp_r, and RSA were measured. Precipitation in Experiment 2 was constantly at 0 mm per day in late August, which led to a continuous decline in SWP at a depth of 30 cm beginning on August 30, reaching around -60 kPa on September 9 (Fig. 1B). LTH, IRAT109, and Rexmont flowered before the start of the decline in SWP, while KDML105, Swarna, and FR13A flowered after the declining period (Fig. 1B). Except for FR13A, which flowered extremely late, each genotype was harvested between September 28 and October 4, 2013.

Shoot dry weight reduction by drought stress

In both experiments, SDW was not significantly reduced by the drought treatment (Fig. 2), indicating a relatively mild degree of drought stress. However, genotypes varied in SDW reduction caused by drought, which was significant in Experiment 1 (Fig. 2A). In both experiments, Swarna and KDML105 had a relatively low reduction in SDW while Rexmont had a relatively high reduction in SDW. Interestingly, many genotypes showed an increase in SDW under drought relative to the control treatment (all except Rexmont and IRAT 109 in Exp. 1 and IRAT 109 in Exp. 2), as indicated by negative average values for % SDW reduction (Fig. 2). Some genotypes showed no reduction in SDW

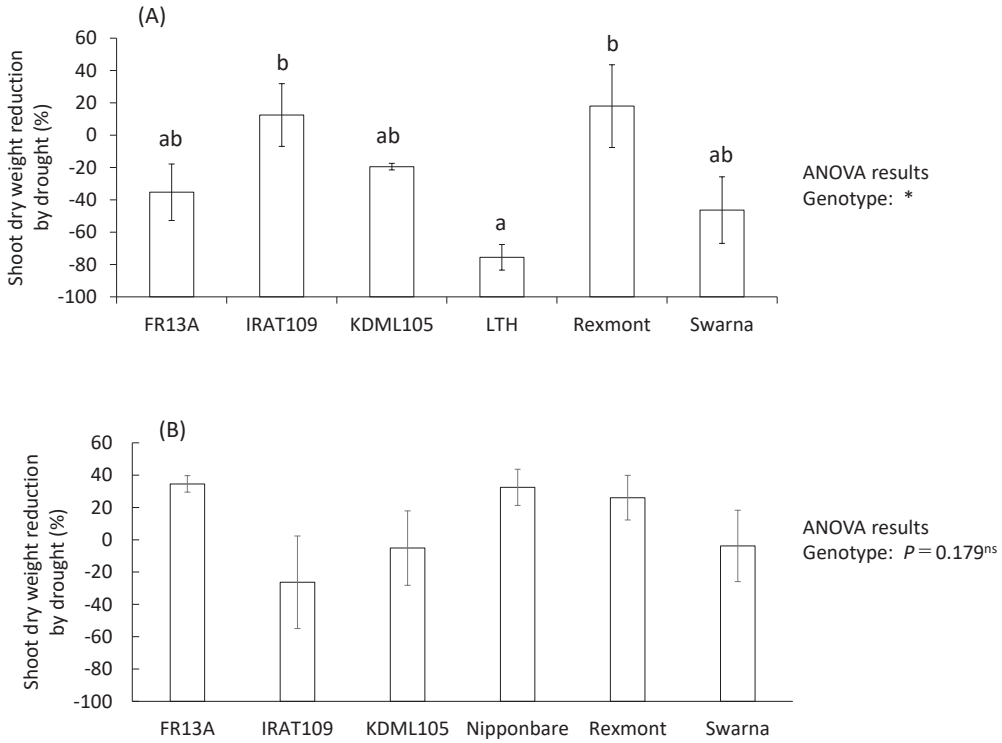

Fig. 2. Shoot dry weight (SDW) reduction in the drought treatment as compared to the control treatment (A) at the end of Experiment 1 (21 days after sowing, phytotron experiment), and (B) at the final bleeding rate measurement date (89 days after sowing) in Experiment 2 (field experiment). SDW reduction was calculated as (SDW control – SDW drought) / SDW control x 100. Bars indicate the standard error. Significance level among treatments is $*P < 0.05$. Letter groups indicate significant differences ($P < 0.05$).

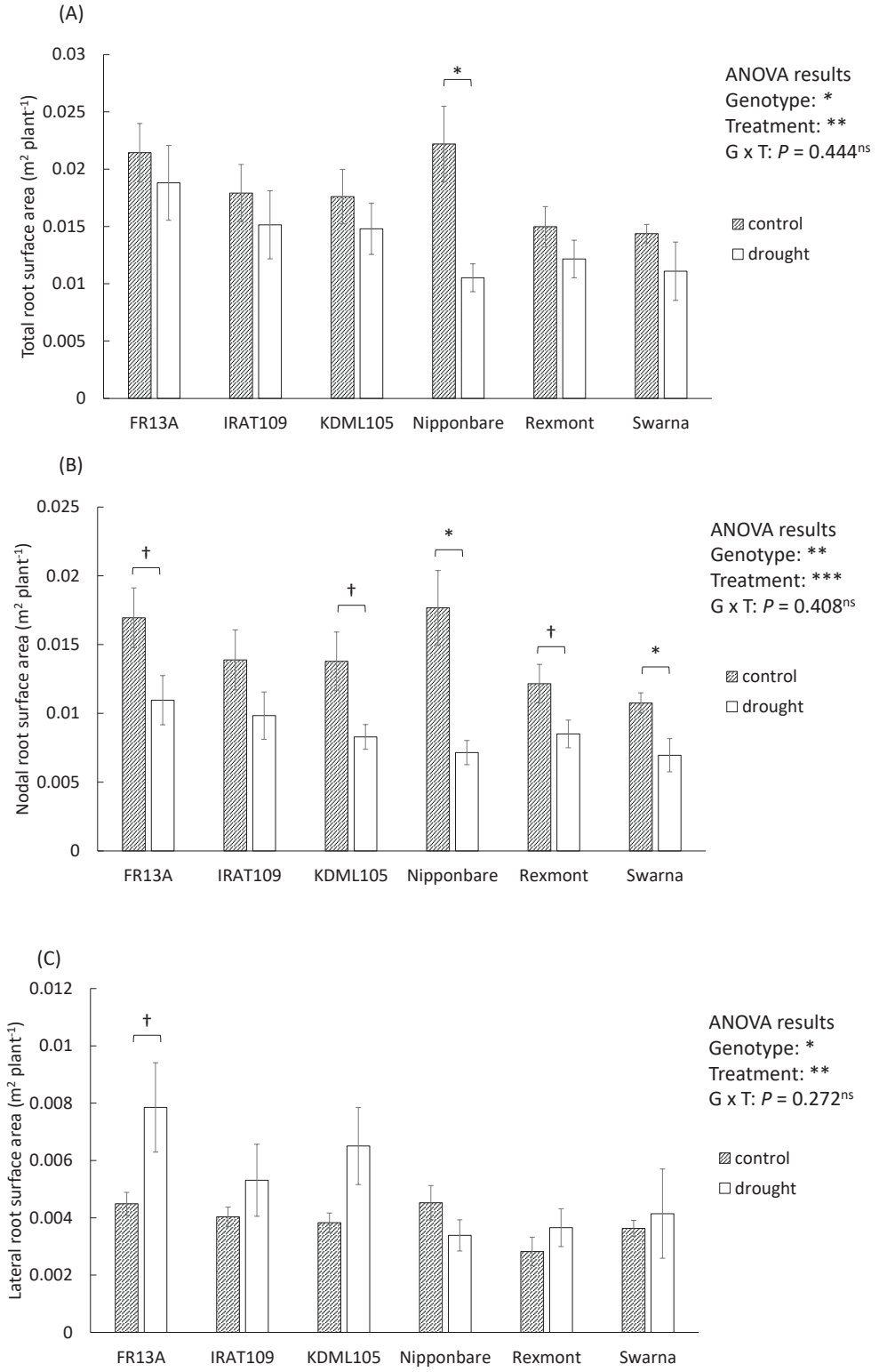

Fig. 3. Total root surface area (A), nodal root surface area (B), and lateral root surface area (C) from Experiment 1 (phytotron experiment). Bars indicate the standard error. Significance levels among treatments are $*P < 0.05$ and $\dagger P < 0.10$.

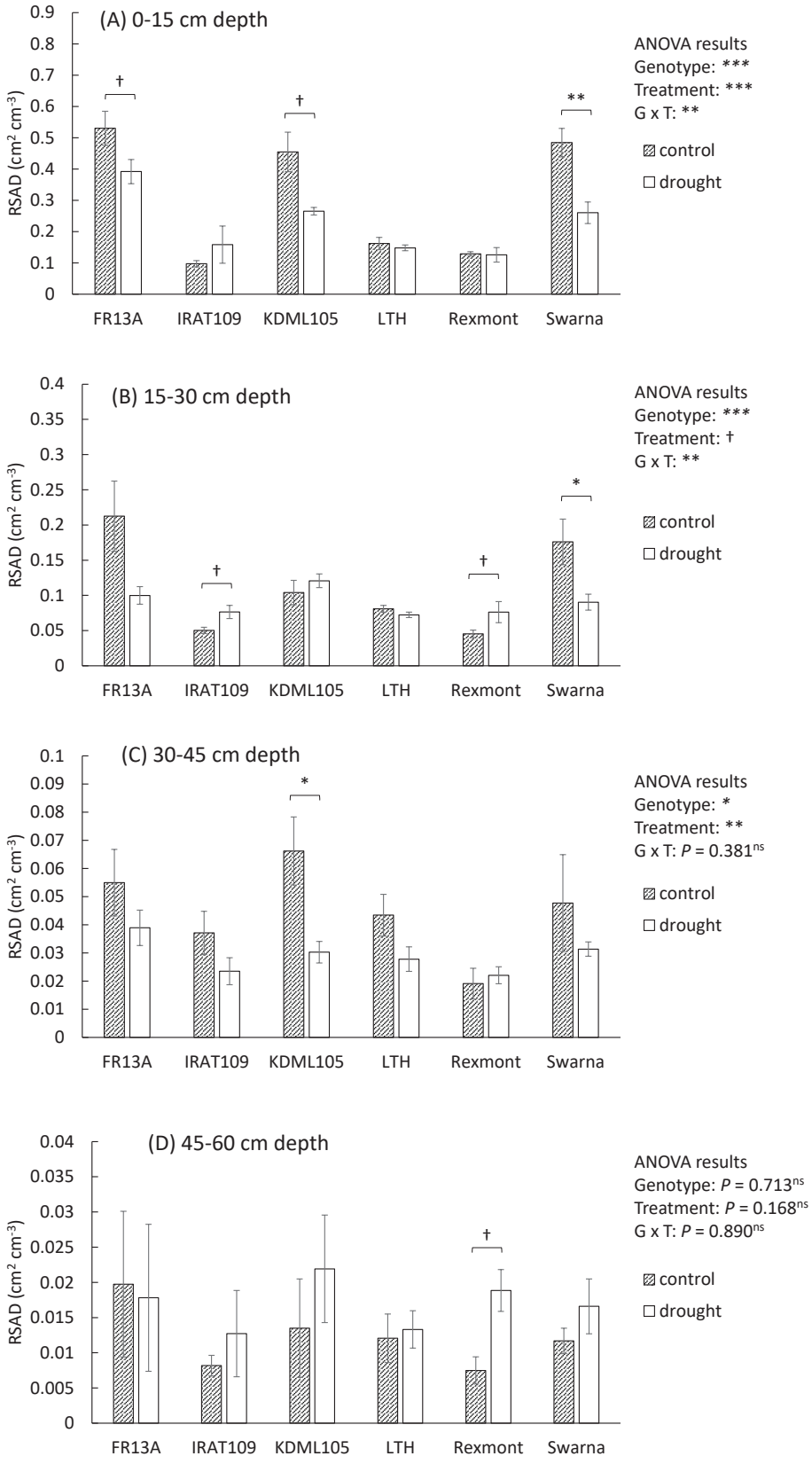

Fig. 4. Root surface area density (RSAD) in Experiment 2 (field experiment). Soil depth intervals were 0-15 (A), 15-30 (B), 30-45 (C), and 45-60 cm (D). Bars indicate the standard error. Significance levels among treatments are $^{**}P < 0.01$, $^{*}P < 0.05$ and $^{\dagger}P < 0.10$.

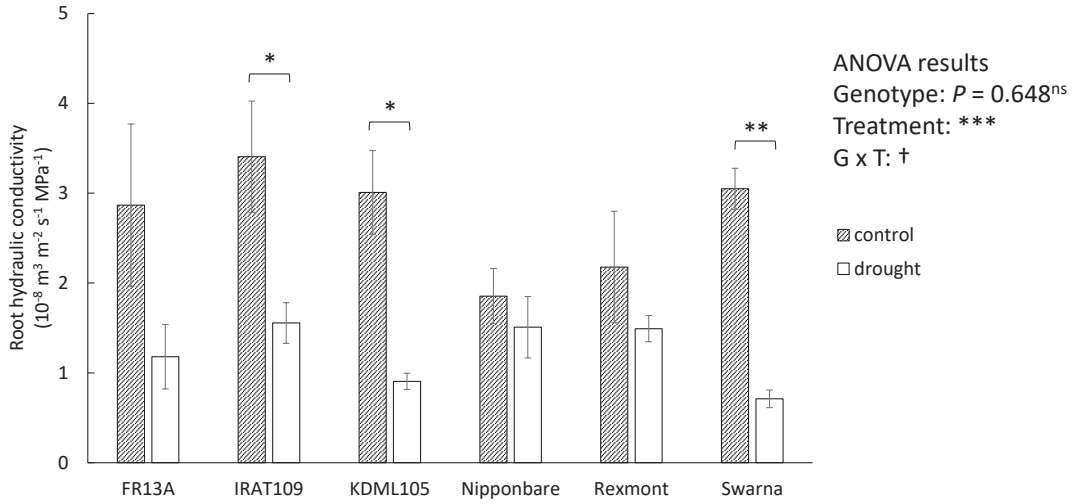

Fig. 5. Root hydraulic conductivity (Lp_r) in Experiment 1 (phytotron experiment). Bars indicate the standard error. Significance levels among treatments are *P < 0.05 and †P < 0.10.

and even showed an increase in SDW under drought relative to the control treatment.

Root surface area

In Experiment 1, Swarna showed some of the lowest RSA values in the drought treatment, and its lateral root surface area (LRSA) was not affected by drought, whereas the LRSA of several other genotypes appeared to be promoted under the drought compared to the control treatment (significant for FR13A; Fig. 3). In Experiment 2, root surface area density (RSAD) of each genotype decreased with soil depth in both soil moisture conditions (Fig. 4). There was a significant interaction between genotype and water treatment for RSAD at the 0-15 and 15-30 cm soil depths. At 0-15 cm soil depth, the RSAD of FR13A, KDML105, and Swarna was significantly reduced in the drought treatment compared to the irrigated paddy field (control) (Fig. 4A). At 15-30 cm soil depth, only the RSAD of Swarna was significantly lower in the drought compared to the control, while the RSAD of IRAT109 and Rexmont was significantly increased in the drought compared to the control (Fig. 4B). There was no significant interaction between genotype and water treatment on RSAD at 30-45 and 45-60 cm soil depths (Fig. 4C, 4D).

Root system activity

In Experiment 1, Lp_r was reduced in the drought treatment and the degree of reduction in Lp_r differed among genotypes (Fig. 5). The Lp_r of IRAT109, KDML105, and Swarna was significantly decreased by drought compared to the control treatment. In Experiment 2, there were significant differences in

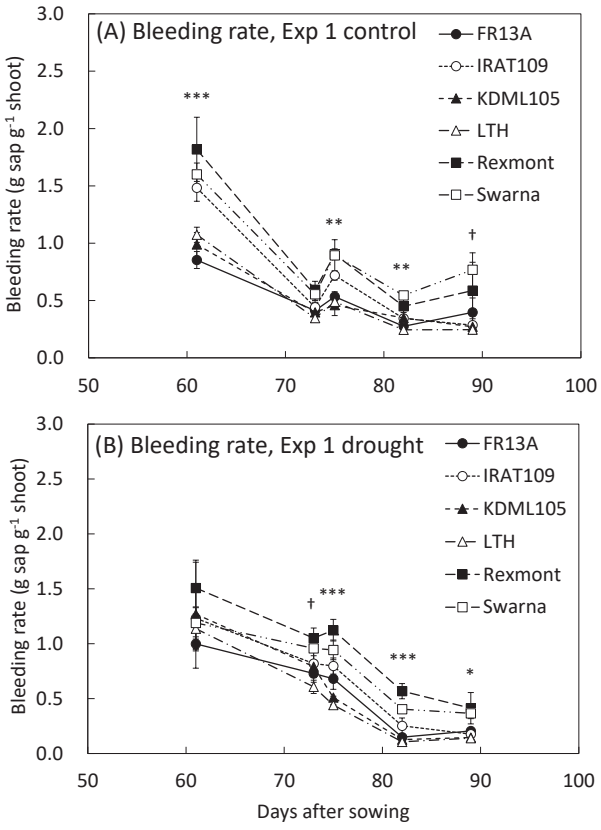

Fig. 6. Bleeding rates for Experiment 2 (field experiment) [(A) control treatment and (B) rainfed drought treatment]. Bars indicate the standard error. Significance levels among treatments are ***P < 0.001, **P < 0.01, *P < 0.05 and †P < 0.10.

bleeding rate measured from 61 to 89 DAS for both the control and drought treatments (Fig. 6). Under both conditions, Swarna and Rexmont showed higher bleeding rates than the other genotypes, and

Table 1. Correlation coefficients between root traits

(A)

RSA (Exp. 1) vs Lp_r (Exp. 1)			
control		drought	
r	*P*-value	r	*P*-value
-0.302	0.561	-0.0136	0.980

(B)

date	Lp_r (Exp. 1) vs Bleeding rate (Exp. 2)			
	control		drought	
	r	*P*-value	r	*P*-value
12-Aug	-0.346	0.559	0.361	0.551
24-Aug	-0.608	0.279	0.093	0.881
26-Aug	-0.354	0.557	0.327	0.591
2-Sep	-0.233	0.722	0.239	0.698
9-Sep	-0.398	0.526	-0.008	0.990

(C)

date	soil depth	RSAD (Exp. 2) vs Bleeding rate (Exp. 2)			
		control		drought	
	(cm)	r	*P*-value	r	*P*-value
12-Aug	0-15	-0.495	0.318	-0.693	0.127
	15-30	-0.471	0.346	-0.213	0.686
	30-45	-0.800	0.056 †	-0.813	0.049 *
	45-60	-0.834	0.039 *	0.295	0.570
24-Aug	0-15	-0.069	0.896	-0.225	0.669
	15-30	-0.075	0.887	-0.076	0.886
	30-45	-0.608	0.200	-0.405	0.426
	45-60	-0.486	0.329	0.345	0.503
26-Aug	0-15	-0.236	0.652	-0.235	0.653
	15-30	-0.156	0.768	-0.345	0.503
	30-45	-0.703	0.119	-0.381	0.456
	45-60	-0.607	0.202	0.069	0.897
2-Sep	0-15	0.143	0.788	-0.401	0.431
	15-30	0.051	0.924	-0.383	0.454
	30-45	-0.289	0.578	-0.510	0.302
	45-60	-0.442	0.381	0.116	0.826
9-Sep	0-15	0.277	0.595	-0.175	0.740
	15-30	0.330	0.523	-0.268	0.608
	30-45	-0.323	0.533	-0.252	0.631
	45-60	-0.165	0.754	0.204	0.699

Correlation coefficients between (A) RSA (Experiment 1) and Lp_r (Experiment 1); (B) Lp_r (Experiment 1) and Bleeding rate (Experiment 2); (C) RSAD (Experiment 2) and Bleeding rate (Experiment 1). RSAD: Root surface area density; Lp_r: Root hydraulic conductivity; RSA: Root surface area. *p*-Values area indicated by asterisk and dagger: $^{*}P < 0.05$ and $^{\dagger}P < 0.10$.

the trend was consistent throughout the measurement period (61 to 89 days after sowing) (Fig. 6).

Correlation analysis between root architecture and root activity measurements in Experiment 2 indicated that only bleeding rate at 61 DAS was correlated with RSAD at 30-45 cm in the drought treatment and with RSAD at 45-60 cm in the control treatment (Table 1C). In Experiment 1, Lp_r was not correlated with RSA or bleeding rate (Table 1A, 1B). Genotypic variation in bleeding rate in Experiment 2 was not correlated with the variation in Lp_r in Experiment 1 (Table 1B).

Leaf water status

The drought treatment reduced the LWP measured in Experiment 1, but no significant genotypic differences were observed (Fig. 7A). There were no significant genotypic differences in stomatal conductance measured on 77 DAS in Experiment 2 in the drought treatment on August 28, 2013 (Fig. 7B).

Biomass and grain yield at harvest

There were no significant differences between water treatments in terms of straw biomass or grain yield at the time of harvest in Experiment 2 (Fig. 8). The SDW reduction percentage at the last bleeding rate measurement date (89 DAS) in Experiment 2 showed a negative correlation with the Lp_r reduction percentage in Experiment 1 ($P = 0.087$), and with the RSA at soil depths of 15 to 30 cm ($P = 0.066$) and 45 to 60 cm ($P = 0.092$), as well as a significant positive correlation Lp_r under drought in Experiment 1 ($P = 0.050$; Table 2A, 2B), while it showed no significant correlation with the bleeding rate on any date measured in Experiment 2 (Table 2C).

Discussion

The ability of the rice plant to maintain shoot biomass under drought can be an important indicator of vegetative stage drought tolerance (Cal et al. 2019). In this study, both root architectural and root hydraulic traits were measured to understand genotypic variation in the ability to maintain shoot

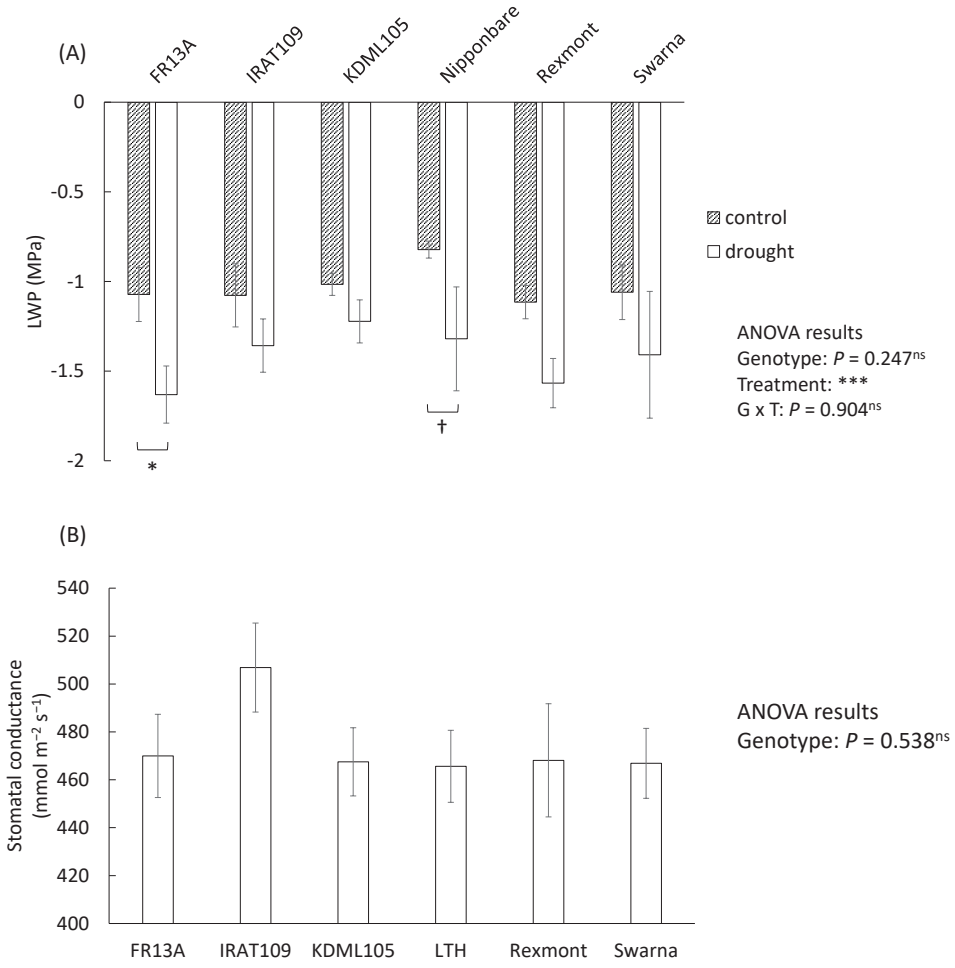

Fig. 7. Plant water status A) leaf water potential (LWP) from Experiment 1 (21 days after sowing, phytotron experiment), and B) stomatal conductance in the drought treatment at 77 days after sowing in Experiment 2 (field experiment). Bars indicate the standard error. Significance levels among treatments are $*P < 0.05$ and $\dagger P < 0.10$.

biomass under short-term drought conditions during the vegetative stage. Of the seven genotypes studied, the most contrasting ability to maintain shoot biomass under short-term drought conditions was consistently observed between Rexmont (relatively higher SDW reduction), and Swarna and KDML105 (relatively lower SDW reduction). Although Swarna was included in this study for its reported susceptibility to drought at the reproductive stage (Venuprasad et al. 2009, Vikram et al. 2015), it is frequently observed to perform well at the vegetative stage. The results of this study corroborate the previous reports that both KDML105 and Swarna are at least moderately tolerant to short-term drought during the vegetative stage.

In terms of Lp_r in Experiment 1, Swarna, KDML105, and IRAT109 showed low Lp_r under the drought treatment, which was significantly reduced from that in the control treatment. The Lp_r of Rexmont and Nipponbare was not reduced under the drought treatment. On the other hand, bleeding rate did not appear to be related to SDW reduction in this study since both Rexmont and Swarna showed the highest bleeding rates in both treatments. Similar to the results of this study, Swarna has consistently shown the greatest bleeding rates in all treatments of the field experiment in both wet and dry seasons in previous studies (Henry et al. 2012). Henry et al. (2015) reported that genotypic differences in bleeding rates were more evident in the dry season than in the wet season and that no consistent genotypic differences were observed under control conditions. Based on the results of previous studies and the present study, it is suggested that the association between bleeding rate and drought tolerance may be weaker under relatively mild drought conditions, such as during the wet season.

Among root traits in this study, the shallow RSAD was more reduced in Swarna, KDML105 and FR13A, but not in Rexmont and IRAT109 in

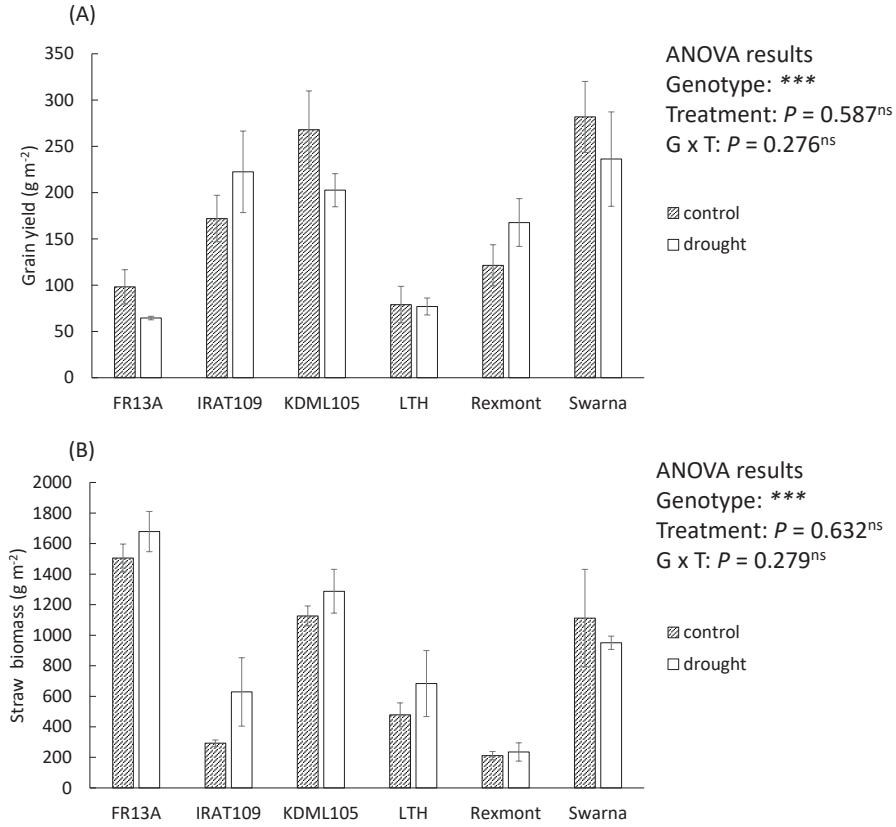

Fig. 8. Harvest data from Experiment 2 (field experiment): A) Straw biomass and B) grain yield. Bars indicate the standard error.

Table 2. Correlation coefficients between SDW reduction at the final bleeding rate measurement date (89 days after sowing) in Experiment 2 and root traits in both experiments

	(A)					
	vs Lp_r (Exp. 1)					
	control		drought		Lp_r reduction	
	r	*P*-value	r	*P*-value	r	*P*-value
	-0.280	0.648	0.8781	0.050 †	-0.8229	0.087 †
(B)						
soil depth	vs RSAD (Exp. 2)					
	control		drought		RSAD reduction	
(cm)	r	*P*-value	r	*P*-value	r	*P*-value
0-15	-0.376	0.462	-0.284	0.586	-0.527	0.283
15-30	-0.494	0.319	-0.043	0.935	-0.782	0.066 †
30-45	-0.477	0.339	-0.541	0.268	-0.465	0.353
45-60	-0.514	0.297	0.216	0.682	-0.741	0.092 †
(C)						
date	vs Bleeding rate (Exp. 2)					
	control		drought		bleeding rate reduction	
	r	*P*-value	r	*P*-value	r	*P*-value
12-Aug	0.539	0.270	0.670	0.145	0.293	0.573
24-Aug	0.519	0.291	0.649	0.163	-0.335	0.516
26-Aug	0.459	0.360	0.617	0.192	-0.643	0.169
2-Sep	0.295	0.571	0.549	0.259	-0.654	0.159
9-Sep	0.077	0.884	0.346	0.502	-0.700	0.122

Correlation coefficients between (A) Lp_r (Experiment 1); (B) RSAD (Experiment 2); (C) Bleeding rate (Experiment 2). RSAD: Root surface area density; Lp_r: Root hydraulic conductivity; RSA: Root surface area. Reduction was calculated as (control – drought) / control x 100 †: $P < 0.10$.

Experiment 2. In experiments with greater degrees of drought, Swarna and KDML105 developed an extensive surface root system (Kano-Nakata et al. 2013, Kameoka et al. 2016), but in the milder drought conditions of this study, both varieties suppressed surface root system development. Therefore, in this study, reducing shallow root growth and Lp_r in response to drought appeared to confer the best ability to maintain shoot biomass under short-term drought conditions during the vegetative stage, as evidenced by the correlations in Table 2.

Interestingly, the root distribution and physiology traits appeared to affect vegetative stage shoot growth, but not plant water status (based on stomatal conductance and LWP). Furthermore, this study indicated that even soil drought stresses that do not lead to yield reductions can cause distinct changes in root system development, and that there are significant genotypic differences observed. At 77 DAS, when SWP began to decrease, there were no significant genotypic variations in stomatal conductance, suggesting that no genotype experienced significant water uptake inhibition at this time (Fig. 7B). Likewise, few genotypes showed a treatment effect on LWP in Experiment 2 (Fig. 7A). However, by that point in the drought stress treatments, the root system distribution of each genotype had already been specifically altered by the drought treatment, and the amount of change differed among genotypes (Fig. 4).

Although it is likely that root architecture and hydraulics interact, there was no significant correlation between bleeding rate and RSAD in this study, regardless of treatment, genotype and measurement date (Table 1). Grondin et al. (2016) reported that bleeding rate was positively and significantly correlated with stele diameter and metaxylem diameter, particularly under well-watered conditions. Swarna showed a different trend in response to soil drying for bleeding rate and Lp_r: under both treatments, Swarna maintained a higher bleeding rate than the other genotypes, while its Lp_r decreased to a greater degree than the other genotypes (Fig. 5, 6). Although there was no clear relationship between Lp_r and bleeding rate in this study, the physiological significance of each trait on water absorption deserves additional research.

Another genotype that stood out in terms of the root architecture vs hydraulics comparison was KDML105. In a study by Henry et al. (2012), KDML105 maintained a relatively low bleeding rate and Swarna maintained a relatively high bleeding rate, similar to the results of this study. The Lp_r of IRAT109, KDML105, and Swarna was significantly reduced by the drought treatment (Fig. 5). KDML105 has been reported to exhibit plasticity in root system development in response to mild soil drought stress (Bañoc et al. 2000a, 2000b, Kano-Nakata et al. 2013, Kameoka et al. 2015, 2016). Root architectural plasticity plays a key role in rice productivity under drought-prone rainfed lowland conditions (Suralta et al. 2018, Sandhu et al. 2016, Xie et al. 2021). Our study suggests that plasticity in root hydraulics may also be a key trait.

In summary, this study showed that there are significant genotypic differences in the effects of relatively mild or short-term soil drought stress on root system distribution and root system activity and that combinations of these traits can help rice plants grow better under vegetative stage drought. We conclude that in this study, the combination of reduced shallow root growth and reduced Lp_r under drought were most beneficial for maintaining shoot biomass under drought conditions.

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