Genotypic variation in rice varieties screened for deep rooting under field conditions in West Africa

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ABSTRACT

In this study, to identify deep rooting accessions, we assessed the differences in root depth based on the length of the longest primary root among 586 different rice accessions: 511 *Oryza sativa* and 75 *O. glaberrima*. Malagkit Pirurutong and Binicol were identified as the two rice accessions with deepest roots through four field experiments conducted at two different locations in West Africa. For these two accessions, root depths reached 35.6 and 41.4 cm, respectively, in the first experiment at Bamako; on the other hand, their depths only reached 22.6 and 18.6 cm, respectively, in the second and third experiments at Ibadan, leading to inconsistent genotypic ranking based on root depth between the two locations. However, Malagkit Pirurutong was identified as deep rooting in both locations; in addition, it showed deep rooting in the fourth experiment in a 20-mm irrigation treatment, even when compared with the deep rooting reference Azucena. Nonetheless, this pattern was not found under a 10-mm irrigation treatment. Malagkit Pirurutong kept developing deep roots even following 60 days after sowing (DAS), whereas other shallower rooting accessions ceased deepening by 60 DAS. The longer period for deepening roots would be beneficial for terminal drought stress.

In Africa, rice has become increasingly important both as a food source and as an economic commodity. Among several rice-growing environments, rainfed uplands occupies a key place, representing about 40% of the area in sub-Saharan Africa (Balasubramanian et al., 2007) and about 30% of the production in West and Central Africa (WARDA, 2002). Although increasing upland rice production is crucial, grain yield in this environment remains low, at about 1 t ha^{-1} in West Africa (Somado et al., 2008). The low productivity is partly attributable to drought, because rainfall is unstable and is the sole water source for most small-scale farmers in the region. Breeding for drought resistance is one of the ways to achieve stable yield of upland rice.

In West Africa, rainfed upland rice cultivation is a common practice in the Rain Forest zone (characterized by high rainfalls and presenting a mean monthly rainfall varying from <20 mm to 300 mm with a short dry period usually in August), Derived Savanna zone (1300–2000 mm of annual rainfall from April to October), Southern Guinea Savanna zone (bimodal rainfall of 1200–1500 mm per year with a wet season of 6–8 months), Northern Guinea Savanna zone (monomodal rainfall of 1000–1200 mm per year, usually from April to October), and to some extent the Sudan Savanna zone (monomodal rainfall of 600–1000 mm per year with a wet season of 4–6 months) (WARDA, 2002). Rainfall patterns are highly variable in sub-Saharan Africa, and the type of drought stress can vary accordingly (Sié et al., 2008). Rainfed upland rice cultivation in West Africa, which is the target cropping and location of this study, can experience several types of drought stress, including that mainly influencing the vegetative-stage, intermittent mid-season stress, and terminal droughts. Compared to other cereal crops, upland rice presents a relatively shallow root system, limiting its capacity to extract water from deeper soil layers, partially explaining the low resistance to drought stress exhibited by this crop (Kondo et al., 2000). Deep roots promote drought resistance by allowing the plant to extract water from otherwise inaccessible deep soil layers (Lilley & Fukai, 1994; Yoshida & Hasegawa, 1982). Thus, the ability to rapidly develop a deep root system is essential to survive intermittent mid-season and late terminal droughts. Deep rooting rice varieties are required as sources

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Table 1. Summary of the four experiments.

Site	Description of site	Experiment	Type	Year and season	Watering	Number of accession
Bamako(Mali)	Sudan Savanna zoneDeep sandy soil	1	Screening	2004 WS	Sprinkler irrigation	586
Ibadan(Nigeria)	Rain Forest zoneSandy loam surface horizon changing into a clayey subsurface horizon with a gravelly horizon between 15 and 30 cmHard layer between 15 and 20 cm below the soil surface	2	Screening	2005 WS	Rainfed	100
		3	Screening	2006 WS	Rainfed	17
		4	Evaluation	2006 DS–2007 WS	Two irrigation rates by hand-watering	5

WS and DS indicate wet and dry seasons, respectively.

of deep rooting traits in breeding drought-resistant varieties. In fact, rice varieties are known to significantly differ in root depth (Kato et al., 2006; Kondo et al., 2003; Lilley & Fukai, 1994), justifying the efforts made to screen rice cultivars for deep rooting traits.

Information from repeated screening under targeted field conditions is required because root traits are affected by growth conditions, including water stress (Fischer & Fukai, 2003; Kondo et al., 2003; Liu et al., 2004; Oyanagi, 1998) and soil mechanical impedance (Cairns et al., 2004; Passioura, 2002). Reliable screening information about deep rooting traits of rice under the field conditions of West Africa is limited because comparison of root depth under field conditions using many accessions is laborious and time-consuming. In this study, three field screening experiments were performed in West Africa to screen rice accessions for deep rooting under targeted conditions, representing the optimal characteristics of a late terminal drought. The effect of irrigation rate on root depth of the selected deep rooting accessions was evaluated in a fourth experiment.

Materials and methods

In this study, we conducted three field screening experiments and one field experiment using two different levels of irrigation (Table 1).

Screening accessions for deep rooting

In the first screening (Experiment 1) conducted in an upland field in Bamako (Mali) during the 2004 wet season (WS), 75 accessions of *Oryza glaberrima* and 511 accessions of *O. sativa* were evaluated. The basis for the selection of the *O. glaberrima* was their availability in the Genebank of Africa Rice Center, Cotonou (Benin). The 233 *O. sativa* accessions were supplied by the International Rice Research Institute, Los Baños (Philippines), and 278 accessions were supplied by National Institute of Agrobiological Sciences, Tsukuba (Japan). The *O. sativa* accessions originated from 38 countries and regions and were intended to cover the worldwide genetic diversity of the species (Supplemental Table 1). Based on the results of Experiment 1, seven *O. glaberrima* and 93 *O. sativa* were selected for

Table 2. Mean temperature and rainfall of every 10-day period in Experiments 2 and 3 at Ibadan in the 2005 and 2006 wet seasons, respectively.

Days after sowing	Mean temperature (°C)		Rainfall (mm)	
	2005	2006	2005	2006
Before 10	25.3	27.4	159	16
11–20	24.6	26.3	80	27
21–30	23.9	25.7	56	71
31–40	24.7	26.3	55	45
41–50	23.8	25.4	1	1
51–60	23.4	26.0	1	101
61–70	23.5	25.3	0	23
71–80	23.7	24.7	51	91
81–90	24.9	24.3	49	4
91–100	25.5	24.4	25	45
101–110	25.1	24.6	122	190
111–120	24.9	25.1	54	53
121–130	25.7	25.4	33	98
131–140	26.3	26.8	12	57
141–150	26.7	27.5	4	11
151–160	28.1	27.4	0	23
161–170	27.9	27.4	0	27
171–180	27.5	28.2	25	10

Table 3. Data of days to heading, shoot dry weight, and root depth of Azucena from the preliminary experiments conducted at Ibadan.

Year	Season	Watering	Days to heading	Shoot dry weight[†](g)	Root depth[†](g)
2005	Dry	5-mm irrigation	138	27.1	15.4
		7-mm irrigation	131	28.3	14.8
		10-mm irrigation	118	36.8	15.8
2006	Wet	Rainfed	114	48.6	20.6

[†] Measurement was conducted 2 weeks after heading

the second screening (Experiment 2) in an upland field at Ibadan (Nigeria) in the 2005 WS. Furthermore, one *O. glaberrima* and 16 *O. sativa* accessions were selected based on the average ranking for root depth in Experiments 1 and 2. In the 2006 WS, these 17 accessions were used in the third screening (Experiment 3) conducted in an upland field adjacent to that of Experiment 2.

Each accession was direct seeded at a rate of 5 seeds per hill, with a spacing of 25 cm × 25 cm. Each plot was 75 cm × 250 cm and contained 30 hills. Thinning was not conducted to prevent root damage. The sowing date was July 12, 2004 in Experiment 1, June 17, 2005 in Experiment 2, and May 25, 2006 in Experiment 3. Plots were laid out in a completely randomized design with no replication for Experiment 1 and in a completely randomized block design with four replications for Experiments 2 and 3. Fertilizer was applied before sowing at 267 kg ha^{-1} of complex fertilizer so that N, P_2O_5, and K_2O were applied at 40, 40, and 40 kg ha^{-1}. Hand weeding was performed as required. In Experiment 1, sprinkler irrigation was applied two or three times per week throughout the growth season to avoid an early season or a mid-season drought at Bamako, which is located in the Sudan Savanna zone. In Experiments 2 and 3 at Ibadan, which is in the Rain Forest zone, rice plants were grown under rainfed conditions. However, seven late-maturing accessions received sprinkler irrigation two or three times per week from 153 days after sowing (DAS) in Experiment 2 following the end of WS. The mean temperature and rainfall of every 10-day period during Experiments 2 and 3 are shown in Table 2 (temperature and rainfall were not recorded in Experiment 1). The Bamako field had deep sandy soil and the Ibadan field had a sandy loam surface horizon changing into a clayey subsurface horizon with a gravelly horizon between 15 and 30 cm depth. The soft soil layer was interrupted by a hard soil layer approximately 15–20 cm deep at Ibadan. In the same site at Ibadan, penetrometer resistances at a depth of 20 cm were greater than 3.0 MPa (Kayombo & Lal, 1986).

Days to heading was recorded from each hill based on daily observation. After cutting the shoots at the surface level, the remaining roots immediately below ground for each hill were extracted using a soil core of 5 cm in diameter and 50 cm in length (DIK-162D, Daiki Rika Kogyo Co., Kounosu, Japan). Three hills were harvested per plot in

Experiment 1 and five in Experiments 2 and 3. We collected soil root samples at each hill at 2 weeks after heading under the assumption that the root system had fully developed by that time. The roots were washed and the length of the longest primary root of each hill was measured. We measured primary root depth, because collection of fine lateral roots was difficult in field experiments involving a root washing process. The shoots and roots were dried at 70 °C for 3 days and weighed.

This type of sampling with soil cores only provides data from a fraction of the entire root system presenting clear limitations; however, this method is considerably less time-consuming than alternative methods such as the monolith method or the trench profile method, potentially allowing the inclusion of a larger number of samples. In this study, we sampled fraction of a root system from a hill foot, assuming that the deepest roots in the hill were grown directly downward.

Correlations between Experiments 1 and 2 and between Experiments 2 and 3 for root depth and plant growth parameters [days to heading, shoot dry weight (DW), root DW, and number of panicles] were calculated using Microsoft Excel. For the 17 accessions screened in Experiments 2 and 3, two-way analysis of variance was performed using SPSS to identify significant effects of accession, year, and their interaction on root depth and plant growth parameters. Broad sense heritability (H^2) for the 100 accessions included in Experiment 2 and the 17 accessions included in Experiment 3 was computed as $H^2 = \delta2g(\delta2g + \delta2e)$, where $\delta2g$ and $\delta2e$ represent the estimate of genetic and residual variances, respectively, derived from the expected mean squares of analysis of variance. Root depths between the accessions were compared using Tukey's HSD method.

Effect of irrigation on root depth

An upland field experiment (Experiment 4) was performed at Ibadan from the middle of the 2006 dry season (DS) (December 8, 2006) to the 2007 WS (March 8, 2007) to evaluate the effect of irrigation rate on root depth of the selected deep rooting accessions. We used five accessions for this experiment, including four screened in Experiments 1–3 and Azucena as a reference deep rooting cultivar (Price et al., 1997). From the four accessions previously screened,

the deep rooting accessions at Ibadan were Malagkit Pirurutong and Khao Dam and the shallow rooting accessions at Ibadan were Nam Sa-Gui 19 and IR29. Malagkit Pirurutong and Nam Sa-Gui 19 had similar numbers of days to heading in our screening, as did Khao Dam and IR29.

The five accessions were grown with irrigation of 10 or 20 mm per day throughout the experiment, i.e. from sowing to 90 DAS. These treatments were used to simulate water stress (10-mm irrigation) and moderate wet conditions (20-mm irrigation). Hereafter, the plots irrigated with 10 or 20 mm of water per day are referred to as 10-mm plots and 20-mm plots, respectively. Preliminary results from Ibadan showed that the growth of Azucena was a bit reduced under the simulated drought conditions (10-mm irrigation) during the dry season compared to rainfed conditions during the wet season (Table 3). The 20-mm irrigation was deemed sufficient to ensure rice growth without intense stress even during the dry season at Ibadan. To simulate water stress conditions, we irrigated all plots with 10 mm of water every morning at 0730, with an additional 10 mm applied to the 20-mm plots every afternoon at 1600. The 20-mm plots were irrigated twice to avoid the risk of losing significant amounts of water through runoff if the irrigation was only performed once. A watering pot was used to avoid uneven sprinkler irrigation. The irrigation rates were applied to main plots in a split-plot design with no replication for the main plot factors. A main plot contained four blocks, and the genotypes were randomly allocated to subplots within a block. During the experiment, there was no rainfall before 80 DAS, but there was heavy rainfall after 80 DAS (data not recorded). Daily mean temperature ranged from 21.8 to 29.6 °C throughout the experiment.

Each accession was direct seeded at a rate of five seeds per hill with a spacing of 25 cm × 25 cm. Each plot was 100 cm × 225 cm and contained 40 hills. Fertilizer was applied before sowing at the rate of 267 kg ha^{-1} of complex fertilizer: N, P$_2$O$_5$, and K$_2$O were applied at 40 kg ha^{-1} each.

The volumetric soil water content of the 6-cm surface layer was measured daily from 17 to 90 DAS in all plots, using a soil moisture meter (DIK-311C, Daiki Rika Kogyo Co., Ltd. Kounosu, Japan). We used the built-in calibration curve for mineral soils to convert voltage to the volumetric soil moisture content. The measurements were carried out at 0800, just after watering, to record the highest soil water content, and at 1530, just before watering, to record the lowest soil water content.

Three hills per plot were sampled at 30, 60, and 90 DAS. Shoot DW and root depth of each hill were determined using the same method as in the screening. Two-way analysis of variance was performed using SPSS to identify significant effects of accession, irrigation, and their interaction on root depth and shoot DW on each sampling day. The simple main effect of accession at each irrigation rate on root depth and shoot DW was tested by Bonferroni-adjusted multiple pairwise comparisons.

Results

Screening accessions for deep rooting

The growth of the 586 accessions varied widely. Heading of the earliest accession began at 45 DAS and that of the latest at 178 DAS (Figure 1(a)). Shoot DW varied from 2 to 133 g per hill (equivalent to 32 and 2128 g m^{-2}, respectively) (Figure 1(b)), and root DW varied from .1 to 6.3 g per hill (equivalent to 2 and 101 g m^{-2}, respectively) (Figure 1(c)). These wide variations in growth confirmed the large genetic diversity of the accessions used in this study.

The root depth of the 586 accessions ranged from less than 10 to more than 40 cm (Figure 1(d)). The root depths of the top 100 accessions in Experiment 1 ranged from 16.6 to 41.4 cm. Only a Filipino variety, Binicol, had a root depth greater than 40 cm. The root depths of five accessions, namely, a Chinese variety (Yun 83–149); a Myanmar variety (Bunt Kardu); two Filipino varieties (Malagkit Pirurutong and Macan Binundok); and a Bangladeshi variety (DA5) were greater than 30 cm. The number of accessions with root depth from 20 to 30 cm and from 16.6 to 20 cm was 78 and 16, respectively.

The 100 selected accessions were evaluated in Experiment 2 at Ibadan in 2005. Among the accessions, days to heading ranged from 55 to 163 in Experiment 1 and from 57 to 181 in Experiment 2 (Figure 2(a)). Although days to heading correlated positively and strongly between the locations, heading of the 100 accessions tended to be delayed at Ibadan (Figure 2(a)). Shoot DW (Figure 2(b)) and root DW (Figure 2(c)) correlated positively and weakly between the locations. The maximum shoot DW and root DW of the 100 accessions in Experiment 1 was 108 and 6 g per hill (equivalent to 1728 and 96 g m^{-2}), respectively (Figure 2(b) and (c)). These values were greater than those in Experiment 2 [35.7 and 3.0 g per hill (equivalent to 571 and 48 g m^{-2})], respectively (Figures 2(b) and (c)).

The more severe growth conditions at Ibadan restricted deep root development of the 100 accessions (Figure 2(d)). The deepest root length observed was 41.4 cm in Experiment 1 and 23.8 cm in Experiment 2. The number of accessions with roots deeper than 20 cm was only 23 in Experiment 2, but 84 in Experiment 1 (Figure 2(d)). Root depth of the 100 accessions was not correlated between the locations (Figure 2(d)). For selecting deep rooting accessions in different locations, the average ranking of the accessions for root depth in Experiments 1 and 2 was used as the screening criterion, resulting in the selection of 17 accessions, including an *O. glaberrima* accession, for Experiment 3 at Ibadan in 2006.
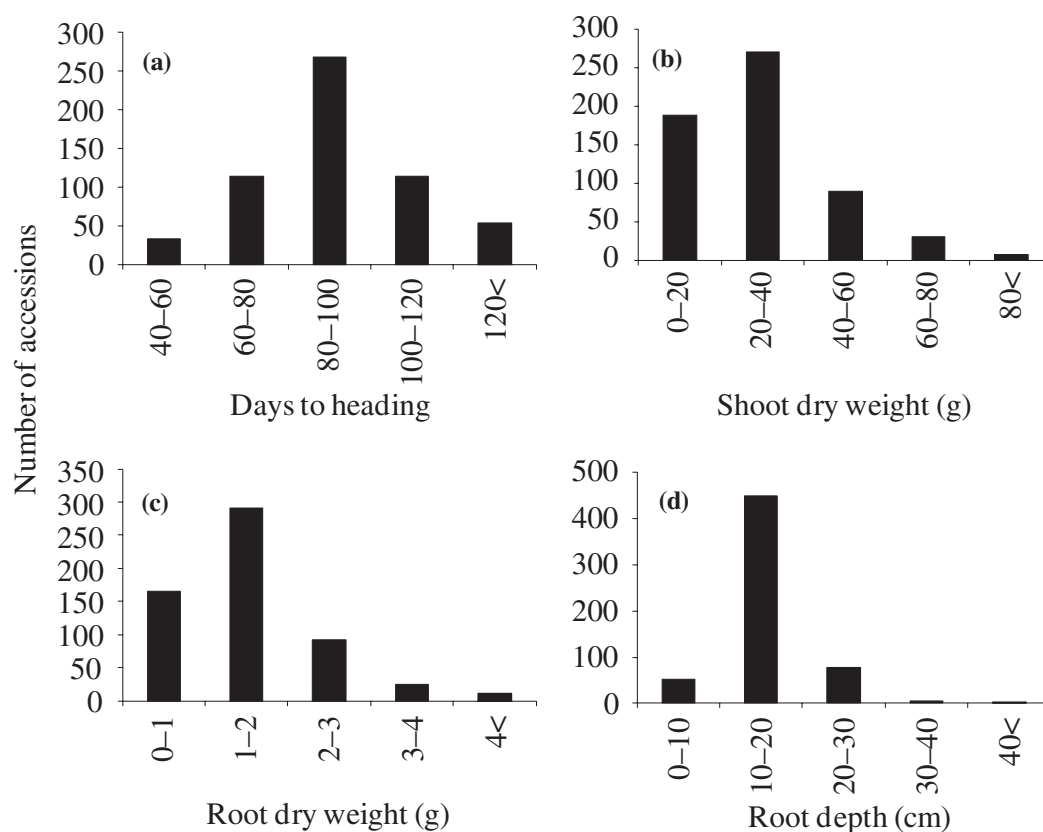
Figure 1. Frequency histogram of (a) days to heading, (b) shoot dry weight, (c) root dry weight, and (d) root depth for 586 accessions measured in Experiment 1 at Bamako in the 2004 wet season.

Days to heading of the 17 accessions were not significantly different ($p > .05$) between years, and accession × year interaction was significant ($p < .01$) (Table 4) but correlated strongly between years ($r = .969, p < .001$) (Figure 3(a)). Significant differences in shoot DW ($p < .001$), root DW ($p < .001$), root depth ($p < .01$), and number of panicles ($p < .05$) of the 17 accessions were observed between 2005 and 2006, and accession × year interaction was not significant ($p > .05$) for these parameters (Table 4). For all parameters, the correlation between years was significant ($r = .654–.867, p < .01$ or $.001$) and positive (Figure 3(b)–(e)). All the parameters, including root depth, were significantly different ($p < .001$) between the accessions (Table 4). Therefore, the method for determining root depth of upland rice could consistently distinguish the 17 accessions under relatively similar growth conditions in Experiments 2 and 3.

Malagkit Pirurutong was the top-ranking accession for root depth in Experiments 2 and 3 at Ibadan, according to the average of the 2 years (Table 5). Although the root depth of Malagkit Pirurutong was not significantly different ($p > .05$) from that of the second to eleventh accessions, it was the only accession that had a significantly deeper root system ($p < .05$) than the twelfth and lower ranking accessions (Table 5). The eleventh ranking accession at

Ibadan was Binicol, which had the greatest root depth in Experiment 1 at Bamako. The *O. glaberrima* accession TOG5484 ranked last for root depth in Experiments 2 and 3 at Ibadan (Table 5).

The correlation between days to heading and root depth was significant ($r = .251, p < .001$) in Experiment 1; however, the correlation was not significant ($p > .05$) in Experiment 2 ($r = .138$) (Table 6). Shoot DW and root depth ($r = .315–.391, p < .001$) and root DW and root depth ($.406–.601 p < .001$) were significantly correlated in Experiments 1 and 2 (Table 6). The correlation coefficient between the number of panicles and root depth was negative but not statistically significant ($p > .05$) in Experiments 2 and 3 (Table 6). Correlations between root depth and other parameters were also not significant in Experiment 3 ($r = −.337–.439, p > .05$) (Table 6).

Broad-sense heritability for root depth was similar to that measured for shoot and root DWs, although it was much lower than that measured for days to heading and number of panicles in Experiments 2 and 3 (Table 7).

Effect of irrigation on root depth

The volumetric soil water content was lower in the 10-mm plots than in the 20-mm plots throughout the
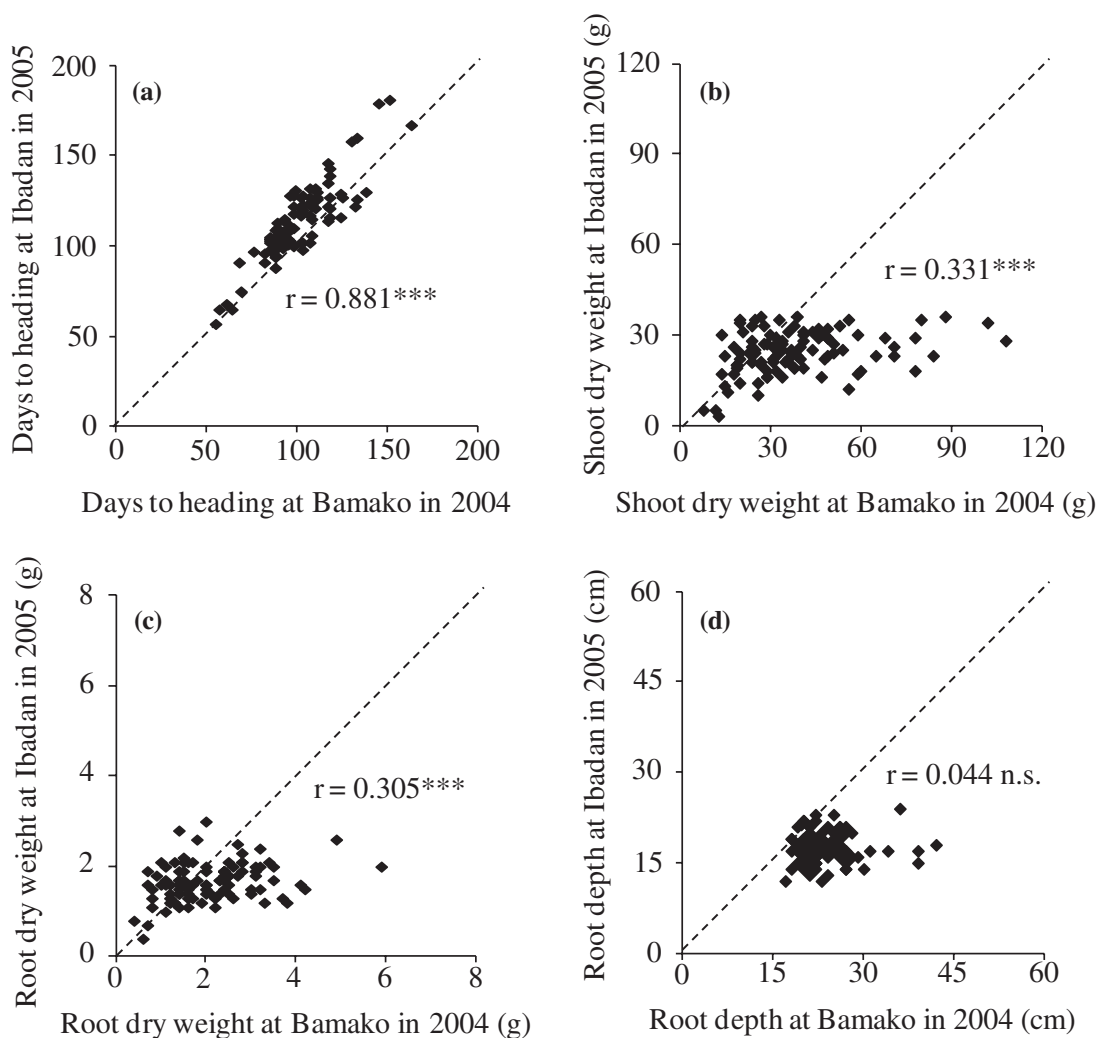
Figure 2. Year-to-year correlations of (a) days to heading, (b) shoot dry weight, (c) root dry weight, and (d) root depth for 100 accessions in Experiment 1 at Bamako in the 2004 wet season and in Experiment 2 at Ibadan in the 2005 wet season. The dashed lines represent the 1:1 relationships. *** indicates significance at a .001 probability level; n.s. indicates not significant.

Table 4. Two-way analysis of variance for days to heading, shoot dry weight, root dry weight, root depth, and number of panicles for 17 accessions in Experiments 2 and 3 at Ibadan in the 2005 and 2006 wet seasons, respectively.

Source	df	Mean square				
		Days toheading	Shoot dryweight	Root dryweight	Root depth	Number of panicles
Accession	16	2169***	392***	1.07***	28.3***	44***
Year	1	46 n.s.	2780***	4.86***	75.5**	16*
Accession × year	16	35**	133 n.s.	0.20 n.s.	5.1 n.s.	4 n.s.
Error	102	14	114	0.21	5.9	3

***, **, and * indicate significance at .001, .01, and .05 probability levels, respectively. n.s. indicates not significant.

measurement period (data not shown). At 0800, it ranged from 9.5 to 22.5% in the 10-mm plots and from 15.2 to 28.3% in the 20-mm plots. At 1530, it ranged from 7.4 to 18.2% and from 14.1 to 28.4%, respectively. Thus, water stress was more severe in the 10-mm plots than in the 20-mm plots.

Shoot DW per hill of the five accessions at 90 DAS ranged from 15.9 to 23.3 g (equivalent to 398 and 583 g m^{-2}) in

the 10-mm plots and from 21.4 to 27.1 g (equivalent to 535 and 678 g m^{-2}) in the 20-mm plots (Table 8). In the 10-mm plots, differences in shoot DW among the five accessions were not significant throughout the experiment ($p > .05$) (Table 8). Similar results were observed in the 20-mm plots, where differences in shoot DW among the five accessions were not significant at 30 and 90 DAS ($p > .05$) (Table 8).
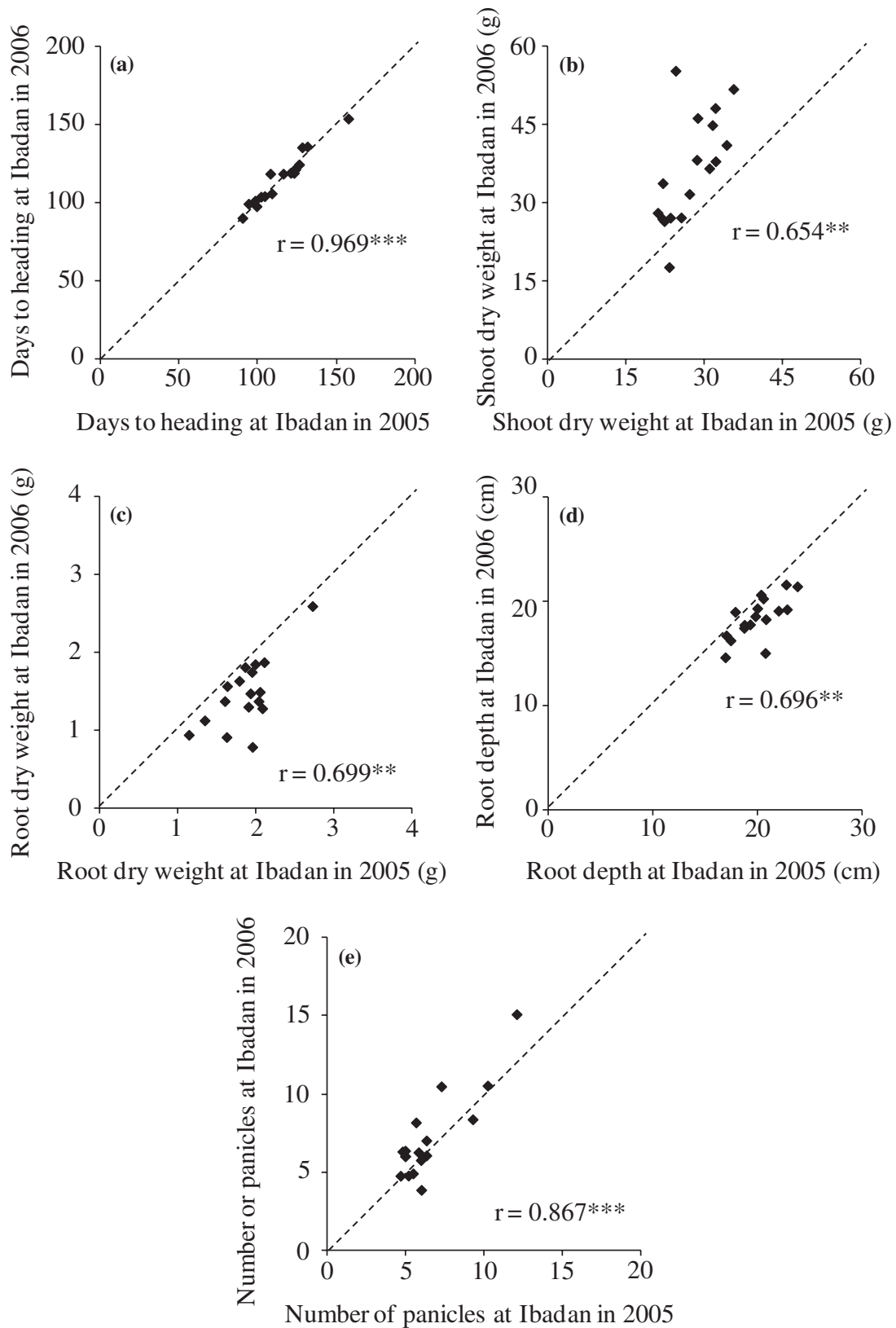
Figure 3. Year-to-year correlations of (a) days to heading, (b) shoot dry weight, (c) root dry weight, (d) root depth, and (e) number of panicles as the mean of five hills for 17 accessions in Experiments 2 and 3 at Ibadan in the 2005 and 2006 wet seasons, respectively. The dashed lines represent the 1:1 relationships. *** and ** indicate significance at a .001 and .01 probability level, respectively.

Table 5. Values and ranking of 17 accessions for root depth in Experiment 1 at Bamako in the 2004 wet season and in Experiments 2 and 3 at Ibadan in the 2005 and 2006 wet seasons, respectively.

Accession	Root depth (cm)		Ranking	
	Bamako	Ibadan[†]	Bamako[‡]	Ibadan[†]
Malagkit Pirurutong	35.6	22.6 a[§]	4	1
Godawee	21.5	22.2 ab	58	2
Dam Ngo	24.2	21.1 abc	29	3
Sharial	21.7	20.6 abc	54	4
Cuba65	23.6	20.5 abc	35	5
Khao Dam	26.5	20.5 abc	11	6
Padi Kenikir Puti	26.5	19.7 abcd	12	7
Rathal	25.0	19.6 abcd	25	8
Calutos	27.2	19.2 abcd	10	9
Cere Air	24.7	18.6 abcd	26	10
Binicol	41.4	18.5 abcd	1	11
Binatangan 2	25.4	18.3 bcd	23	12
Davao	24.1	18.1 bcd	30	13
Kahei	26.0	17.9 bcd	20	14
Bunt Kardu	38.2	16.9 cd	3	15
Manan Elata	25.5	16.9 cd	22	16
TOG5484	26.3	15.8 d	14	17

[†]Mean of the 2 experiments.
[‡]Ranking of 586 accessions used at Bamako.
[§]Values in the same column followed by the same letter are not significantly different at $p = .05$ (Tukey's HSD).

Table 6. Correlation between root depth and other parameters in Experiment 1 at Bamako in the 2004 wet season and in Experiments 2 and 3 at Ibadan in the 2005 and 2006 wet seasons, respectively.

Parameter	Experiment 1(586)[†]	Experiment 2(100)[†]	Experiment 3(17)[†]
Days to heading	0.251***	0.138	−0.024
Shoot dry weight	0.315***	0.391***	0.255
Root dry weight	0.406***	0.601***	0.439
Number of panicles	nd[‡]	−0.114	−0.337

***indicates significance at .001 probability level.
[†]Values in parenthesis are the number of accessions included.
[‡]No data was determined.

Table 7. Broad-sense heritability for days to heading, shoot dry weight, root dry weight, root depth, and number of panicles among 100 accessions in Experiment 2 and 17 accessions in Experiment 3 at Ibadan in the 2005 and 2006 wet seasons, respectively

Parameter	Experiment 2	Experiment 3
Days to heading	0.970	0.985
Shoot dry weight	0.490	0.408
Root dry weight	0.389	0.460
Root depth	0.473	0.529
Number of panicles	0.781	0.728

The effects of accession, irrigation, and their interaction on root depth were significant at 90 DAS ($p < .001$) (Table 8). However, in the 10-mm plots, the differences in root depth among the five accessions were not significant ($p > .05$) throughout the experiment and the root depth was less than 15.1 cm, irrespective of accession (Table 8). In the 20-mm plots, Malagkit Pirurutong and Khao Dam had deeper roots than IR29 and Nam Sa-Gui 19 at 60 and 90 DAS (Table 8). The root depth of Malagkit Pirurutong and Khao Dam were greater than that of Azucena at 90 DAS (Table 8). The difference in root depth between the 10- and 20-mm plots at 90 DAS was 10.4, 9.6, and 5.2 cm for Malagkit Pirurutong, Khao Dam, and Azucena, respectively. Thus, the deep rooting traits of the selected deep rooting accessions were confirmed in the 20-mm plots, where the water stress was relatively mild.

Discussion

Screening accessions for deep rooting

In this study, we used soil core samples to determine the root depth of different rice cultivars in hill settings. This method does not allow the evaluation of the entire root system, presenting its main limitation; however, using

Table 8. Shoot dry weight and root depth of five accessions with the 10- and 20-mm irrigation treatments per day in Experiment 4 at Ibadan from the middle of the 2006 dry season to the beginning of the 2007 wet season.

DAS[†]	Accession	Shoot dry weight (g)		Root depth (cm)	
		10 mm	20 mm	10 mm	20 mm
30	Malagkit Pirurutong	0.5 a[‡]	0.5 a	8.8 a	8.6 a
	Khao Dam	0.5 a	0.6 a	9.6 a	9.5 a
	Azucena	0.7 a	0.6 a	10.8 a	11.9 a
	IR29	0.5 a	0.4 a	10.4 a	10.1 a
	Nam Sa-Gui 10	0.5 a	0.7 a	9.6 a	10.4 a
	ANOVA				
	Irrigation (I)	n.s.		n.s.	
	Accession (A)	*		n.s.	
	I × A	n.s.		n.s.	
60	Malagkit Pirurutong	10.6 a	7.2 c	13.0 a	18.2 ab
	Khao Dam	11.0 a	10.7 abc	12.3 a	19.6 a
	Azucena	12.2 a	16.3 ab	14.1 a	18.8 ab
	IR29	9.0 a	9.9 bc	13.7 a	16.1 ab
	Nam Sa-Gui 10	11.6 a	17.6 a	12.1 a	14.1 b
	ANOVA				
	Irrigation (I)	n.s.		***	
	Accession (A)	**		n.s.	
	I × A	n.s.		n.s.	
90	Malagkit Pirurutong	15.9 a	21.9 a	14.4 a	24.8 a
	Khao Dam	20.1 a	26.1 a	14.4 a	25.0 a
	Azucena	17.2 a	27.1 a	13.8 a	19.0 b
	IR29	19.9 a	21.4 a	15.1 a	15.8 bc
	Nam Sa-Gui 10	23.3 a	26.9 a	12.8 a	12.7 c
	ANOVA				
	Irrigation (I)	**		***	
	Accession (A)	n.s.		***	
	I × A	n.s.		***	

***indicate significance at a 0.001, 0.01, and 0.05 probability level.
**indicate significance at a 0.01 probability level.
*indicate significance at a 0.05 probability level.
n.s. indicates not significant.
[†]Days after sowing
[‡]Values in the same column at each sampling day followed by the same letter are not significantly different at *P* = 0.05 (Bonferroni adjusted multiple pairwise comparisons).

this technique, we were able to identify wide variations in root depth for the 586 accessions assessed, which ranges from less than 10 cm to over 40 cm in Experiment 1 (Figure 1(d)). In Experiments 2 and 3 under relatively similar experimental conditions, root depth was found to be strongly positively correlated (*r* = .696, *p* < .01) between years (Figure 3(d)) and the interaction of accession × year was insignificant for root depth (*p* > .05) (Table 4) for 17 accessions. These results confirm the repeatability of the measurements using core sampling, an essential condition to warrantee data robustness. Our data might still include repeated incorrect measurements; however, it is reasonable to assume that the root depth data obtained using core sampling can be used to compare different accessions.

The wide range for root depth at Bamako (Figure 1(d)) suggests opportunities for genetic improvement in the deep root system under the growth conditions in West Africa. The root depths of six accessions, Binicol, Yun 83–109, Bunt Kardu, Malagkit Pirurutong, Macan Binundok, and DA5, were greater than 30 cm at Bamako. Rice roots below 30 cm depth are considered deep roots and are assumed to be important for water uptake under drought conditions (Azhiri-Sigari et al., 2000; Kamoshita et al., 2002; Kamoshita et al., 2004; Kato et al., 2006; Kato et al., 2007; Kondo et al., 2003; Shen et al., 2001; Yoshida & Hasegawa, 1982; Yue et al., 2005; Yue et al., 2006). Although Experiment 1 was conducted with no replication, owing to the large number of accessions, the six accessions with root depth greater than 30 cm would be candidate deep rooting accessions at Bamako.

Including the six accessions, the top 100 deep rooting accessions were selected in Experiment 1 at Bamako. Two accessions, Kinandang Patong and OS4, reported as being deep rooting (Price et al., 1997; Uga et al., 2011), were included in the 100 accessions. Their root depths were 21.0 and 23.1 cm, respectively (Supplemental Table 1). However, some accessions reported as being deep rooting, such as Azucena (Price et al., 1997), Moroberekan (Price et al., 1997), IAC25 (Price et al., 1997), and IRAT109 (Kato et al., 2006), were not included in the 100 deep rooting accessions. These findings indicate that deep rooting varieties in one environment do not always develop deep root systems in another environment. It is thus possible that accessions with shallow roots at Bamako would exhibit deep rooting

under other conditions. Note that additional irrigation was conducted at Bamako and the effects of early season and mid-season droughts, which are expected to occur more frequently under rainfed conditions, on the root depth of the accessions remain unknown.

The differences in growth conditions between Bamako and Ibadan led to a considerable change in the ranking of accessions for root depth, and root depth for the 100 accessions did not correlate between locations (Figure 2(d)). However, two accessions showed deep rooting at both locations (Table 5). Malagkit Pirurutong developed the deepest root systems at Ibadan and its root depth was greater than 30 cm at Bamako (Table 5), indicating that the deep rooting trait of Malagkit Pirurutong was independent of the growth conditions. The other accession was Binicol. It ranked eleventh at Ibadan, but there was no significant difference in root depth between Binicol and Malagkit Pirurutong ($p > .05$) (Table 5); at Bamako, only Binicol exhibited a root depth greater than 40 cm (Table 5). Binicol and Malagkit Pirurutong would be useful deep rooting accessions under several growth conditions.

The selected deep rooting accessions at both locations originated from South-East Asia. The materials from outside West Africa could contribute to root deepening rice varieties bred in West African conditions. In contrast, although we used 75 accessions of O. glaberrima for the screening, only one, TOG5484, passed Experiments 1 and 2. In Experiments 2 and 3, among the 17 accessions, this accession ranked the lowest in root depth (Table 5). Thus, our screenings identified no O. glaberrima accessions with deep rooting.

Root depth correlated significantly with days to heading ($p < .001$) in Experiment 1; however, these two parameters showed very weak correlations in Experiments 2 and 3 ($r = -.024–.138$), suggesting that days to heading cannot predict root depth in the selected populations (Table 6). Other parameters, such as shoot DW and root DW, correlated significantly ($r = .315–.601$, $p < .001$) for the 586 accessions in Experiment 1 and the 100 accessions in Experiment 2 (Table 6). Therefore, these parameters could be used as root depth substitutes to eliminate low potential accessions during the first rough selection. The use of each of these parameters is associated with different advantages and disadvantages. Shoot DW is relatively easy to determine because it does not require root sampling. However, compared with the correlation between root DW and root depth, shoot DW only weakly correlate with root depth throughout the three experiments (Table 6). Determination of root DW is less laborious than that of root depth. However, the direct determination of root depth appears to be indispensable to identify deep rooting accessions, judging from the nonsignificant correlations ($p > .05$) between root depth and root DW in the final screening in Experiment 3 (Table 6). Judging from the broad-sense heritability (Table 7), deep root screening was proven to be at least as efficient as ordinary screening techniques that are based on shoot DW and root DW. However, we should be careful to select the traits of shoot DW, root DW, and root depth, since environmental effects on them were large. It would be effective to modify days to heading and number of panicles genetically (Table 7).

Environmental effects on root depth of the selected varieties

The root depth of the 17 accessions was limited to about 20 cm or less in Experiments 2 and 3 at Ibadan, whereas some of these accessions had root depths of 35 cm or more in Experiment 1 at Bamako (Table 5). The maximum root depth reached by Azucena in Experiment 4 was 19 cm (Table 8), whereas the same variety reached root depths in soil layers deeper than 30 cm under field conditions at Mbé (Côte d'Ivoire) (Cairns et al., 2004). The differences in root depth may be attributed to the soil hardness in the locations. Although soil hardness was not determined in this study, the clayey subsurface horizon with a gravelly horizon between 15 and 30 cm depth at Ibadan was much harder than the deep sandy soil at Bamako, based on the difficulty in penetration by the core samplers during root sampling. The hard layer was observed between 15 and 20 cm below the soil surface at Ibadan. In the same site at Ibadan, penetrometer resistances at a depth of 20 cm were greater than 3.0 MPa when maize roots were observed between 0 and 21 cm depth (Kayombo and Lal, 1986). An impedance of 3.0 MPa is high enough to inhibit rice root elongation (Bengough & Mullins, 1990; Hasegawa et al., 1985; Price et al., 2002). Azucena root density declined sharply below 20 cm in high penetration resistance (PR) sites but not in medium PR sites at Mbé (Côte d'Ivoire) (Cairns et al., 2004).

The hard layer at Ibadan may restrict the drainage of rain water, resulting in a higher soil water content above it. Root systems of rice plants may remain in the layer with high water content without penetrating deeper layers. Mambani and Lal (1983) investigated the effect of depth of water table below the soil surface on the root development of 10 rice varieties under normal field conditions at Ibadan. Their results showed a lateral deflection of vertical root growth caused by high (shallow) water table, whereas in sites with low (deep) water table, roots primarily grew downward toward the wet soil.

It is not clear whether water stress is the only reason behind the lack of a clear expression of deep rooting traits in Malagkit Pirurutong and Khao Dam observed for the 10-mm plots (Table 8). The clayey subsurface horizon at Ibadan may harden under water stress conditions;

however, while the effect of soil texture on soil PR, measured as an index of soil hardness, is reduced under wet conditions and the resistance drastically increases in clayey soil under dry conditions (Vaz et al., 2011). Therefore, further studies should aim to understand the influence that the rate of irrigation has on root depth of accessions at Bamako, whose sandy soil is less harder than the soil at Ibadan even under dry conditions to clarify the effect of water stress on deep root traits.

Environmental conditions strongly influenced root depth in the selected deep rooting accessions, Malagkit Pirurutong and Binicol (Tables 5 and 8). Less hard soil may be required for the expression of their rooting traits to develop root depths >30 cm; however, the soil hardness threshold that prevents root penetration is still not clear for these accessions. In addition, deep rooting traits appeared to be expressed in the absence of water stress during early and middle growth stage, at least for Malagkit Pirurutong. This type of deep rooting traits would be beneficial for alleviating water stress late in the growing season, if the late terminal drought occurs without early and mid-season droughts. The continuous increase in root depth of Malagkit Pirurutong and Khao Dam even after 60 DAS (Table 8) also confers an advantage in preparation for late water stress. However, rainfall patterns vary substantially among sub-Saharan African regions (Sié et al., 2008), implying that early and mid-season droughts, inducing soil hardening, may also occur depending on the region. The locations included in this study differ in their soil hardness, as suggested by the comparison between Bamako and Ibadan. Thus, it may be risky to entirely rely on deep rooting traits to breed drought-resistant varieties under the variable environments in West Africa.

Conclusion

Six accessions had deep roots (deeper than 30 cm) at Bamako. Two of these had deep roots at two locations, namely Bamako and Ibadan. The accessions selected at Ibadan showed relatively deeper roots but were not effectively deep. The development of deep roots appeared to be prevented by the hard soil and water stress at Ibadan. This indicates that root depth can be significantly influenced by environmental factors. Therefore, the observation of rooting traits of the selected accessions under different environments is necessary for future studies. Other varieties not included in this study could also express deep rooting traits under field conditions in West Africa, and therefore screening other germplasm banks, including local varieties is highly recommended. The root depths of rice varieties screened in this study in a target environment provide important information for breeding of drought-resistant varieties in West Africa.

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