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Research article

Rice (*Oryza sativa* L.) breeding for a combination of shallow and deep root traits derived from lowland × upland for alternate wetting and drying systems

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Abstract

Drought stress is one of the major abiotic stresses caused by climate change that limits rice yield production worldwide. To overcome this situation, alternative wetting and drying (AWD) systems may be a solution to reduce the water supply required for rice production in irrigation areas. In addition, the architecture, morphology and anatomy of a root under mild-drought conditions are considered key traits driving the adaptive response of rice to water deficit. Thus, the combination of shallow and deep roots derived from lowland rice (Homchonlasit) crossed with upland rice (Pa-yah Leum Gaeng) was performed based on pedigree selection. The results showed that the mean (115 ± 4) number of deep roots (DR) was successful in combining with shallow roots (SR) (97 ± 5) in four selected lines that contained a ratio of deep roots (RDR) of 1.19 ± 0.06, while the lowland parent had 24 ± 2 DR and 41 ± 3 SR that accounted for an RDR of 0.58±0.02 for AWD conditions. Notably, the xylem area, cortex area and epidermis thickness of DR in breeding lines were similar to those of the upland rice parents and they were larger than those of the lowland rice parents. Finally, the mean (± SE) values for grain yield (4.44 ± 0.37 t/ha) and water use efficiency (WUE, 0.55 ± 0.05) of the breeding lines were higher than those of their parents (3.23 ± 0.24 t/ha, 0.40 ± 0.03 WUE, respectively) under AWD conditions by 28% and 37%, respectively. Thus, the breeding program was successful in combining shallow and deep root traits suitable for the AWD system and the breeding lines could be used as donor parents in future breeding programs.

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Introduction

Climate change is increasing the frequency and severity of abiotic stress in plants that is threatening food production around the world (Pandey and Shukla, 2015). Among the abiotic factors, rice production in rainfed ecosystems is the most imperative and major limitation, affecting 20% of the total rice-growing area in Asia (Pandey and Bhandari, 2009). Rice (*Oryza sativa* L.) is one of the major staple food crops that is very sensitive to mild water deficits (Centritto et al., 2009). Sandhu et al. (2012) estimated that by 2025, 15 out of 75 million ha of Asia's irrigated rice crop will experience water shortages. In Thailand, the central region is the largest irrigated rice-producing area where water scarcity has a severe effect in the dry season (Silalertruksa et al., 2017), and the Thai government has restricted the growth of rice in this season (Ngammuangtueng et al., 2019). Therefore, improvement of water management techniques and breeding programs to overcome the water deficit situation under climate change should be considered in irrigated areas.

Rice roots play an important role in the absorption of water and nutrients and in drought stress tolerance (Singh et al., 2013). Thus, root architectural and anatomical traits are key to breeding strategies aimed at drought avoidance (Gowda et al., 2011) and are one of the most important strategies for maintaining crop yields in water-limited environments (Lynch et al., 2014). Nodal roots comprise a large proportion of the root system based on the length, number and angle of roots (Harada and Yamazaki, 1993) and are responsible for the greatest amount of water absorption (Yoshida and Hasegawa, 1982). In general, rice cultivation has two major land growing systems, uplands and lowlands. These two systems differ greatly in their potential yields because of soil characteristics that affect root growth and plant responses to drought conditions (Ling et al., 2002). Lowland rice cultivars that are suitable to grow in flooded soil have shallow, finer roots, a large number of roots and many tillers, whereas upland rice is typically characterized by a deep (defined as roots below a depth of 30 cm) and coarse root system, tall stature, thicker stems and fewer tillers (Gowda et al., 2011). Thus, changes in the plant root system architecture may allow the selection of an ideal root system for different environments with better water uptake capacity, which would allow the maintenance of yield levels even under adverse weather conditions (Lynch, 2007).

Root angle distribution, which is regulated by the growth angle of the nodal roots, is one of the root morphological traits used to determine the area of soil over which roots capture water and nutrients (Uga et al., 2015). Deep roots are useful for extracting more water from the soil and minimizing drought stress (Uga et al., 2015), while shallow roots help plants absorb oxygen near the soil surface and help avoid hypoxic conditions (Hanzawa et al., 2013). Several lowland experiments (Samson et al., 2002; Gowda et al., 2011) reported that 69–94% of roots are located in the top 10 cm of the soil (shallow roots), with very few roots found below 30 cm (deep roots). In addition, Wang et al. (2019) reported that rice with a deep root system tolerated drought better than rice with a shallow root system. Ho et al. (2005) reported the importance of dimorphic root systems having both deep and shallow roots that can adapt to multiple resource limitation factors, such as water and nutrition. Thus, introducing deep rooting characteristics into shallow-rooting cultivars is considered one of the most promising breeding strategies to avoid a case of lack of water in the root zone (Uga et al., 2013).

With changing climate and rainfall, there is a gradual shift in the system of rice cultivation from traditional anaerobic to semi-irrigated aerobic methods, such as alternate wetting and drying (AWD) methods (Chidthaisong et al., 2018). The "safe AWD" recommendations of the International Rice Research Institute, which are intended to minimize yield reductions, state that the soil should be dried until the soil water depth has reached 15 cm below the surface and that the field should be reirrigated to a standing water depth of approximately 5 cm (Bouman and Tuoung, 2001). AWD irrigation management resulted in increased rice yield, rice water use and nitrogen use efficiency and reduced amount of irrigation compared to continuous flooding (Sibayan et al., 2018). In addition, AWD is a technique that could reduce CH_4 emissions from paddy fields (Chidthaisong et al., 2018; Sibayan et al., 2018). However, suitable cultivars are needed to sustain yield levels (Dharmappa et al., 2019), and the root system should be adapted to wet and dry soil conditions during the vegetative stage. Thus, increasing the deep root ratio in lowland rice should enhance the growth in an AWD system better than in lowland rice with a higher shallow roots ratio when the rice is grown under dry conditions.

Developing rice genotypes that maintain high productivity under AWD systems is a major challenge. Therefore, the goals of this project were to combine shallow roots with deep roots in new breeding lines derived from lowland rice (Homchonlasit; HCS) crossed with upland rice (Pa-yah Leum Gaeng; PLG). The improved lines should be well suited for use in irrigated lowlands and for adaptation to AWD conditions.

Materials and Methods

Plant material and growth conditions

The experiment was conducted from 2016 to 2020 at the Rice Research Station, Kasetsart University, Kamphaeng Saen, Nakhon Pathom province, Thailand (14°01′ N, 99°58′ E, 10 m above sea level). The HCS and PLG varieties were used as female and male parents, respectively. The progenies from this cross were selected based on the pedigree method until F_5.

The rice plants were seeded in a field nursery. After 15 d, each seedling plant was transplanted into a plastic basket (top diameter of 15 cm, bottom diameter of 8 cm, height of 5 cm and mesh size of 0.5 mm) and then placed in a pot experiment or breeding field. The soil properties consisted of 3.82% organic matter, 0.10% total N, 29.45 mg/kg available P, 59.30 mg/kg exchangeable K, 1120.20 mg/kg exchangeable Ca and 69.73 mg/kg exchangeable Mg, with 0.48 ds/m electrical conductivity and pH 5.80. Additionally, basal fertilizer was applied 15 d after planting at a rate of 33.7 kg N/ha (diammonium phosphate) and 41.3 kg of P_2O_5/ha. The second split of fertilizer was applied at the booting stage (65 d) at a rate of 57.5 kg N/ha. Other management practices were performed in accordance with conventional high-yielding cultivation approaches (Rice Department, 2021). The weather data (air temperature, relative humidity and amount of rainfall in the field) were measured every 3 h/d using a data logger (WatchDog 2000 Series Micro Stations; Spectrum Technologies, Inc.; USA).

Basket method to identify root angle

The basket method of Hanzawa et al. (2013) was used to investigate root angle distribution, with modifications. The plastic mesh baskets were buried under the soil surface to a depth of 10 cm. Each 15-day-old seedling plant was sown at the center of each basket at a depth of 1 cm from the soil surface. A 60-day-old (tillering stage) plant was dug out carefully, and the growth angles of the nodal roots emerging from each basket were counted at various angles. The angle was determined based on the hole that the root penetrated. Nodal roots penetrating the basket in the range 0–50° from the horizontal were defined as shallow roots (SR), while nodal roots in the basket in the range >50–90° from the horizontal were defined as deep roots (DR). The total number of roots was determined by counting all nodal roots that extended out of the basket. The root architecture graphics (RAG) program v.1.0 (Kasetsart University, Thailand) was used to identify the angles of roots. The ratio of deep roots (RDR) was defined as the number of deep roots divided by the total number of roots (Uga et al., 2009). In addition, the RDR and the total roots (TR) in each generation (F_2–F_4) were presented in the segregation data.

Preliminary test of root traits in parent varieties

The parent varieties HCS (lowland rice) and PLG (upland rice) were evaluated for root traits (SR, DR and TR) under two soil conditions. Pot (30 cm in height and 25 cm in diameter) experiments as part of a factorial completely randomized design with three replicates (five pots/treatment) were carried out in 2016. The first factor applied was soil condition (dried or flooded soil conditions). Under the dried soil conditions, a tensiometer was installed in each pot at 20 cm depth to maintain the soil tension force at -50 kPa. In contrast, the flooded soil treatment maintained the water level at 5 cm above the soil surface. The second factor was HCS and PLG varieties. Each pot was filled with 8 kg of sieved loamy clay soil. The root angle distribution was investigated at age 60 d.

Breeding schemes and alternative wetting and drying management

The breeding program to combine shallow and deep roots is shown in Fig. 1. The two parents were crossed to obtain F_1 seeds. The resulting F_1 plants were selfed to produce F_2 seeds. After that, pedigree selection was used for selection from F_2 to F_4. An RDR in the range 40–60%, together with a TR greater than 100, were used as the criteria for phenotypic selection. The selected progenies from F_2 to F_4 were grown in flooded conditions. In F_5, the roots, agronomic traits and grain yield were evaluated based on a comparison with those of their parents under AWD and flooding conditions in the field.

The yield trial (F_5) was conducted in the early rainy season (March–July 2020) at the same location as the breeding program. The experimental plots were laid out in accordance with split plots in a randomized complete block design with three replications. The main plot was AWD and flooded conditions, while the subplot was the four breeding lines with their parents. The plot size for each flooded and AWD treatment was 21 m × 11 m (231 m^2). The distance between the flooded and AWD treatments was 2 m. Bunds and canals were constructed between treatments and a plastic lining was installed along each bund at a 40 cm depth in each plot to prevent water seepage between treatments. The subplot size for

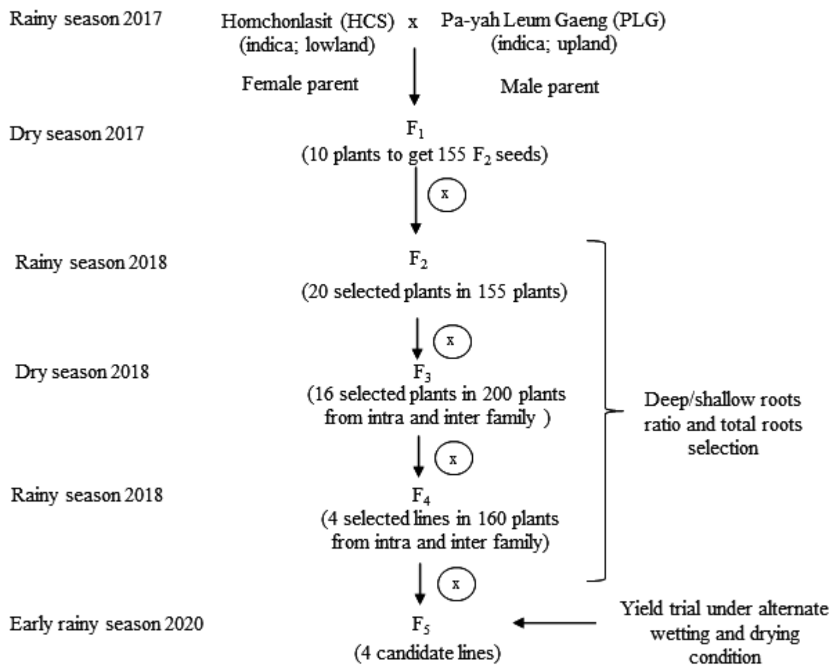

Fig. 1 Schematic diagram of the breeding program for combination of shallow and deep root traits derived from lowland × upland for alternate wetting and drying systems from wet season 2017 to early rainy season 2020

each treatment was 2.5 m ×2.5 m (6.25 m^2) and the transplanting method was applied with a spacing of 25 cm × 25 cm. In addition, five rice plants in each subplot were grown in plastic mesh baskets.

Under AWD conditions, the water levels of AWD in this study were modified from Bouman and Tuoung (2001). The water was applied to a level 10 cm above the soil surface and allowed to infiltrate the soil surface to -15 cm (10/-15 cm). The water was reirrigated again to a level 10 cm above the soil surface. This protocol was repeated until the booting stage. After that, the water depth was maintained at 10 cm and the field was dried 14 d before harvest. In contrast, the flooded condition involved continuous flooding by maintaining a water depth of 10 cm above the soil surface and the water level was not allowed to be more than 5 cm above the soil from 15 d after transplanting to 14 d before harvest. The rice cultivation practices in the AWD and flooded fields followed the recommendations of Ruensuk et al. (2021).

Water use measurement

Five water tubes were installed in the AWD fields after transplanting. The water levels were measured once per day between 0800 hours and 0900 hours using a watermark in a water tube. A 2.5 cm diameter calibrated analog flow meter was installed on the irrigation pump and was used to monitor the volume of irrigation applied to each plot (Malumpong et al., 2020). The water used in the AWD and flooded treatments was calculated as the sum of all irrigation applied plus the total rainfall from the day of transplanting until 14 d before harvest. The soil moisture content in the flooded soil and dried soil (-15 cm) in the AWD field was measured using the gravimetric soil moisture method.

Evaluation of agronomic and root traits in yield trial

The agronomic traits examined were plant height, number of tillers per plant, number of panicles per plant, 1,000-grain weight and grain yield. Plant height, number of tillers per plant and number of panicles per plant were measured at maturity. The grain yield in each subplot was determined by finding the area harvested per 6.25 m^2. The grain moisture was adjusted to 14% and then the grain yield was reported in units of kilograms per hectare. Following threshing, the grains were weighed to obtain the 1000-grain weight. The weight of the grain yield divided by the total water volume used during the growth period was calculated to determine the water productivity. In addition, the SR, DR and TR from each subplot were collected at age 60 d.

The anatomical features were observed of 60-day-old DR from selected lines with their parents. The root cross section was located 5 cm above the root tip and sectioned using a Leica Vibratome model VT 1000S. Each root section was observed and photographed using a stereomicroscope (Leica, model

ICC50 HD; Switzerland). The area, diameter and thickness of each root tissue were measured using the GNU Image Manipulation Program (GIMP 2.10; University of California, Berkeley, CA, USA). The root diameter, xylem area, xylem number, cortex area, stele diameter, epidermis thickness and endodermis thickness were measured.

Statistical analysis

All data were analyzed using analysis of variance facilitated by the R program version 3.6.1 (R Core Team, 2014). The means were compared using Duncan's multiple range test. All tests were considered significant at $p < 0.05$.

Results

Observed root traits in parent varieties

The HCS and PLG that were used as parents were investigated for SR, DR and TR in flooded and dried soil conditions. The TR in flooded soil of both varieties was higher than that of dried soil. In addition, HCS had more SR than PLG in both conditions, while PLG had more DR than HCS. Interestingly, PLG, which is upland rice, can grow well and produce DR under flooded conditions (Fig. 2). Thus, the DR trait in PLG was controlled by genetics rather than the environment and it was suitable for use as a parent for introgressing DR in this breeding program.

Early generation from F_2 to F_4

After HCS was crossed with PLG in the 2017 wet season, 10 F_1 plants were grown and selfed to obtain 155 F_2 seeds. Pedigree selection began at F_2 until F_4 to select the RDR and TR from the segregated population. The RDRs of their parents (HCS and PLG) were 19% and 81%, respectively. These values indicated that HCS (lowland rice) had more shallow roots than deep roots, while PLG (upland rice) had more deep roots than shallow roots. In addition, the RDR distributions of the F_2, F_3 and F_4 populations displayed normal distribution patterns and showed transgressive segregation in the range 10–90% (Fig. 3A). The TR was right skewed in F_2 and displayed a normal distribution in F_3 and F_4. In addition, the TR was in the range 25–250, which also showed transgressive segregation, while their parents (HCS and PLG) were 119 and 106, respectively (Fig. 3B). Thus, the RDR and TR were identified as quantitative traits.

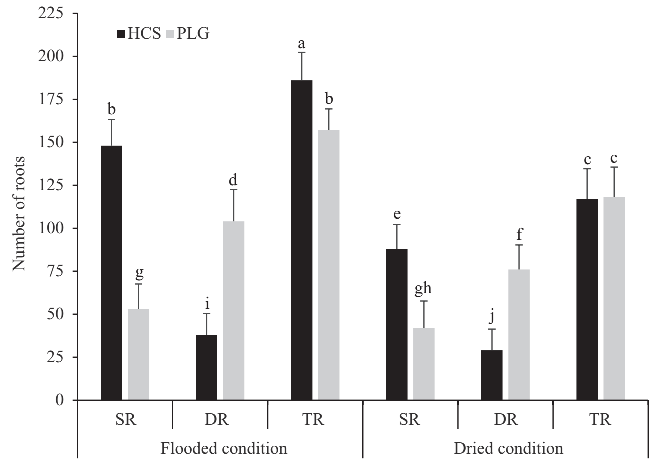

Fig. 2 Comparison of deep root (DR), shallow root (SR) and total root (TR) traits of Homchonlasit (HCS) and Pa-yah Leum Gaeng (PLG) under flooded and dried conditions, where different lowercase letters above columns indicate significantly different ($p < 0.05$). Error bars indicate + SD

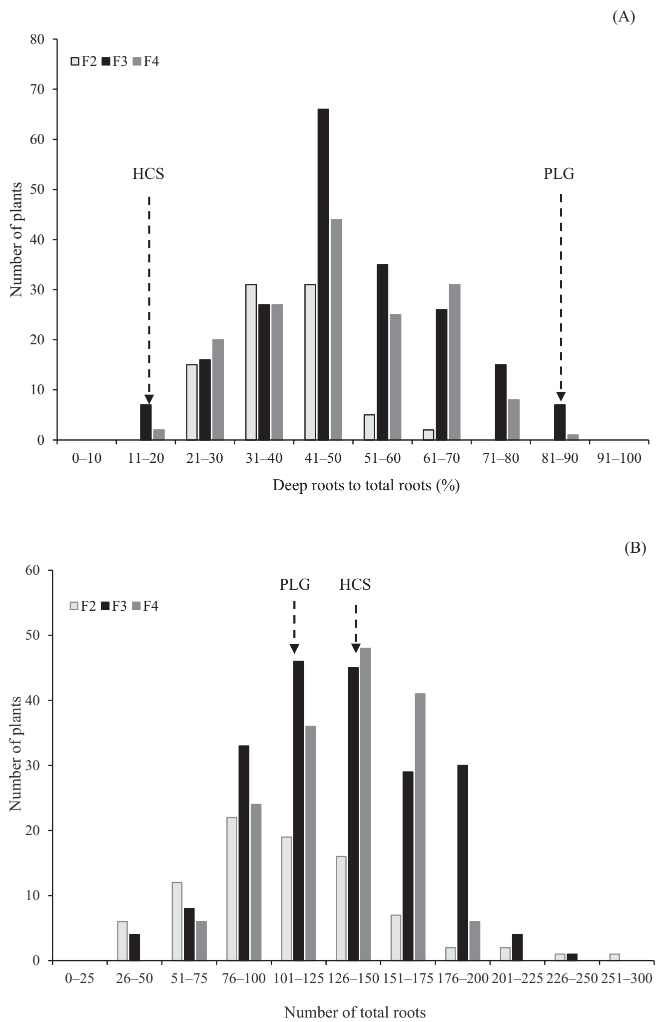

Fig. 3 Segregation of (A) deep root ratio; (B) total roots in F_2, F_3 and F_4 breeding lines and their parents, Homchonlasit (HCS) and Pa-yah Leum Gaeng (PLG)

In the F_2 generation, 20 of 155 plants that had an RDR in the range 40–60% together with TR greater than 100 were selected; F_3 seeds from the 20 selected plants were grown as panicles per row (10 plants/row). After that, the 16 selected intra- and interfamily plants were selected using the same criteria as for the F_2 generation. Next, the F_4 seeds of the 16 selected plants were grown as panicles per row and the four selected lines were selected and continued through to the F_5 generation under AWD and flooded conditions.

Advanced generation under alternative wetting and drying

Weather and water conditions

The weather data in the early 2020 rainy season is shown in Figs. 4A–B. The duration of the growth period in this experiment was from late March to July, which was in the early rainy season in Thailand. Ambient air temperature was steady throughout rice growth from transplanting to harvesting. The mean daytime temperature was 31.7°C and the mean nighttime temperature was 26.7°C. In addition, the average air temperature was 29.2°C and the average relative humidity was 74.9%. Therefore, no upper or lower extreme values for relative humidity were recorded. Thus, heat stress was not a confounding factor. The amount of rainfall in the 4 mm from transplanting to harvesting was 63.10 mm or 631 m^3/ha. The amount of rainfall from the seedling to tillering stage was less than that from the flowering to harvesting stage. In addition, the amount of rainfall in the range of AWD management (tillering stage) was 21.9 mm or 219 m^3/ha.

The field experiment and water levels compared between AWD and flooded conditions are shown in Figs. 5A–5C. In this experiment, the irrigation volume in the tillering period was managed using AWD two times. The duration of water decrease from 10 cm above the soil to -15 cm was 15 d for both times. The soil moisture at a soil depth of -15 cm in the AWD treatment was 29.29% by weight and 20.10% by weight for the first and second dried soil conditions, respectively. On the other hand, the soil moisture of flooded soil was 60.00% by weight. The amount of irrigation water in the AWD treatment was 17,600 m^3/ha, while that in the flooded treatment was 25,000 m^3/ha. Therefore, the total water used (irrigation + rainfall) in the AWD treatment (18,231 m^3/ha) was less than that for the control treatment (25,631 m^3/ha). Therefore, the AWD condition reduced the water use by 29% compared with the flooded condition.

Evaluation of root traits

In the F_5 generation, the four selected lines were grown with HCS and PLG in AWD and flooded treatments. The 3-dimensional (3D) representations of the roots of these lines/varieties are shown in Fig. 6. The number of DR of the four selected lines with PLG in the AWD condition was significantly higher than that for the flooded condition (approximately 13%), and line F_5-82-5-8-1 had the highest DR in the AWD condition (112 roots). In contrast, HCS had the lowest DR under both AWD and flooded conditions (96 and 88 roots in AWD and flooded, respectively), as shown in Fig. 7A. The four selected lines with HCS had more SR in flooded conditions than in AWD conditions (approximately 18%). In addition, the HCS had the highest SR number under flooded conditions (110 roots), but the lowest SR under AWD conditions (41 roots). Furthermore, the SR number in PLG was lower than that of the four selected lines for both conditions (Fig. 7B). However, each line/variety (except F_5-82-5-8-1 and HCS) was not significantly different in TR between AWD and flooded conditions. This suggested that increasing DR in breeding lines did not increase the TR. In contrast, the TR of HCS in the AWD condition (65 roots) was significantly lower than that for

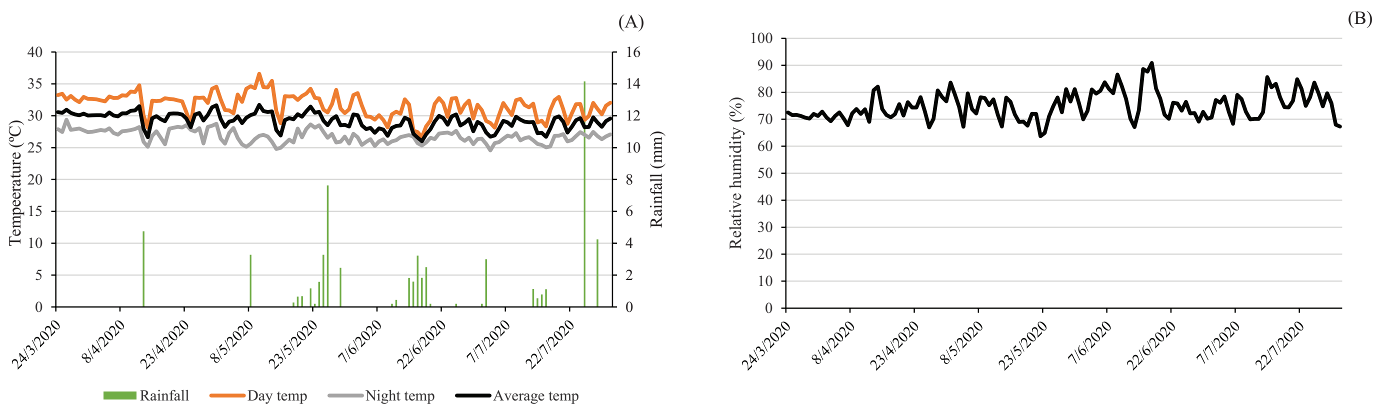

Fig. 4 Weather data of early rainy season 2020 (March–July): (A) air temperature and amount of rainfall; (B) relative humidity in the F_5 yield trial

the flooded condition (139 roots) and was the lowest compared with the other selected lines and PLG. In addition, the TR of the four selected lines was higher than that of the parents for both conditions (Fig. 7C). The RDR of the four selected lines under AWD conditions was significantly higher than that under flooded conditions. This indicated that in the AWD condition, there was greater development of DR in the selected lines than for SR. Surprisingly, the RDR of PLG in flooded conditions was the highest, while that of HCS was the lowest, especially in flooded conditions (Fig. 7D).

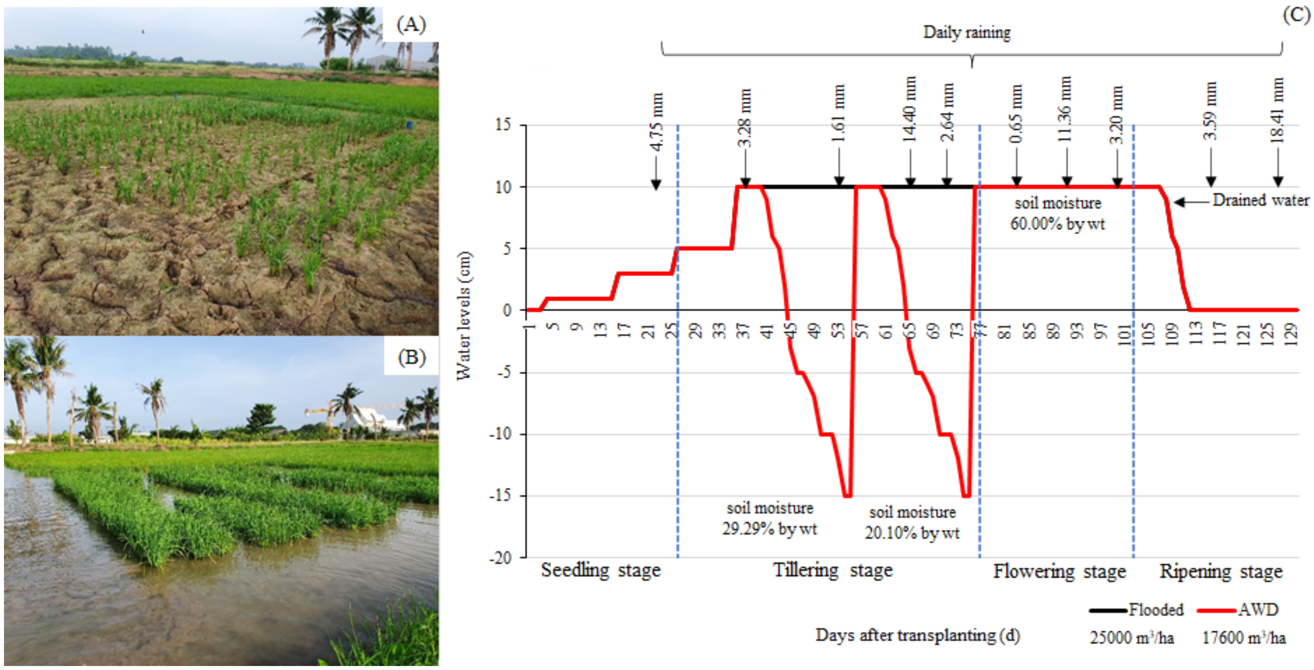

Fig. 5 Figures showing the experimental paddy fields where two planting conditions were practiced: (A) alternate wetting and drying (AWD) conditions; (B) flooded conditions; (C) soil moisture content, amount of rainfall and total water used from planting to harvesting in early rainy season 2020

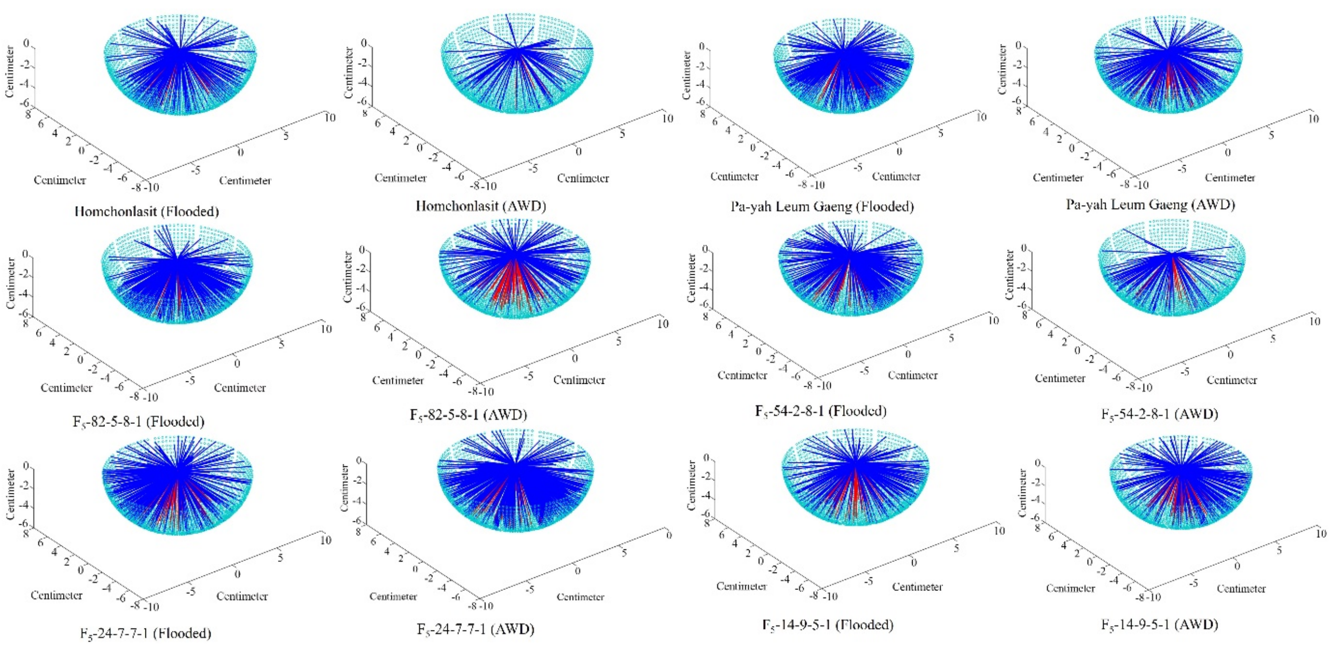

Fig. 6 Three-dimesional representations of roots of four breeding lines and their parents compared between flooded and alternate wetting and drying (AWD) conditions in early rainy season 2020, where blue represents shallow roots and red represents deep roots

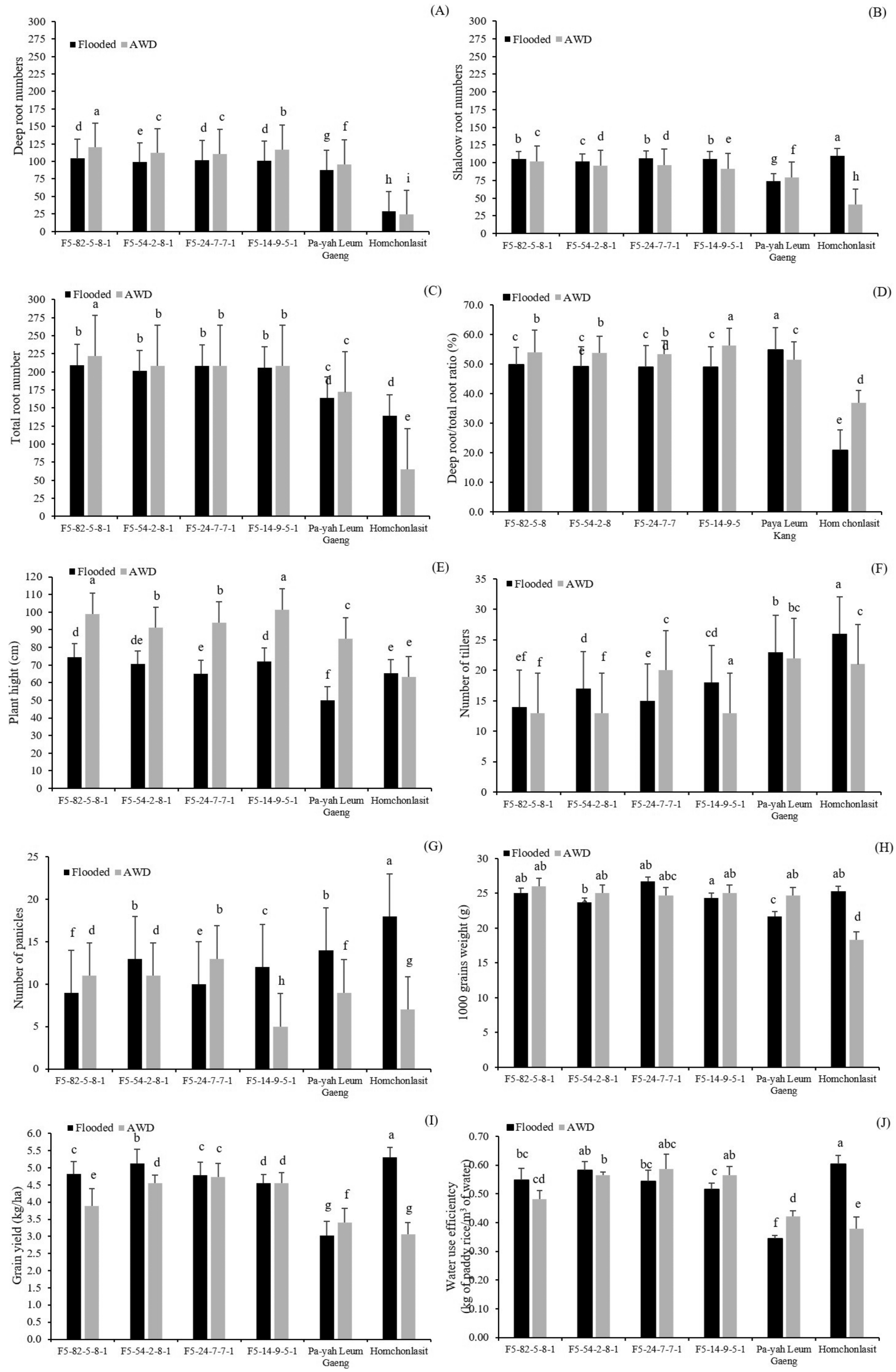

Fig. 7 Root traits (A–D) and agronomic traits and grain yield (E–J) of four breeding lines and their parents compared between flooded and alternate wetting and drying (AWD) conditions in early rainy season 2020, where different lowercase letters above columns indicate significantly different ($p < 0.05$). Error bars indicate + SD

Root anatomy assessment

The root anatomy compared among the four breeding lines and their parents is shown in Table 1 and Fig. 8. The results clearly distinguished upland rice (PLG) and lowland rice (HCS). The xylem area in PLG (710 µm^2) was larger than that of the lowland rice parent HCS (160 µm^2). Notably, the xylem areas of breeding lines F_5-82-5-8-1 and F_5-54-2-8-1 (860 µm^2 and 640 µm^2, respectively) were similar to that of PLG. However, the xylem number of breeding lines was not significantly different from that of their parents, which had xylem numbers in the range 5–7. In addition, the stele diameter located in the vascular bundles (xylem and phloem) in breeding lines was larger than that of HCS, with the stele diameter of F_5-82-5-8-1 being the largest (640 µm). F_5-54-2-8-1 had the largest cortex area (11.62 × 10^5 µm^2), followed by F_5-82-5-8-1 (8.68 × 10^5 µm^2) and PLG (7.44 × 10^5 µm^2). However, F_5-54-2-8-1 had a cortical aerenchyma area similar to HCS, while PLG and the other breeding lines had a few cortical aerenchyma (Fig. 8). Furthermore, the epidermis of PLG was the thickest (55 µm), while HCS and F_5-14-9-5-1 were the thinnest (38 µm and 36 µm, respectively). Thus, the DR anatomy of breeding lines was closer to that of the upland parents than that of lowland rice, especially regarding the xylem area and cortex area.

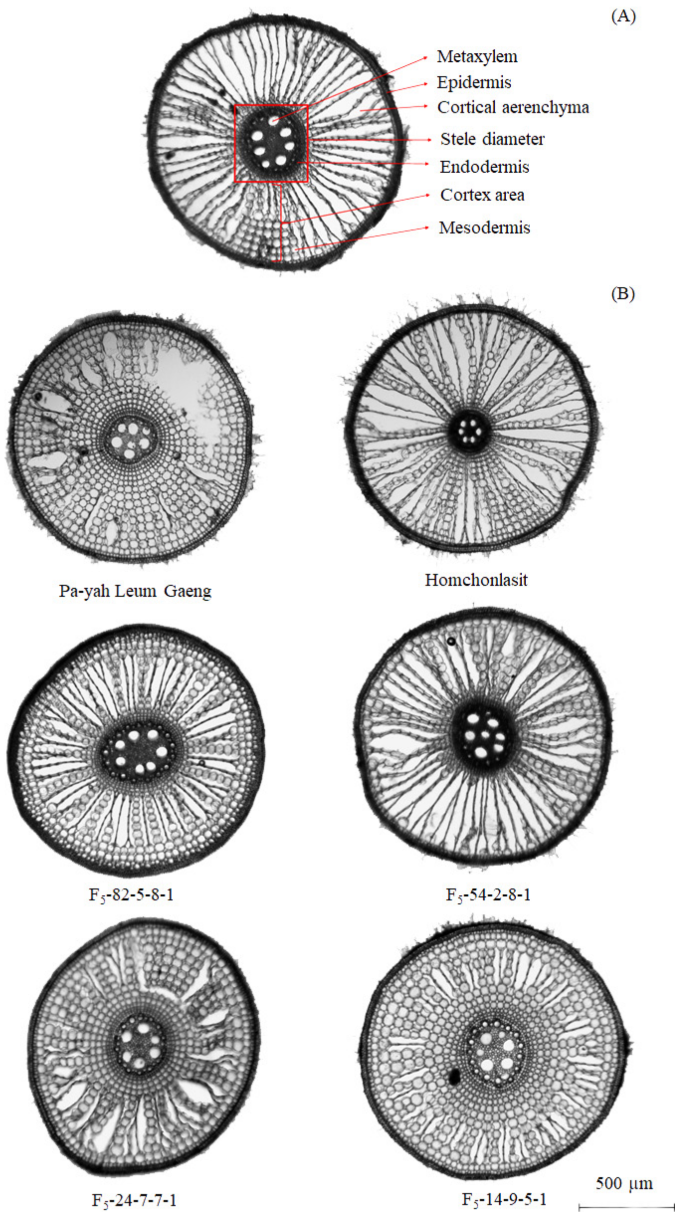

Fig. 8 Rice deep root anatomy at age 60 d: (A) schematic representation of radicle transverse section of root; (B) comparison of root anatomy among four breeding lines and their parents

Table 1 Anatomical traits of deep roots of candidate lines at age 60 d compared with their parents

Line/variety	Xylem No.	Xylem area (μm^2)	Stele diameter (μm)	Endodermis thickness (μm)	Cortex area (μm^2)	Epidermis thickness (μm)	Root diameter (μm)
F_5-82-5-8-1	7^{a}	860^{a}	640^{a}	19^{b}	8.68×1^{05b}	44^{b}	$1,098^{ab}$
F_5-54-2-8-1	7^{a}	640^{abc}	470^{bc}	27^{a}	11.62×10^{5a}	43^{b}	$1,215^{a}$
F_5-24-7-7-1	6^{ab}	550^{c}	510^{b}	17^{bc}	5.16×10^{5c}	41^{b}	855^{c}
F_5-14-9-5-1	5^{b}	330^{d}	440^{c}	16^{c}	4.73×10^{5cd}	36^{c}	849^{c}
Pa-yah Leum Gaeng	6^{ab}	710^{ab}	310^{d}	13^{d}	7.44×1^{05b}	55^{a}	$1,055^{b}$
Homchonlasit	7^{a}	160^{e}	250^{e}	19^{b}	5.92×10^{5c}	38^{c}	907^{c}
F-test	*	*	*	*	*	*	*
Coefficient of variation (%)	7.96	23.64	21.9	15.89	12.4	14.47	7.1

Different lowercase superscripts in each column indicate significantly different ($p < 0.05$)

Evaluation of agronomic traits and grain yield

The plant types of the four selected lines with their parents under AWD and flooded conditions are shown in Fig. 9. The plant height of the four selected lines and PLG in the AWD condition was taller than that for the flooded condition (approximately 34 cm), while the plant heights of HCS between the AWD and flooded conditions were not significantly different. In addition, the plant height of PLG in flooded conditions was the shortest (50 cm), as shown in Fig. 7E. The numbers of tillers/plant and panicles/plant of all lines/varieties for the flooded condition were higher than for the AWD condition, except for line F_5-24-7-7-1. In addition, both tillers/plants and panicles/plant of HCS had the highest values for the flooded condition (Figs. 7F–G). The grain weight of the four selected lines was slightly different between the AWD and flooded conditions (Fig. 7H). However, the grain yield of HCS was the highest under flooded conditions (5.31 t/ha) and lowest under AWD conditions (3.06 t/ha), while PLG under flooded conditions was the lowest (3.03 t/ha). Therefore, HCS was not suitable to grow under AWD condition, while PLG was not suitable to grow under flooded conditions. The grain yields of F_5-82-5-8-1, F_5-54-2-8-1 and HCS under flooded conditions were higher than those under AWD conditions. On the other hand, F_5-24-7-7-1 and F_5-14-9-5-1 did not have significantly different grain yields between the AWD and flooded conditions (Fig. 7I). Thus, these two selected lines showed good performance in terms of yield under both conditions. In addition, the grain yield of PLG for the AWD condition was higher than that for the flooded condition. Finally, the water use efficiency (WUE) showed the same trend as the grain yield results, except that the WUE values of F_5-24-7-7-1 and F_5-14-9-5-1 for the AWD condition were better than that for the flooded condition (Fig. 7J).

Discussion

In climate change situations, semi-irrigated aerobic systems, such as AWD systems of rice cultivation, have been suggested as a means of potential water-saving in agronomy to increase crop productivity. However, it has not become popular because currently available rice varieties are not suitable to sustain yield levels under AWD (Sandhu et al., 2017; Dharmappa et al., 2019). The modification of the root system architecture of presently cultivated rice genotypes with plasticity in their root systems in response to changes in the ecosystem may increase the chances of improving water savings as well as yield under AWD (Sandhu et al., 2017). Therefore, breeding for rice with equal SR and DR densities should be considered an efficient water management strategy in which roots capture water and nutrients in both the shallow and deep soil layers. In this study, the breeding program combined DR from upland rice varieties (PLGs) and SR from lowland rice varieties (HCSs) and the RDR and TR were used as the main criteria for selection based on the pedigree method.

In the early generation, the RDR and TR were segregated in the same pattern and same range, presenting a normal distribution. In addition, the RDR and TR showed transgressive segregation. This segregation pattern showed the same result as Uga et al. (2011, 2015). Furthermore, the condition to select root traits in this study was conducted in flooded soil in which HCS produced more shallow roots (149 SR roots, 38 DR roots), while PLG had more deep roots (53 SR roots, 104 DR roots), as shown in Fig. 2. It has been confirmed that upland varieties are typically characterized as deep root systems, while lowland varieties have shallow roots (Ling et al., 2002). This result suggested that the root angle distribution in the F_2–F_4 populations was the result of transgressive segregation

Fig. 9 Plant types of four breeding lines and their parents compared between flooded and alternate wetting and drying conditions (AWD)

through the interaction of genetic factors from the HCS and PLG varieties. In addition, the mean TR in three generations between HCS (119 roots) and PLG (106 roots) was not much different, but the segregation in the F_2–F_4 generations showed more transgressive segregation than that of DR (Fig. 3). Therefore, the variations in the RDR and TR were controlled by several genetic factors (Uga et al., 2015; Tomita et al., 2017). However, Oyanagi et al. (1993) suggested that root angle was also affected by several environmental factors, such as gravity, light and water potential.

The basket method together with the root 3D program can help breeders to identify the root angle, although it is more laborious and time-consuming to evaluate root traits than aboveground traits (Uga et al., 2009). The present study highlighted that DR was successful in combining with SR in the four selected lines based on the basket method with RDRs of approximately 49% and 55% for the flooded and AWD conditions, respectively, in the four selected lines. In addition, the DR for the AWD condition increased significantly compared with the flood condition in all selected lines and their parents (Figs. 7A–B). Ruangsiri et al. (2021) reported that the proportion of nodal roots that were more elongated vertically increased under drought stress by 21%, while the RDR under AWD in the present study increased by 6% (Fig 7D).

In terms of root anatomy, the xylem area of breeding lines (330–860 μm^2) was larger than that of HCS (160 μm^2), while the xylem area of PLG was 710 μm^2 (Table 1). This confirmed that larger xylem vessels and thicker roots are characteristics of upland rice . In addition, these results indicated that PLG had the largest stele and metaxylem diameters that corresponded with the results of Kondo et al. (2000). Yambao et al. (1992) suggested that the xylem diameter was likely to be related to the maintenance of conductivity and an increased xylem vessel size has been hypothesized to be a useful trait for improving water extraction from deeper soil layers. However, Phule et al. (2019) observed that the formation of fewer aerenchyma, thickened roots and larger xylem areas were critical anatomical traits associated with aerobic adaptation compared to anaerobic conditions. A reduced aerenchyma but a high proportion of cortex area was observed in the breeding lines (Table 1, Fig. 8). Thus, the breeding lines are more suited to AWD conditions, with a lower soil moisture period than lowland parents. However, Lucob-Agustin et al. (2021) reported that drought reduced the plant's ability to produce aerenchyma following a sudden O_2 deficiency caused by waterlogging. Therefore, the growth of the breeding lines may have been affected by this. As a result, the plant height of the breeding lines in the flooded condition (65–75 cm) was lower than that in the AWD condition (91–101 cm), as shown in Fig. 7E. However, it was surprising that the tillering ability of the breeding lines in the flooded condition was better than in the AWD condition (Fig. 7F).

It has been reported that the AWD system is successful in increasing grain yields (by approximately 10%) in rice production (Sibayan et al., 2018). In some studies, AWD has been shown to provide similar rice yields to those of flooded systems (Yao et al., 2012) or slightly lower yields (Yadav et al., 2012). In the present study, the agronomic and grain yields differed for each line/variety compared between the AWD and flooded conditions. The grain yields of the two lines F_5-82-5-8-1 and F_5-54-2-8-1 and HCS under flooded conditions (4.83 t/ha, 5.13 t/ha and 5.31 t/ha, respectively) were higher than that under AWD conditions (3.88 t/ha, 4.56 t/ha and 3.06 t/ha, respectively). In contrast, the grain yields of the other two lines F_5-24-7-7-1 and F_5-14-9-5-1 were not significantly different between the two conditions, while the grain yield of PLG for the AWD condition was higher than that for the flooded condition. However, the grain yield of the four breeding lines was higher than that of PLG for both conditions. Notably, the grain yields of the four selected lines under AWD (3.88–4.74 t/ha) were higher than that under HCS (3.06 t/ha), as shown in Fig. 7I, indicating that a new breeding line combining SR with DR could be adapted to the AWD system rather than the lowland variety. There was no single good plant type in all lines (Fig. 9). This breeding strategy aimed to combine SR and DR from lowland and upland rice varieties. The selection criteria from F_2 to F_4 did not focus on agronomic traits or plant type. In addition, the root traits were not associated with plant type. However, these selected lines must be continued until F_7–F_8 and they can be used as donor parents in the next breeding program.

Conclusion

Improving breeding lines to produce one that is suitable for AWD conditions by introducing DR characteristics into SR varieties was considered one of the most promising breeding strategies in this study. Four breeding lines were successful in integrating the SR and DR from lowland and upland parents. In addition, the anatomy of DR in the breeding lines was close to that of the upland parent. However, the plant type of these breeding lines was not acceptable. Therefore, these breeding lines must be continually improved for yield and plant type or they could be used as donor parents for the next drought tolerance breeding program.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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