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Research Article Phenotypic Diversity of Root Characteristics in Recombinant Inbred Lines of Cross Between Lowland and Highland Rice Varieties for Drought Tolerance Potential

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Abstract

Background and Objective: Breeding between highland and lowland rice varieties is one of the strategic breeding of lowland rice for enhancing drought-tolerant capacity through root structure improvement. The objective of this study was to evaluate the phenotypic diversity of rice root traits in pot screening compared to the lowland parent. **Materials and Methods:** The basket method was utilized in pot cultivation to evaluate the 100 of F7 Recombinant Inbred Lines (RILs) derived through single seed descent method from a cross between lowland rice, RD49 variety and upland rice, Payaleumgaeng (PLG) variety. The two parents and F7 progenies were evaluated for the number of shallow roots (SRN) and the number of deep roots (DRN), together with other traits which were the number of total roots (TRN), the Ratio of Deep Rooting (RDR), maximum Root Length (RL), Root Dry Weight (RDW), Shoot Dry Weight (SDW), the ratio of Root to Shoot Weight (RSR) and Plant Height (PH). **Results:** The result showed that PLG had significantly higher SRN, DRN, TRN and RDR than RD49. The distribution of these traits showed slightly positive skewness in DRN, RDR, RDW, SDW and RSR and negative skewness in SRN, TRN, RL and PH. However, some lines in this RIL population displayed better performance of root traits compared to both parents. Principal Component Analysis (PCA) of DRN, SRN, TRN and RDR in this population showed a distinctly different pattern among both parents. Most of the selected lines had superior RDR over RD49 and had various root characteristics patterns due to the diverse PCA coordinates. The yield trial of some breeding lines in this cross show superior yield over RD49 under drought-prone cultivation area. **Conclusion:** This study showed broad phenotypic diversity in the population constructed through single seed descent selection for enhancing deep root structure in rice for drought adaptation.

Key words: Basket method, deep root, the ratio of deep rooting, single seed descent, shallow root

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice (Oryza sativa L.), the major source of carbohydrate worldwide especially in Asia, has a less deep rooting structure compared to other cereal crops which cause severe yield losses in the rain-fed areas, particularly in dry season¹⁻⁴. Water deficit problems frequently and increasingly occurred in rice due to climate change in the rain-fed regions⁵. By the causes of soil characteristics and water availability, the rain-fed ecosystem can extremely diverge and which go through extremely higher levels of abiotic stress due to water shortage or crop submergence than in irrigated ecosystem. According to cultivation methods, the formation of rice roots can vary from upland rice which is grown directly and has a deeper root system than in lowland rice¹. For this reason, improving the number of deep roots related to drought resistance in rice is a significant factor to alleviate rice production in rain-fed areas.

Drought is one of the major abiotic stresses reducing plant production because of the effect of the water-limiting environment and changing climate. Drought can either be the cause of major agricultural production losses or be a factor of ecological damage, land desertification and soil erosion⁶. Plants exploit different drought resistance mechanisms when they occur with drought stress^{7,8}, specifically, drought avoidance, the capability to maintain the plants' fundamental normal physiological processes under mild or moderate drought stress conditions⁹⁻¹¹, drought tolerance, the ability to survive a particular level of physiological activities under critical drought stress condition throughout the regulation of thousands of genes and series of metabolic pathways to reduce or repair the resulting stress damage^{9,12-13}, drought recovery, the capability to revive later after a period of severe drought which causes the whole cease of growth, a full loss of turgor and leaf desiccation¹⁰ and drought escape, the capacity to fulfil the plant life cycle before the most severe period of drought initiating by speeding the reproductive stage and hence, fasten the life cycle^{12,14}.

Drought avoidance is the firstly against the morphological mechanism to respond to drought stress and plays an important role in promoting plants' drought resistance¹². Amongst the four drought response mechanisms, drought avoidance is generally related to the approach of drought resistance with root characteristics. The constituent traits of drought avoidance involve deep roots with high levels of branching and penetration, a high root to shoot ratio, the elasticity of leaf rolling, early stomatal closure and high cuticular resistance¹⁵⁻¹⁷. The plant roots are the principal organ

to absorb and translocate water and nutrients from the soil to the plant and therefore the ability to cope with drought stress is mostly subjected to their root architecture of which deep roots can access water from deeper soil layers and so that the plants can avoid from drought stress^{4,18-19}. Hence, the modification of the root distribution of rice from shallow rooting to deep rooting is the strategic approach for a drought resistance breeding program.

Deep rooting is a complicated trait and involves the combination of root growth angle and maximum root length. Thus deep-rooting phenotypes should be evaluated for potential use in the improvement of more drought-resistant crop varieties. To date, the most extensively used method to examine deep rooting is the basket method and the evaluation index of deep rooting is the Ratio of Deep Rooting (RDR)^{20,21}. This method allows the roots to straight growth and quantitative evaluation of shallow and deep rooting²². The roots emerging from the bottom of the hemispherical mesh basket is determined as deep rooting from the frequency of high root growth angle (50-90° as regards to the horizontal) in rice and the sides of the basket mesh as shallow rooting²³. This study evaluated the distribution of the root characteristics in F7 progenies of recombinant inbred lines of a cross between RD49 and Payaleumgaeng (PLG) rice varieties for selection of lines with root characteristics for drought response potential.

MATERIALS AND METHODS

Study area: This experiment was carried out at Rice Laboratory greenhouse, Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand from January-May, 2019.

Plant materials: The two rice varieties, RD49 and PLG as parents and 100 lines of F7 progenies were used in this experiment for evaluation of root traits. RD49 is a lowland rice variety, photoperiod insensitive with high yield, good economic traits and developed by Rice Research Center and got breeding certificate thorough consideration from the research and development committee, Rice Research and Development Office, Thailand on 7th February, 2013²⁴.

PLG is a traditional upland Thai rice variety with good cooking quality and is mainly planted for consumption because of its glutinous, soft and delicious taste²⁵. F7 progenies were developed from the cross between RD49 and PLG through the method of single seed descendent from F2 progeny.

Basket method: For root traits evaluation, F7 progenies were divided into four sets together with two parents in every set and three replications for each set, were used by using the basket method with Randomized Complete Block Design (RCBD). In the basket method, the top width, the bottom width and the height of the basket were 15, 8.5 and 5 cm, respectively and the mesh size is 2 mm and the size of the pot used was 20, 13 and 17 cm for the top width, the bottom width and the height of the pot, respectively. Every set contained 2 parents and 25 lines with 3 replications. The first set of 10 seeds per line were soaked in tap water for 12 hrs and incubated for 72 hrs at room temperature. Pre-germinated seeds were raised into the plastic tray with holes filling with paddy soil and compost and let for 14 days. At the same time, the plastic basket-mesh with the field soil and dipped into the plastic pot which was filled with the same soil. Then, 14 daysold seedlings were transplanted into the basket-mesh inside the pot. Watering every day as required soaking clay soil. Pesticide spraying, (propagate) 20 g per 20 L water, was completed for all sets at two weeks after transplanting and fertilizer application, (N-P₂O₅-K, 15:15:15) 3.35 g per pot, was achieved by date for each set. The ambient temperature was ranging from 31.18-37.10°C during daytime and ranging from 23.80-27.90°C during the nighttime with relative humidity from 64.75-71.13% from January-May, 2019.

Root phenotyping: Sixty days after sowing, the roots of plants were carefully washed to separate the basket from pot and, the parameters of Plant Height (PH) and the maximum Root Length (RL), the length of the longest root penetrating from the bottom of the basket mesh and with the basket height were measured and the number of shallow roots (SRN), the number of deep roots (DRN) and the number of total roots (TRN) were counted as the roots emerging from the bottom of the hemispherical mesh basket was determined as deep rooting (root angle from 50-90°) and roots emerging from the side of the basket mesh as shallow rooting (root angle from 0-50°). Furthermore, the shoot and roots parts were separated and under sun-drying in the greenhouse with paper bags to get Root Dry Weight (RDW), Shoot Dry Weight (SDW) and Root to Shoot weight Ratio (RSR), the dry weight of the total roots divided by the total dry weight of the shoot (upper ground parts). Furthermore, the Percentage ratio of deep root to the total root (RDR) was evaluated as the number of deep roots that emerged from the bottom of the hemispherical basket mesh divided by the number of total roots extending from the whole side of the basket mesh as modified from deep root to shallow root ratio in Nwe et al.25:

$$RDR = \frac{DRN}{TRN} \times 100$$

Frequency distribution analysis with histograms for the traits was done by Microsoft Excel version 2010 and Pearson's correlation and Principal component analysis for the traits was performed with Statistical Tool for Agricultural Research (STAR) version 2.0.1 (IRRI, Los Banos, Laguna, Philippines).

Agronomic characteristics, yield components and grain yields of RILs and female parent: Additional six lines of F7 progenies from 100 lines in the pot test were planted at the Lopburi Research Station, Kasetsart University of upland field condition and collected the agronomic characteristics, yield components and grain yield. Female parent, RD49 was also planted along with these populations as check variety for yield performance.

RESULTS

The root architectures of RILs and two parents were with different performances showed in Fig. 1. The phenotypic values of the RIL population and parents' varieties, RD49 and PLG were shown in Table 1. The phenotypic mean value of RILs in each trait was between the parental values, except for the SRN, RSR and PH. The mean shallow root number (SRN) of the RIL population fell to 119.96 roots, whereas the lowest SRN parent, RD49 had 122.58 roots on averagely. The mean value



Fig. 1: Different root system architecture of RD49, PLG and F7 progenies

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Table 1.1 Herioty	pic description of seven root		and mill population		
	Parents		100 RIL population		
Traits	 RD49	PLG	 Mean±SD	Minimum	Maximum
SRN	122.58	157.33	119.96±37.65	29.67	231.33
DRN	20.42	48.92	26.41±10.14	7.67	55.33
TRN	143.00	206.25	146.11±42.41	38.33	262.67
RDR	14.00	24.00	19.00土7	7.00	38.00
RL	17.25	19.54	17.97±3.14	10.00	24.67
RSR	0.46	0.44	0.38±0.10	0.20	0.63
RDW	2.89	4.40	3.14±1.29	0.93	7.74
SDW	8.06	10.60	8.17±2.42	3.30	14.00
PH	87.28	79.20	106.79±19.69	66.67	144.83

Table 1: Phenotypic description of seve	n root-related traits and two shoot	t traits in two parents	and RIL population
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RIL population: Recombinant inbred lines from RD49×PLG, SD: Standard deviation, SRN: Number of shallow roots, DRN: Number of deep roots, TRN: Number of total roots, RDR: Ratio of deep rooting (%), RL: Maximum root length (cm), RSR: Root to shoot weight ratio, RDW: Root dry weight (g), SDW: Shoot dry weight (g) and PH: Plant height (cm)

of the ratio of root weight to shoot weight of RIL population was 0.38, while RD49 had this ratio for 0.46 and PLG had for 0.44. In contrast, the average plant height of the RIL population was higher than both parents at 106.79 cm. The shoot traits and root traits of the two parents were different. The male parent, PLG, developed larger root architectures which had higher SRN (157.33>122.58 roots), DRN (48.92>20.42 roots), TRN (206.25>143.00 roots), RDR (24>14%), RL (19.54>17.25 cm), RDW (4.40>2.89 g) and SDW (10.60>8.06 g), except RSR (0.44<0.46) and PH (79.20<87.28 cm) because of higher shoot of the female parent, RD49.

For the RIL population, the average SRN of lines in this RIL population varied from fewer than 30 roots up to 231 roots per plant. DRN of the population showed the range between 7-55 roots per plant and of which TRN expressed less than 38 and more than 262 roots per plant. RDR of RIL ranged from 7-38%. RL, RSR, RDW, SDW and PH of the F7 population conveyed the lower and upper limit of 10-24 cm, 0.20-0.63, 0.93-7.74 g, 3.30-14.00 g and 66-144 cm, respectively.

The population displayed significant better performance of root traits in some lines compared to both parents. As these lines were developed through the single seed descent method, the selection process has not been performed. Therefore, the distribution of this RIL population reflects the genetic variation of this bi-parental population. The frequency distribution of the evaluated traits is expressed as continuous variation with the negative skewness for SRN in Fig. 2a. The range of SRN frequency distribution was from 29.67-238.48 roots with the highest frequency at 134.07 roots, while the mean value was at 113.85 roots. There was a positive skewness frequency distribution for DRN in Fig. 2b. The range of DRN frequency distribution was from 7.67-58.07 roots with the highest frequency at 32.87 roots, whereas the mean value was at 37.02 roots. The range of TRN frequency distribution in Fig. 2c was from 38.33-279.13 roots with the mode value at 188.83 roots, while the mean value was at 178.22 roots. Therefore, the TRN frequency distribution was negatively skewed. The frequency distribution of RDR in Fig. 2d was positively skewed distribution which had a range of data from 7-38.5% with the highest frequency at 16.0% and the mean value was at 19.3%. The frequency distribution of RDW in Fig. 2e was a slightly positively skewed distribution having the range of RDW from 0.93-8.73 g with a higher mean value (3.60 g) than the mode value (3.53 g). For the frequency distribution of SDW in Fig. 2f, the distribution was also positively skewed ranging from 3.3-15.41 g with a higher mean value (9.02 g) than the mode value (6.76 g). Moreover, the frequency distribution of RSR in Fig. 2g was positively skewed ranging from 0.200-0.698 with a higher mean value (0.424) than the mode value (0.366). However, the frequency distribution of RL in Fig. 2h was negatively skewed ranging from 10-27.335 cm with a lower mean value (18.0 cm) than the mode value (20.4 cm). The frequency distribution of PH in Fig. 2i was also negatively skewed ranging from 66.67-151.27 cm with a lower mean value (121.97 cm) than the mode value (130.12 cm).

RDR trait distribution in this experiment showed positively skewness indicating of more lines tended to have a low deep root ratio as RD49. Interestingly, among 100 RILs, fifteen lines showed more RDR than PLG which was the highland variety. For this reason, these lines were selected as shown in Table 3 for further field investigation for drought response potential.

The coefficients of pairwise correlation between seven root-related traits and two shoot-related traits in the RIL population were calculated in Table 2. Highly significant positive correlations (p < 0.01) were observed among the traits. Pak. J. Biol. Sci., 24 (11): 1152-1161, 2021



Fig. 2(a-i): Frequency distribution for traits in F7 progenies, (a) Number of shallow roots (SRN), (b) Number of deep roots (DRN), (c) Number of total roots (TRN), (d) Ratio of deep rooting (%) (RDR), (e) Root dry weight (g) (RDW), (f) Shoot dry weight (g) (SDW), (g) Root to shoot weight ratio (RSR), (h) Maximum root length (cm) (RL) and (i) Plant height (cm) (PH) Letters, R and P, specify the values of the two parents, RD49 and PLG, respectively. The X-axis indicates the value of the trait and the Y-axis indicates the frequency of values

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Table 2: Coefficients of Pearson' product-moment correlation between seven root-related traits and two shoot traits in the RIL population

Traits	SRN	TRN	RDR	RL	PH	RDW	SDW	RSR
DRN	0.35**	0.55**	0.60**	0.44**	-0.03 ^{ns}	-0.22 ^{ns}	-0.08 ^{ns}	-0.16 ^{ns}
SRN		0.97**	-0.47**	0.56**	0.02 ^{ns}	-0.08 ^{ns}	-0.03 ^{ns}	0.01 ^{ns}
TRN			-0.28**	0.59**	0.03 ^{ns}	-0.13 ^{ns}	-0.05 ^{ns}	-0.02 ^{ns}
RDR				-0.04 ^{ns}	-0.01 ^{ns}	-0.16 ^{ns}	-0.03 ^{ns}	-0.19 ^{ns}
RL					0.03 ^{ns}	-0.20*	0.003 ^{ns}	-0.30**
PH						-0.01 ^{ns}	-0.02 ^{ns}	-0.12 ^{ns}
RDW							0.72**	0.67**
SDW								0.14 ^{ns}

SRN: Number of shallow roots, DRN: Number of deep roots, TRN: Number of total roots, RDR: Ratio of deep rooting (%), RL: Maximum root length (cm), RSR: Root to shoot weight ratio, RDW: Root dry weight (g), SDW: Shoot dry weight (g) and PH: Plant height (cm), **Significant correlation at p<0.01.*Significant correlation at p<0.05 and n®No significant correlation

Table 3: Extreme traits: SRN, DRN, TRN and RDR of selected RIL in the F₇ population

Lines/varieties	SRN	DRN	TRN	RDR	Colour
RD49	122.58	20.42	143.00	14	Red
PLG	157.33	48.92	206.25	24	Blue
F-test	ns	**	**	**	
CV (%)	29.99	31.01	26.23	28.19	
RP3-118-1	231.33 ^{ns}	31.33	262.67 ^{ns}	12	Green
RP3-138-1	189.00 ^{ns}	38.33	227.33*	17	Green
RP3-20-5	202.67 ^{ns}	28.00	230.67 ^{ns}	12	Green
RP3-54-1	182.00**	29.00	211.00**	14	Green
RP3-11-1	145.00	55.33**	200.33	28*	Purple
RP3-108-2	96.33	53.67*	150.00	36*	Purple
RP3-71-2	129.33	45.33	174.67	26 ^{ns}	Black
RP3-78-1	45.33	21.67	67.00	32**	Black
RP3-25-1	78.33	38.67	117.00	33*	Black
RP3-109-3	78.00	29.00	107.00	27*	Black
RP3-50-3	63.67	23.00	86.67	25 ^{ns}	Black
RP3-46-2	54.00	27.00	81.00	45 ^{ns}	Black
RP3-57-3	64.00	38.67	102.67	38 ^{ns}	Black
RP3-98-3	98.67	34.00	132.67	26 ^{ns}	Black
RP3-33-1	61.67	22.00	83.67	26 ^{ns}	Black
RP3-123-1	118.67	44.33	163.00	27*	Black
RP3-26-2	37.33	21.67	59.00	37**	Black
RP3-132-1	139.67	46.33	186.00	25 ^{ns}	Black
RP3-79-1	122.00	47.00	169.00	28**	Black

T-test was performed between the value of RIL and the value of RD49 in the same experimental set. **Significant difference between mean at p<0.01. *Significant difference between mean at p<0.05 and ∞ Significant difference between mean at p>0.05

DRN was significantly correlated with SRN (0.35**). Thus, in this experiment, PLG had both DRN and SRN higher than RD49. SRN was significantly correlated with TRN (0.97**) and RL (0.56**), then negatively correlated with RDR (-0.47**) and not correlated with others. The highest correlation between SRN and TRN (0.97**) was observed indicating SRN represents the most of root structure in this population. Furthermore, PH was not correlated with other root traits and shoot-related traits. RDW, SDW and RSR were not correlated with other root traits but RL was negatively correlated with RDW (-0.20*) in this study. Thus, it can be interpreted that plant with more root length tends to have lower root density. Interestingly, RDW was significantly correlated with SDW (0.72**) which indicates the balance between root and shoot growth under well-watered conditions. Principal component analysis in Fig. 3 showed the different root characteristics, DRN, SRN, TRN and RDR, between PLG and RD49. However, considering RILs with the extreme values as reported in Table 3, they had diverse root characteristics among other ones which are required to be evaluated in the drought condition in the field trial for identification of suitable root characteristics for drought response. The basket method used in this study was effective to assess the plant in greenhouse conditions and easy to measure the root related traits. As all traits in this study showed approximately normal distribution, they might be polygenic traits controlled by multiple genes. Even though RDR distributed in positive skewness, it showed the high number of lines with better performance than PLG. Therefore, nineteen RILs were selected from this



Fig. 3: Principle component analysis of SRN, DRN, TRN and RDR in RIL and two parents PC1 explains 57.5% of total variance and PC2 explains 40% of the total variance. Coordinates with grey colour were unselected lines

screening according to the superior performance of root traits: SRN, DRN, TRN and RDR over both parents as shown in Table 3. Among these selected lines, RP3-118-1, RP3-138-1, RP3-20-5 and RP3-54-1 (the green symbols in Fig. 3) had average SRN and TRN higher than those of RD49 (the red symbol in Fig. 3) and PLG (the blue symbol in Fig. 3). The range of average SRN of these four lines were 182.00-231.33 roots, while SRN in RD49 was 122.58 roots and SRN in PLG was 157.33 roots. The range of average TRN of these four lines were 143.00-206.25 roots, while there were 211.00 roots in RD49 and 262.67 roots in PLG. RP3-11-1 and RP3-108-2 (the purple symbols in Fig. 3) had higher average DRN (53.67 and 55.33 roots) than those of both parents (20.42 roots in RD49 and 48.92 roots in PLG). There were 15 lines (The purple and black symbols in Fig. 3) with higher average RDR (ranged from 25-45%) than those of both parents (14% in RD49 and 24% in PLG). However, in the PCA, these two lines were still indifferent coordinates. Even though there was a correlation between RDR, SRN and DRN, the PCA plot showing no cluster constructed in these RILs. These scattering coordinates indicate the diverse phenotypic root traits in this RIL population.

For investigating the yield potential of a cross between RD49 and PLG, six of F7 RILs in addition to 100 RILs in the pot screening were randomly selected and grown in the upland field in Table 4. Among six progenies and female parent, RD49, all the components were significantly different from each other except panicle length. For grain yield, the RP3-92-3 line demonstrated the highest values among RIL in this yield trial concerning 100-grain weight (2.36 g), seed setting rate (72.39%) and harvest index (0.29), which were relatively comparable to RD49 (Table 4), especially for the 100-grain weight (2.36>2.32 g) and harvest index (0.29>0.24) of RP3-92-3 which tended to be higher than those of RD49 even though they were not significantly different.

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Table 4: Agron	omic characters,	, yield componer	nts and grain yi	eld of recombinant	t inbred lines and low	land parent, RD49					
	Plant	Flag leaf	Panicle	Number of tillers	Number of panicles	Number of spike-	Number of filled	Seed setting	100 grains	Har-vest	Grain yield
Varieties	height	length (cm)	length (cm)	per plant	per plant	let per panicle	grain per panicle	rate (%)	weight (g)	index	per plant (g)
RP3-92-3	52.4 ^b	20.80 ^{bc}	22.60	23.0 ^b	21.20 ^b	78.40^{b}	57.20 ^b	72.39 ^{ab}	2.36^{a}	0.29ª	15.24^{a}
RP3-92-8	49.6 ^{bc}	32.80ª	21.60	17.4 ^b	16.00°	66.80 ^b	39.20 ^{bc}	57.46 ^{bcd}	1.63^{a}	0.17 ^{bcd}	5.99
RP3-92-9	44.2 ^d	35.80ª	19.00	22.8 ^b	19.20 ^b	65.00 ^b	28.60⁵	45.22 ^d	1.78 ^{bc}	0.10 ^d	3.91 ^c
RP3-92-22	46.0 ^{cd}	31.40^{ab}	23.20	33.0ª	28.20ª	73.20 ^b	38.00 ^{bc}	54.55 ^{cd}	1.70 ^{bc}	0.13 ^{cd}	8.87 ^{bc}
RP3-92-29	52.4 ^b	30.20 ^{abc}	23.20	$24.4^{\rm b}$	21.60 ^{ab}	82.40^{b}	52.20 ^{bc}	63.35 ^{abc}	1.61 ^c	0.18 ^{bc}	8.73 ^{bc}
RP3-111-4	50.2 ^{bc}	20.00℃	19.60	$24.4^{\rm b}$	22.60 ^{ab}	72.80^{b}	36.80 ^{bc}	49.25 ^{cd}	2.00 ^b	0.14 ^{cd}	6.39 ^c
RD49	58.8 ^a	35.20 ^a	22.60	20.2 ^b	$16.60^{\rm b}$	111.6 ^a	83.00ª	75.02 ^a	2.32 ^a	0.24 ^{ab}	12.56 ^{ab}
F-test	**	*	ns	**	**	*	**	**	**	**	**
CV (%)	7.11	26.74	11.57	20.78	23.59	28.59	34.41	19.55	12.64	27.93	43.42
*Significant at	0.05 probability	level. ** Significa	int at 0.01 prob.	ability level. Within	a column for each ch	naracter, values follo	wed by the same lette	ers are not signific	antly according	to DMRT (p<0.	15)

DISCUSSION

RDR was reported to be the key trait for the identification of the deep rooting potential of rice in the basket method in hydroponic solution⁴. In this experiment, RIL population and parents were evaluated in basket method as well but under the well-watered condition in the pot to observe the genetic variation of root architecture of RILs because this method will have to classify deep rooting and shallow rooting via the root angle that causes the root passing the side and the bottom of the basket. Therefore, drought stress was not applied in the basket method. Moreover, drought stress was not set for this pot screening to avoid genotype by environment interaction factor to affect root structure.

Among root traits, especially deep roots are crucial role in plant drought avoidance mechanism for water absorbing¹ but there have been only a few studies and needed to know about deep rooting^{4,26-29}. PLG is the deep root variety. It also can express the higher DRN than RD49 in this basket method even though there was no drought condition in this experiment. Therefore, DRN is the trait that expresses due to genotypic variation which makes PLG and its progenies adapted well under drought-prone areas due to the deep root system. There were reports of environmental effects of cultivation method and soil condition^{1,18,29-31} on root structure. Therefore, in this experiment, PLG responded well under this condition and had more shallow roots and deep roots than RD49. Thus, the environmental effect on root characteristics is interesting to be investigated more in future research. DRN was also reported to be twice as much of SRN in the F2 population in Nwe et al.25 which was a cross between Hom Chonlasit variety and PLG variety even though the plant was harvest at sixty days after sowing as in this experiment. It might be due to the higher homozygosity level in the F7 population in this experiment compared to F2 of Nwe et al.25 or because of the different female parents that caused the difference in the average ratio of deep rooting to shallow rooting. If considering overall root numbers, it could not count only the number of deep or shallow roots. Thus, RDR was required to compare among lines/ varieties^{20,21}.

The deep root system was related to PH and inversely related with tiller number in 1081 rice accessions¹⁸. The RL was related to PH under irrigated and water stress conditions by using the NIL population with a chromosome segment comprising QTL study for root length (between RM 242 and RM 201) on chromosome 9³⁰. However, in this RIL population, RL did not correlate with PH. This result indicated that

morphological characteristics are different in different populations. Therefore, root traits were quantitative traits that were commonly controlled by many genes as shown in QTLs studied in rice³¹. Moreover, the variations among these traits could be affected by the environment as well. More importantly, this screening condition was in the pot which was different from the drought condition in the field. Thus, this report is the response of the RIL population under the well-watered condition in the pot. The water deficit response of these root traits under drought conditions in the field will be further investigated in the selected RILs from this experiment for lines with high DRN and RDR as the key root structure for drought response potential in the field.

In numerous upland rice experiments of grain yield analysis with or without stress conditions at the reproductive stage, rice lines/varieties exhibited some genomic regions related to yield components³². The higher yield was correlated with higher panicle numbers and separated of total grain weight³³. It suggested the good performance for yield potential of the cross between RD49 and PLG under upland field condition where the selected lines from table 3 will be evaluated in the further experiment to investigate the potential of root structure on yield performance under drought conditions in the field trial.

CONCLUSION

In summary, recombinant inbred lines from a cross between RD49 and PLG showed diverse root characteristics from the basket method which evaluates shallow rooting and deep rooting traits through the angle of the root passing through the basket. Principal component analysis of DRN, SRN, TRN and RDR could be used to distinguish between the root architect of RD49 and PLG. However, for RILs of this bi-parental cross, clusters could not be distinguished according to diverse root characteristics. RDR was suggested for root related trait evaluation in this F7 population even though it had a positive skewness distribution, some lines showed superior RDR over both parents. Thus, the basket method can be used for pot screening of population to evaluate root traits under wellwater conditions and select lines with higher RDR over parents which should be further investigated in the field trial with drought conditions for the drought-responsive potential of these root traits as yield trial of some F7 lines of this biparental cross tended to have higher yield performance over RD49 under upland field condition.

SIGNIFICANCE STATEMENT

This study discovered the diverse phenotypic root characteristics in RILs developed through the single seed descent method that can be beneficial for rice programs for drought tolerance. The methodology in cultivar development and root trait evaluation for the selection of lines with high drought adaptive potential will help the breeders or researchers uncover the critical areas of rice breeding for drought tolerance. In addition, the phenotypic diversity of root characteristics from the population developed in this study is suitable for further study in quantitative trait loci mapping for these root traits.

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REFERENCES

- Gowda, V.R.P., A. Henry, A. Yamauchi, H.E. Shashidhar and R. Serraj, 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crops Res., 122: 1-13.
- Kondo, M., M.V.R. Murty and D.V. Aragones, 2000. Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. Soil Sci. Plant Nutr., 46: 721-732.
- Kondo, M., P.P. Pablico, D.V. Aragones, R. Agbisit, J. Abe, S. Morita and B. Courtois, 2003. Genotypic and environmental variations in root morphology in rice genotypes under upland field conditions. Plant Soil, 255: 189-200.
- 4. Uga, Y., K. Okuno and M. Yano, 2011. Dro1, a major QTL involved in deep rooting of rice under upland field conditions. J. Exp. Bot., 62: 2485-2494.
- Henry, A., A.J. Cal, T.C. Batoto, R.O. Torres and R. Serraj, 2012. Root attributes affecting water uptake of rice (*Oryza sativa*) under drought. J. Exp. Bot., 63: 4751-4763.
- 6. Fang, Y. and L. Xiong, 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. Cell. Mol. Life Sci., 72: 673-689.
- Kooyers, N.J., 2015. The evolution of drought escape and avoidance in natural herbaceous populations. Plant Sci., 234: 155-162.

- Lawlor, D.W., 2013. Genetic engineering to improve plant performance under drought: Physiological evaluation of achievements, limitations and possibilities. J. Exp. Bot., 64: 83-108.
- 9. Luo, L.J., 2010. Breeding for water-saving and drought-resistant rice (WDR) in China. J. Exp. Bot., 61: 3509-3517.
- Araus, J.L., G.A. Slafer, M.P. Reynolds and C. Royo, 2002. Plant breeding and drought in C₃ cereals: What should we breed for? Ann. Bot., 89: 925-940.
- 11. Blum, A., 2005. Drought resistance, water-use efficiency and yield potential-are they compatible, dissonant or mutually exclusive? Aust. J. Agric. Res., 56: 1159-1168.
- 12. Mitra, J., 2001. Genetics and genetic improvement of drought resistance in crop plants. Curr. Sci., 80: 758-763.
- Hussain, S., S. Hussain, T. Qadir, A. Khaliq and U. Ashraf *et al.*, 2011. Drought stress in plants: An overview on implications, tolerance mechanisms and agronomic mitigation strategies. Field Crops Res., 122: 1-13.
- Manavalan, L.P., S.K. Guttikonda, L.S.P. Tran and H.T. Nguyen, 2009. Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell Physiol., 50: 1260-1276.
- 15. Bristiel, P., C. Roumet, C. Violle and F. Volaire, 2019. Coping with drought: Root trait variability within the perennial grass *Dactylis glomerata* captures a trade-off between dehydration avoidance and dehydration tolerance. Plant Soil, 434: 327-342.
- 16. Samson, B.K., M. Hasan and L.J. Wade, 2002. Penetration of hardpans by rice lines in the rainfed lowlands. Field Crops Res., 76: 175-188.
- 17. Wang, H., Y. Inukai and A. Yamauchi, 2006. Root development and nutrient uptake. Crit. Rev. Plant Sci., 25: 279-301.
- Henry, A., 2013. IRRI's drought stress research in rice with emphasis on roots: Accomplishments over the last 50 years. Plant Root, 7: 92-106.
- Kamoshita, A., C.R. Babu, N.M. Boopathi and S. Fukai, 2008. Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crops Res., 109: 1-23.
- Kato, Y., J. Abe, A. Kamoshita and J. Yamagishi, 2006. Genotypic variation in root growth angle in rice (*Oryza sativa* L.) and its association with deep root development in upland fields with different water regimes. Plant Soil, 287: 117-129.
- Uga, Y., E. Hanzawa, S. Nagai, K. Sasaki, M. Yano and T. Sato, 2012. Identification of qSOR1, a major rice QTL involved in soil-surface rooting in paddy fields. Theor. Appl. Genet., 124: 75-86.

- 22. Han, J.H., N.H. Shin, J.H. Moon, J.H. Chin and S.C. Yoo, 2016. A simple phenotyping method for deep-rooting rice grown in pots. J. Plant Biotechnol., 43: 444-449.
- 23. Hanzawa, E., K. Sasaki, S. Nagai, M. Obara and Y. Fukuta *et al.*, 2013. Isolation of a novel mutant gene for soil-surface rooting in rice (*Oryza sativa* L.). Rice, Vol. 6. 10.1186/1939-8433-6-30.
- 24. Rattanarat, A., T. Wangsawang, W. Matthayathaworn, R. Changsri and F. Worede *et al.*, 2020. Combining ability test of parental lines for three-line hybrid rice breeding. Agric. Nat. Resour., 54: 431-438.
- 25. Nwe, Z.M., W. Phormmard, S. Arikit, T. Sreewongchai and C. Malumpong 2020. Phenotypic evaluation of root angle in F2 segregation by crossing between upland and lowland rice (*Oryza sativa*). KHON KAEN Agr. J., Vol. 47.
- Uga, Y., K. Sugimoto, S. Ogawa, J. Rane and M. Ishitani *et al.*, 2013. Control of root system architecture by deeper rooting 1 increases rice yield under drought conditions. Nat. Genet., 45: 1097-1102.
- Uga, Y., Y. Kitomi, E. Yamamoto, N. Kanno, S. Kawai, T. Mizubayashi and S. Fukuoka, 2015. A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of DEEPER ROOTING 1.Rice, Vol. 8. 10.1186/s12284-015-0044-7.
- Kitomi, Y., N. Kanno, S. Kawai, T. Mizubayashi, S. Fukuoka and Y. Uga, 2015. QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of DEEPER ROOTING 1. Rice, Vol. 8. 10.1186/s12284-015-0049-2.
- 29. Lou, Q., L. Chen, H. Mei, H. Wei and F. Feng *et al.*, 2015. Quantitative trait locus mapping of deep rooting by linkage and association analysis in rice. J. Exp. Bot., 66: 4749-4757.
- 30. Siopongco, J.D., A. Yamauchi, H. Salekdeh, J. Bennett and L.J. Wade, 2006. Growth and water use response of doubled-haploid rice linesto drought and rewatering during the vegetative stage. Plant Prod. Sci., 9: 141-151.
- Suji, K.K., K.S.J. Prince, P.S. Mankhar, P. Kanagaraj and R. Poornima *et al.*, 2012. Evaluation of rice (*Oryza sativa* L.) near iso-genic lines with root QTLs for plant production and root traits in rainfed target populations of environment. Field Crops Res., 137: 89-96.
- Lafitte, H.R. and B. Courtois, 2002. Interpreting cultivar×environment interactions for yield in upland rice: Assigning value to drought-adaptive traits. Crop Sci., 42: 1409-1420.
- Lafitte, H.R., A.H. Price and B. Courtois, 2004. Yield response to water deficit in an upland rice mapping population: Associations among traits and genetic markers. Theor. Appl. Genet., 109: 1237-1246.