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Root Responses to Water Deficit under Rain-fed Lowland Rice

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ABSTRACT

Drought is a major problem for rice grown under rainfed lowland conditions. The ability of rice plants to tolerate drought stress is associated with root system characters. However, genetic of root traits under lowland condition was uncertain. To determine the performance of root characteristic response to drought tolerance, a total of 220 double haploid lines, their parents (CT9993 and IR62266), and three standard checks (IR20, NSG19 and KDML105) were used in the experiments. The extent of genetic variation in root characters, relative water content, visual leaf rolling and drought injury under different intensities of water deficit were determined. Genotypes with short root were more dehydration tolerant than the longer root genotype, consequently more relatively high water content and delayed leaf rolling and senescence under severe water deficit.

Key words: rice, rainfed lowland, double haploid lines, relative water content, drought

INTRODUCTION

Rain-fed lowland rice is mostly grown in South and Southeast Asia, and more than 50% is under drought-prone conditions (Garrity and O' Toole, 1994). Drought is a major factor determining productivity in rain-fed lowland rice. The incidence of drought was measured by timing, duration and severity at specific locations over several years. In relation to the timing of plant growth and development, drought can be classified as vegetative, reproductive, and terminal. Drought may delay the phenological development of the rice plants and may also affect the physiological processes of transpiration, photosynthesis, respiration, and translocation of assimilates to the grain (Fukai and Cooper, 1995). Drought also strongly affects the morphology of the rice plant. Leaf area development may be hampered due to reduced leaf expansion, leaf rolling, early senescence, suppressed tillering (O'Toole and Namuco, 1983).

Increased soil strength under reduced soil moisture and the presence of hardpans in the subsoil of rain-fed lowlands make it difficult for roots to gain access to deep soil moisture. Under such conditions, roots with higher penetration ability have an advantage for absorbing water from deeper soil layers. Genotypic variation in root penetration and other root traits have been reported in rice (Yu *et al*., 1995; Nguyen *et al*., 1997). Increased rooting depth, root density, root

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shoot ratio, root pulling force and penetration ability through hardpans are reported to be major drought resistance traits associated with the root systems in rice.

Visual leaf rolling score is an efficient method for detecting drought avoidance and this can be used as an indirect estimate of drought resistance. Visual drought scoring by an experienced researcher based solely on leaf desiccation is apparently quite effective in discriminating drought avoidance in rice (O'Toole and Moya, 1978). This field study was conducted to determine the performance of root characteristic response to drought stress conditions in rainfed lowland rice.

MATERIALS AND METHODS

Genetic materials

The rice breeding lines, CT9993-10-1-M and IR62266-42-6-2, differ consistently for a range of traits as expressed under drought stress and non-stress conditions (Babu *et al.*, 2001). These traits include gross root morphology, root penetration index (RPI) and osmotic adjustment (OA). A double haploid line (DHL) population was developed through anther culture from a cross between CT9993-10-1-M (abbreviation as CT9993, an upland japonica ecotype possessing a deep and thick root system and low OA) and IR62266-42-6-2 (abbreviated as IR62266, an indica ecotype with a shallow root system and high OA), at Centro International de Agricultura Tropical (CIAT), Columbia, and International Rice Research Institute (IRRI), Philippines. The 220 DHLs, parental lines and standard checks; IR 20, NSG19, KDML105 were used in this study.

Experimental design and cultural practice

The experiment was conducted under lowland rice conditions at Ubon Ratchthani Rice Research Center (latitude 15° 19' 52.35'' N, Longitude 104° 40' 55.15'' E, altitude 110m), located in Northeast Thailand during the 2000-2001 dry season. The soil texture was sandy loam, acidic, infertile and low in organic matter. The plants were seeded on 22 December 2000. The populations were randomly allocated in 3 replications in a randomized complete block design, and every 7 lines, KDML105 and NSG19 were grown as running checks. Individual plot size was 0.84 m^2, which consisted of 4 rows, 15 cm apart, 1.4 m in length, 14 hills per row. Hills were 0.1m apart within each row.

Surface irrigation was applied until vegetative stage (54 days after sowing, DAS) and the first group of data which represent well water condition was collected before drought stress was applied. To induce drought stress, flooded water was drained out of the field. Then the data were collected again as mild stress and severe stress condition 14 days and 24 days after drought was induced, respectively (68 DAS and 78 DAS). To induce recovery condition, water was pumped into the field as surface flood for 7 days and the data were collected as recovery condition (85 DAS).

Measurements

Relative water content (RWC): At specific time intervals (predawn 01.00 – 05.00 am and midday 10.30 am – 3.00 pm) mature leaf tissue was excised from tillers in each experimental plot for all lines and all water conditions. Three mature, fully expanded leaves were used. The leaves were excised at the base, while the top of each leaf was trimmed to make them equal in length. To determine RWC, the 3 leaf samples were excised about 1 cm^2 in size, and immediately weighed them in a hermetically sealed container, floated in distilled water until fully re-hydrated, weighed, and then dried them until a constant oven-dry weight was obtained. The data obtained was computed for RWC according to Turner (1982) as follows:

$$\text{RWC} = \frac{(\text{Fresh Weight - Dry Weight})}{(\text{Turgid Weight - Dry Weight})} \times 100$$

Leaf rolling and drought score: Plants were evaluated for leaf rolling and drought score, to assess the effects of drought. Evaluation began when the most susceptible entries had tightly rolled leaves at midday (10.00 am - 3.30 pm). A rating of leaf rolling score was visually estimated in each plot using a 1 - 5 scale, where a score of 1 was no rolling and 5 was completely rolled (O'Toole and Moya, 1978). Rating of drought scores (0 - 9) was estimated for each plot based on symptom of leaf drying on the plants. A score of 0 indicated no symptoms of stress, with an increasing score where more leaves die due to water deficit (IRRI, 1975). A score of 5 indicated that 50% of the entire leaves was fully dried. The maximum score of 9 indicated that all plants were apparently dead.

Root mass : Root mass density (RMD) and total root mass were determined after recovery period (90 DAS). The method and technique for the determination of root system was developed by Pantuwan *et al.* (1997). Two adjacent hills were randomly selected before taking measurements. A 38 mm (inner diameter) steel tube was placed next to a hill with less than 1 cm between the closest tiller and the tube. The soil column was sampled at 45 cm deep, collected and cut the soil into three sections at the depth of 0-15, 15-30 and 30-45 cm, respectively. The second soil column was taken from another hill using the same procedures. Soil samples were placed on 1 mm mesh screen and roots were washed to remove soil using tap water. Roots were dried in a hot-air oven at 70°C for 48 h and weighed to determine root dry mass.

Plant height : After recovery period , plant height and tiller number were randomly measured on 10 hills in each plot. The height was measured from the soil surface to the tip of tallest panicle within each hill and tiller numbers were counted on 10 hills sampled independently.

RESULTS

Genotypic variation in root characteristics and plant height

Root mass densities (RMD) of rice genotype were significantly different at depths of 15-45 cm in the soil (Table 1). The highest RMD of rice was located at 0-15 cm soil depth. The parent, CT9993 had higher RMD (0.214 mg cm^{-3}) at this depth than that of IR62266 (0.098 mg cm^{-3}). Mean RMD of the DHLs was 0.150 mg cm^{-3} (0.041 - 0.352 mg cm^{-3}). Three standard checks (IR20, NSG19 and KDML105) revealed that RMD was not significantly different for all depths in the soil. Total root mass (TRM) and root mass distribution (%RMD) was significantly different among DHLs at all depths in the soil. Mean TRM of the DHLs was 131.7 gm^{-2} (68.0-228.0 gm^{-2}). %RMD was 80.95 % (62.33-94.57 %) at the depth of 0-15 cm; 17.18% (5.28-33.56 %) at the depth of 15-30 cm, and 1.84 % (0.08-9.62 %) at the depth of 30-45 cm. These three standard checks did not produce significantly different result.

Mean plant height was 37 cm for IR62266 and 45 cm for CT9993, while mean plant height of the population was 42 cm (SEM = ± 4 cm). There was a positive relationship between plant height and RMD (r = 0.212**, 0.226** and 0.158* for RMD at 0-15, 15-30 and 30-45 cm of soil depth, respectively) and TRM (r = 0.251**) (Figure 1). These relationships suggested that taller plants tended to have larger root systems.

Genotypic variation and consistency in relative water content (RWC)

Significant genotypic variation in RWC was observed for both predawn and midday across water stress condition (Table 2). During mild stress, mean RWC of the DHLs was 89.3% at predawn and 77.0% at midday and decreased when stress was more severe (77.6 and 66.9 % at predawn and midday). They increased again when the DHLs were in the recovery period (77.8%). Mean RWC of their parents, CT9993 and IR62266, as well as the three standard checks were similar under all water conditions, except at the midday

Table 1 Minimum, maximum and mean root mass densities (RMD) (mg cm^{-3}), total root mass (TRM) (g m^{-2}) and RMD (%) determined after drought stress period of double haploid lines (DHL), parents (CT9993 and IR62266) and three standard checks (IR20, NSG19 and KDML105) at Ubon Ratchathani Rice Research Center in 2002 dry season.

Root traits	Depth	DHL			CT9993		IR62266		IR 20		NSG 19		KDML 105		LSD (b)
	(cm)	Min	Max	Mean	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	(5 %)
RMD (mg cm^{-3})	0-15	0.037	1.443	0.071	0.852	± 0.057	0.625	± 0.069	0.621	± 0.032	0.700	± 0.077	0.667	± 0.101	0.377
	15-30	0.041	0.352	0.150	0.214	± 0.026	0.098	± 0.016	0.094	± 0.013	0.131	± 0.020	0.120	± 0.021	0.108
	30-45	0.001	0.093	0.017	0.020	± 0.011	0.016	± 0.007	0.019	± 0.005	0.025	± 0.005	0.009	± 0.007	0.032
TRM (g m^{-2})		68.02	228.0	131.7	162.9	± 10.071	111.0	± 12.471	110.1	± 3.844	128.5	± 13.832	119.6	± 17.692	65.36
RMD (%)	0-15	62.33	94.57	80.95	78.79	± 0.284	84.51	± 1.867	84.42	± 2.327	81.80	± 2.064	83.61	± 1.651	10.41
	15-30	5.280	33.56	17.18	19.34	± 1.853	13.73	± 1.870	12.91	± 1.765	15.24	± 1.602	15.26	± 1.501	9.54
	30-45	0.080	9.620	1.842	1.865	± 1.086	1.755	± 0.709	2.664	± 0.709	2.952	± 0.578	1.128	± 0.822	3.22

[a] = standard error of the mean of parents and checks,
[b] = least significant difference

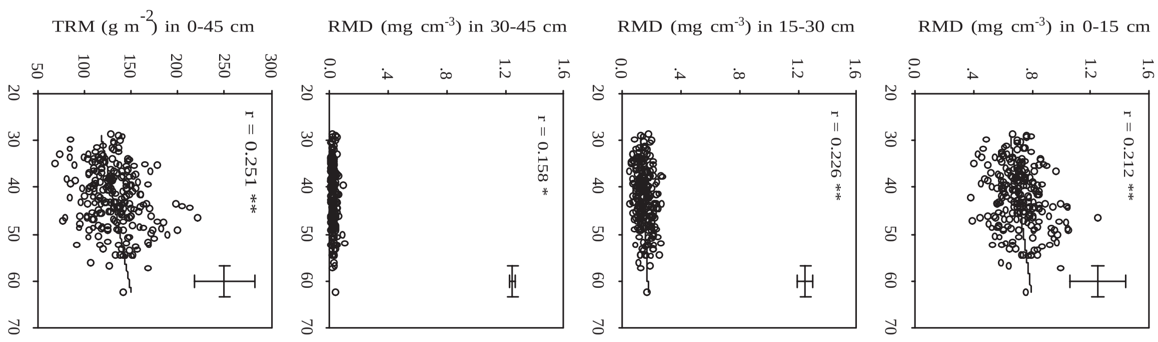

Figure 1 Relationship between root mass density (RMD, mg cm^{-3}) in 0-15, 15-30 and 30-45 cm; and total root mass (TRM, g m^{-2}) and plant height (cm) of double haploid population at Ubon Ratchathani Rice Research Center in 2002 dry season. Horizontal and vertical bars are 5% LSD applicable to differences for root characteristics and plant height among lines.

Table 2 Minimum, maximum and mean relative water contents (%) determined during drought stress period at mild and severe plant water deficit and recovery after drought stress was relieved for five days of double haploid lines (DHL), parents (CT9993 and IR62266), and three standard checks (IR20, NSG19 and KDML105) at Ubon Ratchathani Rice Research Center in 2002 dry season.

		DHL			CT9993		IR62266		IR20		NSG19		KDML 105		LSD[b]
		Min	Max	Mean	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	(5 %)
Mild stress	Predawn	76.2	99.2	89.3	90.7	± 2.15	89.4	± 0.68	87.2	± 3.39	91.7	± 0.75	91.0	± 1.86	10.23
	Midday	56.3	90.0	77.0	74.7	± 1.62	79.6	± 1.88	77.3	± 3.48	81.8	± 1.45	84.0	± 1.14	14.17
Severe stress	Predawn	63.4	92.2	77.6	74.7	± 1.10	80.2	± 0.92	76.9	± 0.70	79.0	± 1.20	82.7	± 1.81	10.43
	Midday	47.0	83.3	66.9	65.6	± 2.28	59.7	± 5.57	70.0	± 1.57	71.5	± 1.11	75.8	± 0.46	11.71
Recovery	Midday	58.5	90.6	77.8	80.1	± 2.39	84.1	± 2.53	73.4	± 1.56	79.1	± 2.65	78.8	± 1.49	12.95

[a] standard error of the mean of parents and checks,
[b] least significant difference

measurement under severe stress treatment, where IR62266 had significantly lower RWC than KDML105.

Genotypic consistency in RWC across water conditions was observed. The correlation genotype means was highly significant across all water condition (Table 3). The correlation coefficient (r) between RWC measured during the water stress (mild and severe stress) and the recovery, was 0.208 ** and 0.199 ** at predawn and midday during mild stress, and 0.411 ** and 0.359 ** at predawn and midday during severe stress.

Genotypic variation and consistency in leaf rolling and death

Mean leaf rolling of the DHLs was 2.9 under mild stress condition, and increased to 3.9 when stress was severe and then decreased to 1.5 thereafter when rice was in recovery period. Mean drought score of the DHLs also increased under mild and severe stress condition and then decreased when rice was in recovery period (3.0, 4.8, 1.7). Highly significant genotypic variation in leaf rolling and death (visual drought score) was observed (Table 4). Although there were significant differences among DHLs, this was not so in their parents. KDML105 had the lowest of both visual scores when compared to the parents and the other standard checks (IR20 and NSG19). As for the leaf rolling score, KDML105 was significantly different from IR62266 only at the mild stress period, and was significantly different from some other cultivars for drought score at all water condition.

The genotypic consistency in visual estimation of leaf rolling and death across water conditions was observed. The relationship for genotype means was significant across all water conditions, except that the relationship between the leaf rolling score under the severe stress and recovery periods (Table 5), indicating that genotype ranking between the two visual symptoms under different intensities of drought was highly consistent.

DISCUSSION

This study has shown the high degree of sensitivity to water deficit in rice and different physio-morphological responses to water deficit among rice genotype examined. After water stress was imposed, although the rice genotypes were somewhat different in root development (root mass density, total root mass and, root mass distribution), most of the root mass distribution was only in the top 0-15 cm layer of the soil (Table 1). This limited root development in shallow top-soil zones in rain-fed lowlands is partly a result of the hardpan that develops through pudding (Pantuwan *et al*.,1997) and, may also due to the limitation of oxygen supply in lower soil depths in anaerobic lowland conditions (Wade *et al.*, 1998). Because of the shallow nature of the root system, genotypic variation in root mass or length is rather limited. Nevertheless, in the parents of DHLs, CT9993 had significantly higher root mass density at 15-30 cm soil depth and, also taller than IR62266. The genotypic differences in root mass density or root length density at 5-30 cm depth were related with differences in both visual estimation of retention of green leaves during a dry period and water extraction (Pantuwan *et al.*,1997). It was anticipated that larger effects of drought resistance could be obtained if genotypes develop deep root systems rather than more shallow roots at the 30 cm deep. A large root system may be able to extract more water from the soil, but this does not necessarily result in higher yield under limited water condition (Fukai *et al.*, 1999). The larger root system may result in more rapid extraction of available water and hence, faster development of water deficit that may have an adverse effect on grain yield.

The results suggested that the DHLs with better root traits had less drought resistance in terms of osmotic adjustment in rainfed lowland conditions. This negative association indicated that there were different strategies (avoidance and tolerance) employed by the rice plant to cope with periods of water deficit. For example, CT9993 has higher root mass density and low osmotic adjustment (Samson *et al.*, 1995), while IR62266 has higher osmotic adjustment and a low root mass density. The differences of the two parental lines was characterized under both stress and non-stress conditions in the greenhouse and in the field (Azhiri *et al*., 2000; Nguyen *et al*.,1997). Under rain-fed lowland conditions where often both flooding and drought alternately occur during crop growth,

Table 3 Phenotypic correlation among predawn and midday relative water content (%) determined during drought stress period at mild and severe water stress and recovery after drought stress was relieved for five days in a double haploid population at Ubon Ratchathani Rice Research Center in 2002 dry season.

environment		Mild stress		Severe stress		Recovery
		Predawn	Midday	Predawn	Midday	Midday
Mild stress	Predawn	1	0.378**	0.239**	0.272**	0.208 **
	Midday		1	0.322**	0.293 **	0.199 **
Severe stress	Predawn			1	0.585 **	0.411 **
	Midday				1	0.359 **
Recovery	Midday					1

**, Significant levels at 1 %

different drought resistance strategies could be combined rather than depending solely on one mechanism (Ludlow, 1989). Yield advances in limited water condition could occur, if high osmotic adjustment and good depth and thickness of roots for exploration of soil water are combined through breeding.

CONCLUSION

These results clearly indicated that between 62-94% of root were distributed in the top 0-15 cm soil depth and very few roots were found below 30-45 cm. The rice genotypes, which had low root mass density, were able to maintain water status, consequently delayed tissue death and leaf senescence in rice under water stress. It is suggested that the ultimate goal to combine high dehydration tolerance with strong root penetration may not be realized in the existing germplasm for rainfed lowland rice.

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Table 4 Minimum, maximum and mean leaf rolling and drought score determined during drought stress period at mild and severe plant water deficit and recovery after drought stress was relieved for five days of double haploid lines (DHL) and their parents (CT9993 and IR62266), and three standard checks (IR20, NSG19 and KDML105) at Ubon Ratchathani Rice Research Center in 2002 dry season.

	DHL			CT9993		IR62266		IR20		NSG19		KDML105		LSD[b]
	Min	Max	Mean	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	Mean	± SEM[a]	(5 %)
Leaf rolling score														
Mild stress	1.7	4.3	2.9	2.5	± 0.22	2.8	± 0.22	2.6	± 0.22	2.6	± 0.11	1.7	± 0.19	1.03
Severe stress	3.0	5.3	3.9	3.8	± 0.08	3.8	± 0.22	4.0	± 0.19	3.8	± 0.11	3.2	± 0.11	0.87
Recovery	1	5	1.5											0.71
Drought score														
Mild stress	1.3	4.7	3.0	2.8	± 0.09	2.9	± 0.21	2.0	± 0.17	2.6	± 0.43	1.6	± 0.13	1.02
Severe stress	3.0	7.3	4.8	5.0	± 0.12	4.5	± 0.22	4.6	± 0.30	4.8	± 0.39	3.5	± 0.39	1.19
Recovery	1.0	5.0	1.7	1.8	± 0.15	1.3	± 0.33	1.0	± 0.00	1.2	± 0.23	1.0	± 0.00	1.54

[a] = standard error of the mean of parents and checks,
[b] = least significant difference

Table 5 Phenotypic correlation among drought score, and leaf rolling score determined during drought stress period at mild and severe water stress and recovery after drought stress was relieved for five days of double haploid lines (DHL) at Ubon Ratchathani Rice Research Center in 2002 dry season.

		Drought score			Leaf rolling		
		Mild stress	Severe stress	Recovery	Mild stress	Severe stress	Recovery
Drought score	Mild stress	1	0.716**	0.376**	0.769**	0.477**	0.171**
	Severe stress		1	0.598**	0.515**	0.365**	0.259**
	Recovery			1	0.180**	0.064 ns[a]	0.385**
Leaf rolling	Mild stress				1	0.615*	0.134*
	Severe stress					1	0.155*
	Recovery						1

[a]; ns, not significant; * and **, Significant levels at 5 % and 1 %, respectively

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