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Proline Accumulation and Rooting Patterns in Rice in Response to Water Deficit under Rainfed Lowlands

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ABSTRACT: Drought is a major impediment to a rainfed lowland rice system. Drought tolerance has been associated with proline accumulation in roots and leaves. However, genetic linkage was uncertain. To determine if there is a genetic correlation between proline accumulation and 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 experiments to determine the extent of genetic variation in root characters, proline accumulation, relative water content, visual leaf rolling and drought injury under different intensities of water deficit. Genotypes with high proline content in leaf tissues were more dehydration tolerant, a relatively high water content was maintained, and leaf rolling and senescence were delayed under severe water deficit. However, the ability of rice roots to penetrate deep into the soil was negatively correlated with proline accumulation in leaf tissue. Rice roots are mostly distributed at 0-30 cm soil depth under lowland conditions. Therefore, the ultimate goal to combine high dehydration tolerance with strong root penetration may not be realized in the existing germplasm.

KEYWORDS: Lowland rice, Proline, Relative water content, Drought.

List of abbreviations: DHLs, double haploid lines; DAS, days after seeding; RMD, root mass density; RWC, relative water contents; TRM, total root mass.

INTRODUCTION

Rain-fed lowland rice is mostly grown in South and Southeast Asia, and more than 50% is under drought-prone conditions.[1] 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.[2] 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. 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.[3]

It is now well established that plants have evolved many adaptations to counteract water deficit. These adaptations are classified into four categories: drought avoidance (developmental and physiological traits), drought tolerance (physiological and biochemical adaptations)[4], drought escape and drought recovery. In term of biochemical changes, several classes of compounds have been observed to accumulate in response to a water deficit. These compounds include sugar alcohol, proline and glycinebetaine.[5,6]

Several traits related to drought tolerance in rice have been identified.[7,8] Among these, a deep root system allows the plant to extract deep soil moisture during drought. 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.[7] Genotypic variation in root penetration and other root traits have been reported in rice.[9] Increased rooting depth, root density, root 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.[7]

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.[10]

Proline accumulation in plant cells exposed to salt or water stress is a widespread phenomenon. Proline is believed to protect plant tissues against stress by acting as a nitrogen storage, as an osmoregulator, and as a protectant for enzymes and cellular structure.[11] Free proline accumulation seems to be a widespread stress response in higher plants such as barley, corn and rice. The pool sizes of several other amino acids are also increased under drought and salt stress but the degree of the accumulation was not comparable to that of proline accumulation, which reached very high levels within a short period after stress induction.[12] Stress-mediated changes in proline biosynthesis, including hydrolysis of proteins and oxidative degradation processes, can result in increased proline levels in plants exposed to different stresses. The degradation of proline was almost completely inhibited in stressed plant materials. The increase in proline content in stressed plant parts is predominantly due to *de novo* synthesis.[13]

Here, we report the proline responses of a genetic population to an imposed water deficit. This study was designed to understand the role of proline accumulation under water stress conditions in 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.[14] These traits include gross root morphology, root penetration index (RPI) and osmotic adjustment (OA). A double haploid line (DH) 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 hight OA), at Centro International de Agricultura Tropical (CIAT), Columbia, and International Rice Research Institute (IRRI), Philippines. The 220 DH lines, 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 after every 7 lines, KDML105 and NSG19 were grown as running checks. Individual Plot size was 0.84 m^2, which consisted of 4 rows, spaced 15 cm apart, 1.4 m in length, 14 hills per row. Hills were spaced 0.1 m apart within each row.

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

Measurements

Proline content

At specific time intervals (predawn 01.00 – 05.00 am and midday 10.30 hr – 15.00 hr) mature leaf tissue was excised from tillers in each experimental plot over all lines and over all water condition. Three mature, fully expanded leaves were used. The leaves were excised at the base, and cut the top of each leaf so that they would all be the same length. The samples were divided into two groups. The first group of samples were approximately 1 cm long and were used to determine relative water content (RWC). The second group of samples was used to determine proline content. Samples for determination of proline were frozen in dry ice, stored at –80°C, and powdered in liquid nitrogen.

The method to determine leaf proline content was essentially as described by Bates.[15] Single aliquots (20-50 mg) of powdered frozen (–80°C) tissue from leaves harvested from each pot were weighed into 1.5 ml centrifuge tubes and the powder suspended in 1.2 ml of 3% (w/v) sulphosallicylic acid to precipitate protein. Samples were vortexed, centrifuged at 12000x *g* for 7 min, and the supernatant transferred to a fresh 1.5 ml tube. An aliquot of 200 ml of supernatant was reacted with the same volume of glacial acetic acid and ninhydrin reagent (2.5% (w/v) ninhydrin (Sigma) in (v/v) glacial acetic acid and 40% (v/v) 6M phosphoric acid) for 1 hour at 100°C before the reaction was stopped by cooling the tubes on ice. The products were extracted with 300 µl of toluene by vortex mixing and the upper (toluene) phase decanted into a glass cuvette. The absorbance was measured at 520 nm. Proline contents were calculated from the absorbances of a set of separately prepared proline stands assayed in the same manner

Table 1. Minimum, maximum and mean root mass density (RMD) (mg cm^{-3}), total root mass (TRM) (g m^{-2}) and RMD (%) determined after drought stress period of the double haploid population, parents (CT9993 and IR62266) and three standard checks (IR20, NSG19 and KDML105).

Root traits	Depth (cm)	DHL			CT9993		IR62266		IR 20		NSG 19		KDML 105		LSD (b) (5 %)
		Min	Max	Mean	Mean	±SEM[a]	Mean	±SEM[a]	Mean	±SEM[a]	Mean	±SEM[a]	Mean	±SEM[a]	
RMD (mg cm^{-3})	0-15	0.037	1.443	0.071	0.852a	± 0.057	0.625a	± 0.069	0.621	± 0.032	0.700	± 0.077	0.667	± 0.101	0.377
	15-30	0.041	0.352	0.150	0.214a	± 0.026	0.098b	± 0.016	0.094	± 0.013	0.131	± 0.020	0.120	± 0.021	0.108
	30-45	0.001	0.093	0.017	0.020a	± 0.011	0.016b	± 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	162.9a	±10.071	111.0a	±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	78.79a	± 0.284	84.51b	± 1.867	84.42	± 2.327	81.80	± 2.064	83.61	± 1.651	10.41
	15-30	5.280	33.56	17.18	19.34a	± 1.853	13.73a	± 1.870	12.91	± 1.765	15.24	± 1.602	15.26	± 1.501	9.54
	30-45	0.080	9.620	1.842	1.865a	± 1.086	1.755a	± 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.

Relative water content (RWC)

To determine RWC, the 3 leaf samples were excised into pieces of about 1 cm^2 in area. The samples were immediately weighed in a hermetically sealed container, floated on distilled water until fully re-hydrated, weighed, and then dried until a constant oven-dry weight was obtained. The data obtained was computed for RWC according to Turner[16] .

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 -15.30 pm). A rating of leaf rolling score was visually estimated in each plot using a 1 - 5 scale, in which a score of 1 indicated no rolling, and 5 complete rollings.[17] Rating of drought scores from 0 - 9, was estimated for each plot based on symptoms of leaf drying on the plants. A score of 0 indicated no symptoms of stress, with an increasing score when more leaves die due to water deficit.[27] A score of 5 indicated that 50% of the entire leaves was fully dried. The maximum score of 9 indicated that all plants are apparently dead.

Root mass

Root mass density (RMD) and total root mass was determined after recovery period (90 DAS). The method and techique for the determination of root system was developed by Pantuwan *et al.*[18] Two adjacent hills were randomly select before taking measurements. A 38 mm (inner diameter) steel tube was placed, immediatedly next to a hill, with less than 1 cm between the closest tiller and the tube, and the soil sample to a depth of 45 cm was collected and cut into three sections, 0-15, 15-30 and 30-45 cm soil depth. The second soil column was taken near the other hill using the same procedures as for the first hill. Soil samples were put on a 1 mm mesh screen and root were washed free of soil using tap water. Roots were dried in a hot air oven at 70^0C for 48 h and weighed to determine root dry mass.

Plant height

After recovery period, plant height was measured on 10 hills randomly sampled in each plot. The height was measured from the soil surface to the tip of tallest panicle within each hill.

Results

Genotypic Variation in Root Characteristics and Plant Height

Root mass density (RMD) of rice genotypes were significantly different at depth of 15-45 cm in the soil (Table 1). The highest root mass density of rice root was

located at 0-15 cm soil depth. The parent, CT9993 (0.214 mg cm^{-3}) had higher RMD at this depth than IR62266 (0.098 mg cm^{-3}). Mean RMD of the DHLs was 0.150 mg cm^{-3} (range from 0.041 to 0.352 mg cm^{-3}). Three standard checks (IR20, NSG19 and, KDML105) revealed 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 g m^{-2} (68.0-228.0 g m^{-2}), %RMD was 80.95 % (62.33-94.57 %) at a depth of 0-15cm; 17.18% (5.28-33.56 %) at a depth of 15-30cm, and 1.84 % (0.08-9.62 %) at a depth of 30-45cm. 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 in 0-15, 15-30 and 30-45cm of soil depth, respectively) and TRM (r = 0.251**) (Fig 1). These relationships suggest that taller genotypes tend to have larger root systems.

Genotypic Variation and Consistency in Proline Accumulation

In this study, proline content was adjusted to 100% RWC to reduced sampling errors which may occur particularly when working with a large number of genotypes which was time–consuming and may effect to lost water of plant.

Proline content was not significantly different among genotypes under well-watered conditions, but was slightly different when drought was introduced (Table 2). Mean proline content of the DHLs at predawn during non-stress period was 0.106 mg g^{-1} fresh weigh of leaf (range from 0.045 to 0.209 mg g^{-1} fresh weigh of leaf). During mild stress and recovery periods, the proline content at midday was higher than at predawn measurement. In contrast, during severe stress period, the proline accumulation at predawn was higher than at midday. Mean proline content of the DHLs during mild stress period was 0.070 mg g^{-1} fresh weigh of leaf (range from 0.053 to 0.105 mg g^{-1} fresh weigh of leaf) at predawn and 0.097 mg g^{-1} fresh weigh of leaf (range from 0.070 to 0.153 mg g^{-1} fresh weigh of leaf) at midday. Mean proline content of the DHLs at predawn and midday increased when stress was severe, and at recovery, the mean proline content decreased at predawn and increased at midday. Among their parents, IR62266 had higher proline content than CT9993 in all water treatments, but differed significantly only at midday under mild stress. The three standard checks were not significantly different under all water treatments.

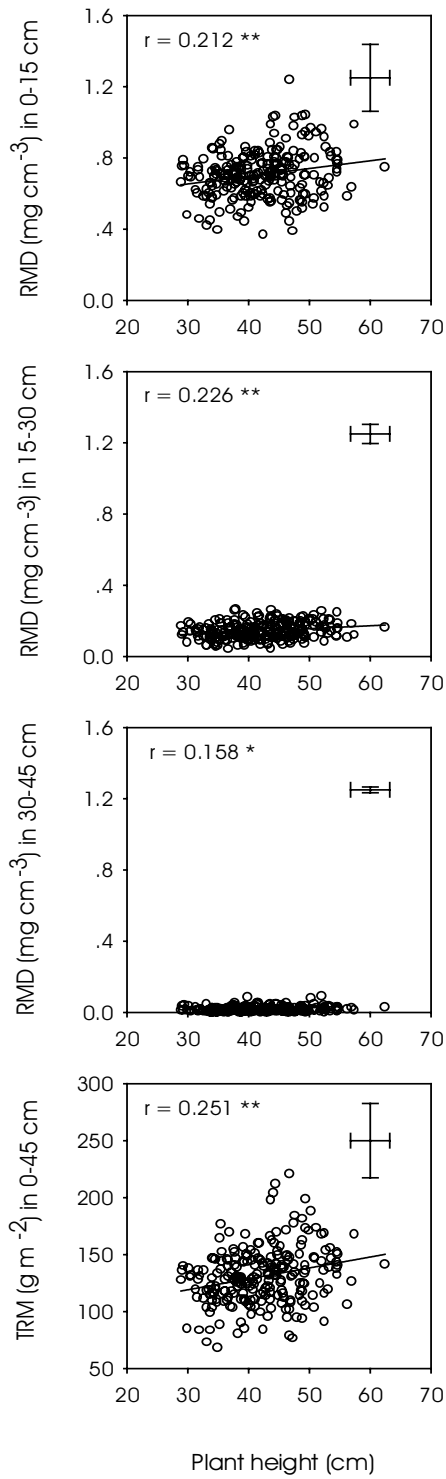

Fig 1. Relationship between root mass density (RMD, mg cm^{-3}) in 0-15, 15-30 and 30-45 cm; and total root mass (TRM, g m^{-2}) and plant height (cm) of the double haploid population. Horizontal and vertical bars are 5% LSD applicable to differences for root characteristics and plant height among lines.

Table 2. Minimum, maximum and mean proline contents (mg g^{-1}fresh weigh of leaf) determined in control before drought stress, during mild and severe water stress, and during recovery of the double haploid population, parents (CT9993 and IR62266) and three standard checks (IR20, NSG19 and KDML105).

		DHL			CT9993		IR62266		IR20		NSG19		KDML		LSD[b]
		Min	Max	Mean	Mean	±SEM[a]	Mean	±SEM[a]	Mean	±SEM[a]	Mean	±SEM[a]	Mean	±SEM[a]	(5 %)
Control	Predawn	0.045	0.209	0.106	0.104 a	± 0.002	0.148 b	± 0.007	0.104	± 0.015	0.109	± 0.011	0.107	± 0.003	0.044
Mild stress	Predawn	0.053	0.105	0.070	0.062 a	± 0.004	0.078 a	± 0.005	0.090	± 0.002	0.073	± 0.012	0.086	± 0.008	0.02
	Midday	0.070	0.153	0.097	0.088 a	± 0.004	0.120 a	± 0.010	0.119	± 0.004	0.114	± 0.019	0.124	± 0.010	0.035
Severe stress	Predawn	0.071	0.282	0.128	0.107 a	± 0.003	0.133 a	± 0.006	0.167	± 0.009	0.129	± 0.003	0.162	± 0.023	0.071
	Midday	0.064	0.231	0.109	0.094 a	± 0.004	0.112 a	± 0.003	0.150	± 0.010	0.099	± 0.006	0.126	± 0.011	0.063
Recovery	Predawn	0.070	0.158	0.107	0.092 a	± 0.002	0.107 a	± 0.004	0.101	± 0.003	0.103	± 0.009	0.116	± 0.005	0.033
	Midday	0.089	0.224	0.134	0.123 a	± 0.008	0.137 a	± 0.005	0.139	± 0.006	0.140	± 0.015	0.122	± 0.002	0.047

a = standard error of the mean of parents and checks.
b = least significant difference.

Proline accumulation of different DH lines across environments was generally consistent. The correlations for genotype means was generally significant across all stress conditions (Table 3).

There was a negative correlation across DH lines between root characteristics (RMD, TRM, and %RMD) and proline content (Fig 2). The genotype that had low RMD or distribution at a depth of 0-15 cm accumulated high proline content under water stress treatments because small roots caused reduced RWC and have high osmotic adjustment thus increased proline accumulation.

Table 3. Correlation coefficients between proline content (μg g^{-1} fresh weight) determined before drought stress, during mild and severe water stress, and during recovery of the double hyploid population and parents (CT9993 and IR62266).

		Control	Mild stress		Severe stress		Recovery	
		Midday	Predawn	Midday	Predawn	Midday	Predawn	Midday
Control	Midday	1	0.008 ns[a]	0.008 ns	0.084 **	0.175 *	0.152 *	0.129 ns
Mild stress	Predawn		1	0.488 **	0.216 **	0.230 **	0.226 **	0.218 **
	Midday			1	0.204 **	0.151*	0.145*	0.175 **
Severe stress	Predawn				1	0.625 **	0.270 **	0.340 **
	Midday					1	0.311 **	0.416 **
Recovery	Predawn							0.657 **
	Midday							1

a, ns = not significant.
* and ** = Significant levels at 5%.

Genotypic Variation and Consistency in Relative Water Content (RWC)

Significant genotypic variation in RWC was observed for both predawn and midday samples across water stress condition (Table 4). 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 measurement under severe stress treatment, where IR62266 had significantly lower RWC than KDML105.

In general, RWCs were consistent and the genotypic correlation were positive across environments (Table 5). 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, respectively.

Table 4. Minimum, maximum and mean relative water content (%) determined during drought stress period at mild and severe water stress and during recovery of the double haploid population (DHL), parents (CT9993 and IR62266), and three standard checks (IR20, NSG19 and KDML105).

		DHL			CT9993		IR62266		IR20		NSG19		KDML		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 a	±2.15	89.4 a	±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 a	±1.62	79.6 a	±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 a	±1.10	80.2 a	±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 a	±2.28	59.7 a	±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 a	±2.39	84.1 a	±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.

Table 5. Correlation coefficients between relative water content of the double haploid population measured at predawn and midday during mild and severe water stress and during recovery.

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%.

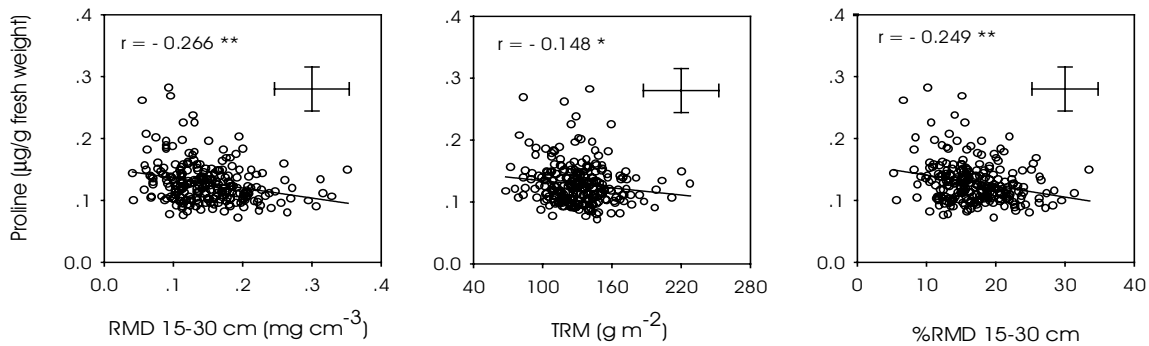

Fig 2. Relationship between root mass density (RMD, mg cm^{-3}), total root mass (TRM, g m^{-2}) , % root mass distribution (%RMD) and proline (µmol g^{-1} fresh weight of leaf) of the double haploid population. Horizontal and vertical bars are 5% LSD.

There was a positive correlation between proline content and RWC among each genotypes across all water conditions (Fig 3). Genotypes which had high proline content maintained high RWC under stress and recovery conditions.

Genotypic Variation and Consistency in Leaf Rolling and Death

Mean leaf rolling of the DHLs was 2.9 under mild stress condition, increased to 3.9 when stress was severe and then decreased to 1.5 thereafter when rice was in

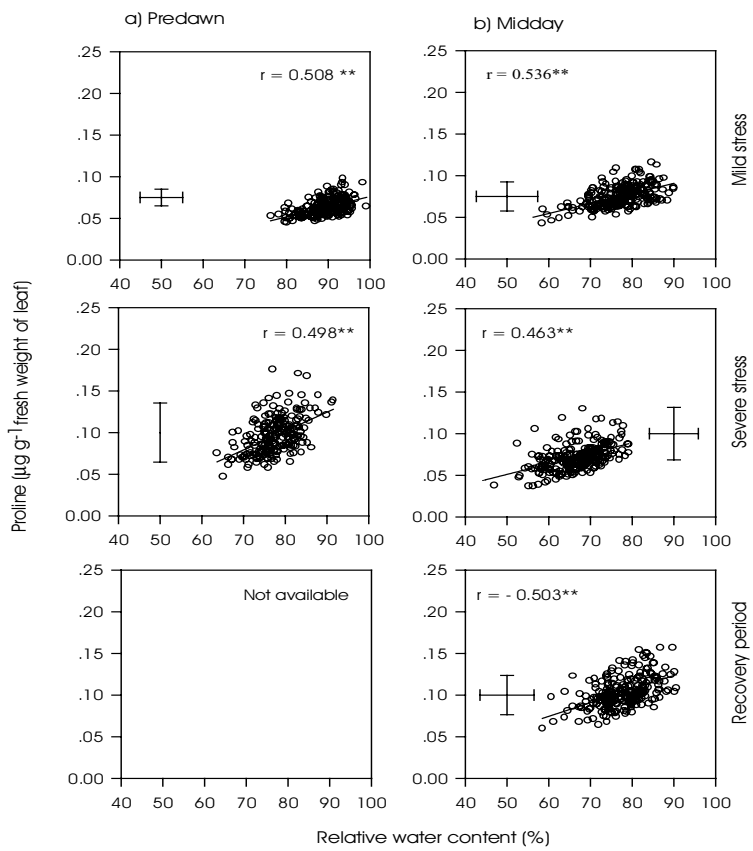

Fig 3. Relationship between relative water content (%) and proline content (µmol g^{-1} fresh weight of leaf) in the double haploid population estimated at a) predawn and b) midday during mild and severe water stress and during recovery. Horizontal and vertical bars are 5% LSD applicable to differences for relative water content and proline among lines in each water condition.

recovery. 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 6). Although there were significant differences among DHLs, this was not so in their parents. KDML105 had the lowest values for 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 during the mild stress period, and was significantly different from some other cultivars for drought score at all water conditions.

Drought score of genotypes determined under mild, severe and recovery periods generally had significantly positive correlation with leaf rolling score, except that

Table 6. Minimum, maximum and mean leaf rolling and drought scores determined during mild and severe water stress and during recovery of the double haploid population (DHL), their parents (CT9993 and IR62266), and three standard checks (IR20, NSG19 and KDML105).

	DHL			CT9993		IR62266		IR20		NSG19		KDML		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 a	±0.22	2.8 a	±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 a	±0.08	3.8 a	±0.22	4.0	±0.19	3.8	±0.11	3.2	±0.11	0.87
Drought score														
Mild stress	1.3	4.7	3.0	2.8 a	±0.09	2.9 a	±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 a	±0.12	4.5 a	±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 a	±0.15	1.3 a	±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 7. Correlation coefficients between drought scores and leaf rolling scores of the double haploid population determined during mild and severe water stress and during recovery.

		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.

the correlation coefficients between drought score under recovery period and leaf rolling under severe stress condition was positive but not significant (Table 7).

There was highly negative correlation between proline content and drought score of DHLs under mild stress condition(r = -0.283 ** and -0.376 ** for predawn and midday, respectively) and severe stress condition(r =- 0.268 ** and -0.384 ** for predawn and midday, respectively). When stress was relieved (recovery period), there was no relationship (Fig 4). There was negative correlation between proline content and leaf rolling scores of DHLs only in mild stress condition (r = -0.171 ** and - 0.243 ** for predawn and midday, respectively).

Discussion

The present study has shown the high degree of sensitivity to water deficit in rice and the different

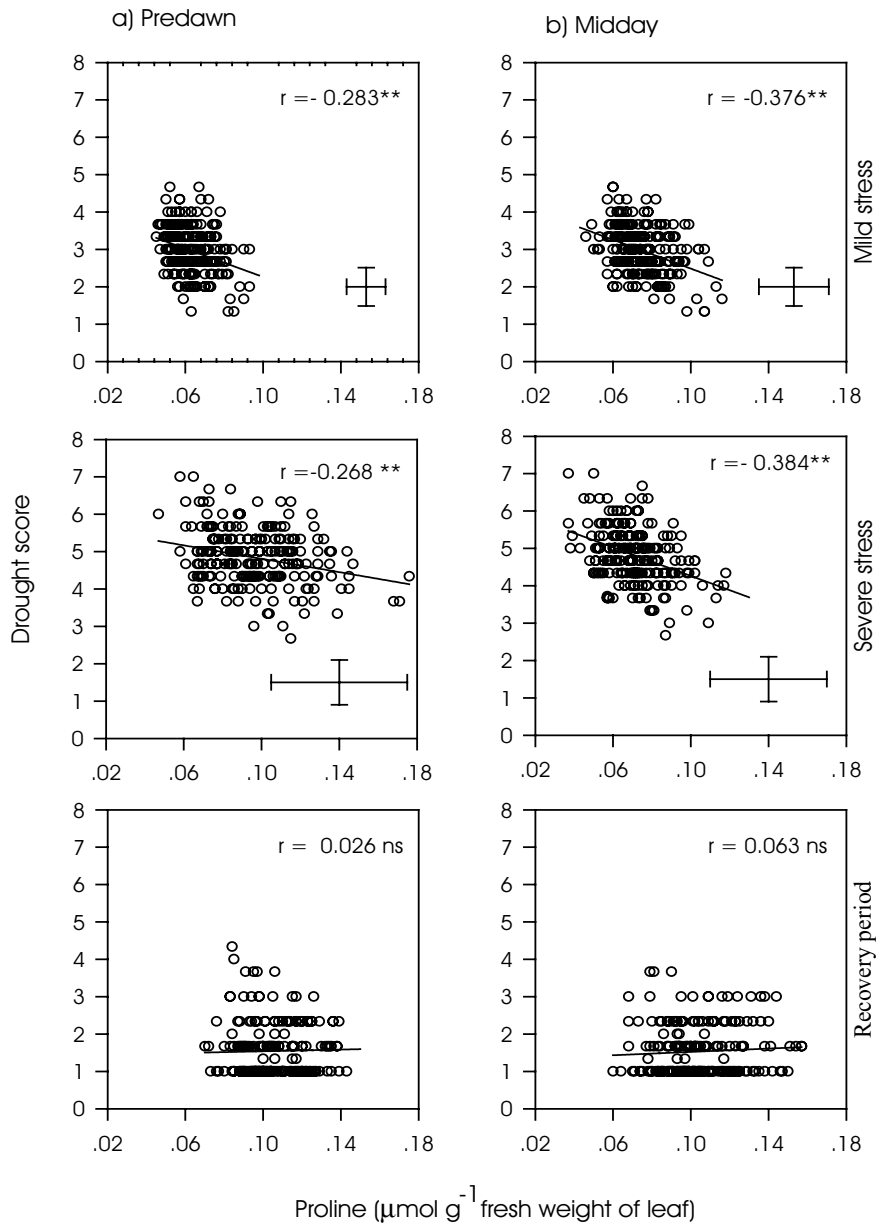

Fig 4. Relationship between proline content (µmol g^{-1} fresh weight of leaf) and visual drought score of the double haploid population estimated at a) predawn and b) midday, during mild and severe water stress and during recovery. Horizontal and vertical bars are 5% LSD applicable to differences for proline and drought score among lines in each water condition.

physio-morphological responses to water deficit among the rice genotypes examined. The differential irrigation for each water treatment was chosen because these are similar to typical rainfalls in this region where rain-fed lowland rice is grown. Although the rice genotypes were somewhat different in root development (root mass density, total root mass and, root mass distribution) after water stress was imposed, most of the root mass distribution was only in the top 0-15 cm layer of the soil (Table 1). This limited development in shallow top-soil zones in rain-fed lowlands is partly a result of the hardpan that develops through pudding[18] and, may also be because the oxygen supply in lower soil depths is limited in anaerobic lowland conditions.[19] 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 was associated with differences in both visual estimation of retention of green leaves during a dry period and water extraction.[18] It may be expected that larger effects of drought resistance can be obtained if genotypes develop deep root systems rather than more roots at the shallow depths down to 30 cm. A large root system may be able to extract water more thoroughly from the soil, but this does not necessarily result in higher yield under limited water condition .[20] The larger root system may result in more rapid extraction of available water and hence, faster development of severe water deficit that may have an adverse effect on grain yield.

Proline plays an important role as an osmo-regulatory solute in plants subjected to water stress.[12] Although all genotypes had similar proline contents under the well watered period, proline accumulated differently when water stress was imposed or, during recovery period (Table 2). This demonstrates that an increase in proline concentration in stressed plants begins when cell injury is evident and elevated levels of proline are maintained for as long as a month after stressed cells are returned to normal osmotic conditions.[21] Thus, it is an inducible or facultative trait rather than a constitutive trait. Proline accumulation in plant tissues provides an adaptive advantage to plants under osmotic stress, as a result of osmotic adjustment. This helps maintain turgor of both shoots and roots as plants experience water stress.[22]

Our results suggest that the DHLs with better root traits have less drought resistance in terms of osmotic adjustment and dehydration tolerance through accumulation of proline. This negative association indicates that there are 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 lower capacity for proline accumulation or low osmotic adjustment,[23] while IR62266 has higher osmotic adjustment through accumulation of proline 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.[24] The rice genotypes exhibiting high proline accumulation had a marked effect on the ability to maintain water status, consequently delayed tissue death and leaf senescence in rice under water stress.[8] The ability to survive during drought and recovery periods affects the yield of rain-fed rice. Therefore, if the dehydration tolerance of plants (the ability of leaves to tolerate desiccation), could be increased, the yield of rain-fed rice should improve or at least stabilize.

Under rain-fed lowland conditions where often both flooding and drought occur alternately during crop growth, different drought resistance strategies could be combined rather than depending solely on one mechanism.[25] Yield advances in limited water condition could occur, if high proline accumulation and good depth and thickness of roots for exploration of deep soil water are combined through breeding. Measuring root traits and proline contents are, however, labor-intensive, slow and not suited to evaluate large number of lines. Mapping quantitative trait loci (QTLs) and regulating those characters may facilitate more rapid development of rain-fed lowland rice cultivars that have wide adaptation to water stress conditions.

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