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Full Length Research Paper

Proline, catalase and root traits as indices of drought resistance in bold grained rice (*Oryza sativa*) genotypes

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The present experiment was carried out with 39 bold grained rice genotypes to study the genetic variability of the traits conferring drought resistance and to screen the drought tolerant rice genotypes with a view to formulate an efficient breeding programme for time bound genetic enhancement. The field experiment was grown during Sali season of 2007 to estimate the genetic variability of eight quantitative traits; root weight (g), number of primary roots, number of tillers/plant, root length (cm), shoot weight (g), root:shoot ratio by length and root:shoot ratio by weight. The data for the quantitative traits were recorded after 60 days of transplanting. The analysis of variance of eight quantitative traits revealed that there was significant genetic variation among the genotypes for the traits conferring drought resistance. The genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2bs) and genetic advance (GA) as percent mean were estimated for all the eight quantitative traits. Small difference between GCV and PCV estimates was observed for shoot length (cm), root length (cm), number of primary roots, shoot weight (g), number of tillers/plant and root:shoot ratio by length suggesting that these characters were little influenced by environment and could be substantially improved through selection breeding program. Out of 39 rice genotypes, 7 genotypes including 2 checks (Ranjit and Monohar Sail) were selected on the basis of morphometric traits for drought resistance. Fifteen day old seedlings of seven selected rice genotypes were subjected to different osmotics of PEG-6000 (0.0, -0.4 and -0.8 MPa) to evaluate the effect of drought stress on proline, protein and antioxidant enzyme catalase. Poline content and catalase activity analyses further suggested that five selected bold grained rice genotypes (excluding two checks) namely Halodhar, George Sail, Kapili Dhan, Karmi Sail and Baodum were potentially drought tolerant.

Key words: Rice, drought tolerance, PEG, proline, protein, catalase.

INTRODUCTION

Dry lands (ca.5.1 billion ha) cover 40% of the world's land surface and serve as the habitat and surface of livelihood for more than 1 billion people. Desertification affects 70% of the world's dry lands, amounting to 3.6 billion ha or one fourth of the land surface. Water stress is one of the main environmental stresses responsible for reducing crop productivity in the dry lands as it affects growth through various physiological and metabolic processes of plant (Bray, 1993). Vital biochemical processes including photosynthesis (Boyer, 1976), respiration (Bell et al., 1971), and protein synthesis (Good and Zapalachinski, 1994) and assimilation of organic nitrogen (Sprent, 1981) have been demonstrated to be adversely affected by water stress. In rain fed agriculture, the short term water stress (10 - 20 days) is very common and it reduces productivity (Christiansen, 1982).

Rice (*Oryza sativa* L.), the domesticated cereal tropical C_3 grass, evolved in a semi aquatic, low radiation habitat. Over half of the world's population depends on rice as staple crop. In Asia, rice supplies 30 - 80% of the daily calories consumed (Narciso and Hossain, 2002). Rice is the staple crop of the entire North Eastern Region (NER) of India. The indigenous rice germplasm of Assam is endowed with rich genetic diversity and represents a wealth of valuable gene system (IRRI, 1974; Das et al., 1981). Rice germplasm maintained at Central Rice Research Institute (CRRI) in Cuttack includes 2054 lines

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from Assam alone out of 12256 collections from all over India (Paroda and Sharma, 1986) and is enough to explain the extent of genetic diversity in Assam. The present stock of rice germplasm in Assam is around 4000 accessions which also possibly include some duplication. Rice carries odd portfolio of tolerances and susceptibilities to abiotic stress as compared to other crops. Drought resistance in plants is the resultant of morphological and biochemical traits. Existence of genetic variation for drought resistance has been demonstrated in many crops, for example, wheat, rice, maize, barley, sorghum, oat, soyabean, rajma and alfalfa (Singh, 2000). The breeding programme for drought resistance in upland rice in USA involved the genetic improvement of root extension and penetration. Sheeba et al. (2005) studied two physiological traits (relative leaf water content and leaf area index) and six other traits (root length, root number, dry root weight, root shoot ratio, days to 50% flowering and biomass yeild) in rice to identify the genotypes resistant to drought. Pradhan et al. (2003) reported that in rice the root length and root number increased due to moisture stress.

Rice plays a major unique role in the genomic era of plant sciences because of the agronomic need for better tolerance to abiotic stress in major rice growing regions. Rice has the smallest genome among the cultivated cereals and it conserves much of the gene content and to some extent, gene order present in other species (Gale and Devos, 2001). The rice gene pool can be best utilized for development of promising or superior varieties, if exhaustive characterization of the various germplasm collections that constitute the rice gene pool is fully characterized to identify the useful genetic diversity. The crosses between the parents with maximum genetic divergence are generally the most responsive approach for genetic improvement. Osmotic adjustment is considered as an important physiological mechanism of drought adaptation in many plants (Subbrao et al., 2000). Osmotic adjustment requires regulation of intracellular levels of several compounds, collectively known as osmolytes (Janardhan and Bhojraj, 1999). Proline is one of the important osmolytes which accumulates during moisture stress condition. It helps to maintain turgor and promotes continued growth in low water potential soils (Mullet and Whitsitt, 1996). Singh and Singh (1983) observed that proline accumulation under drought condition is a good indicator of drought resistance capacity of plants.

At cellular level water stress induces the production of reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$), which ultimately cause membrane damage (Tambussi et al., 2002). One of the applications of water stress tolerant species/cultivars is the dominance of the defense system of antioxidant enzymes. This enzyme includes superoxide dismustases (SOD), which scavenge superoxide radicals and convert them to O_2 and H_2O_2. H_2O_2 is then detoxified by catalase (CAT) and ascorbate peroxidase (APOX). Catalase scavenges H_2O_2 by breaking down directly to form water and oxygen and an increase in its activity is related with increase in stress tolerance (Kraus et al., 1995). Catalase is indispensable for ROS detoxification during stress (Willekens et al., 1997).

Polyethylene glycol (PEG)-6000 appears to be better suited as an external osmoticum to analyse water retention in plants. Polyethylene glycol of high molecular weights has long been used to simulate water stress in plants (Ruf et al., 1967; Kaufman and Eckard, 1971; Corchete and Guerra, 1986). PEG of high molecular weight is a non penetrating inert osmoticum lowering the water potential of nutrient solutions without being taken up or being phytoxic (Lawlor, 1970).

Keeping the above points in mind, the present study was undertaken to estimate genetic variability of root traits and physiological traits that confer drought resistance, and study the influence of moisture stress imposed through different osmotics of PEG-6000 to evaluate the drought stress effects on proline, protein and antioxidant enzyme catalase to screen the drought tolerant genotypes of rice.

MATERIALS AND METHODS

Experimental material

The experimental material consisted of 39 genotypes of rice (*Oryza sativa* L.) collected from Regional Agricultural Research Station (RARS), Karimganj and from the progressive rice farmers in Barak Valley zone.

Field experiment

The experiment was conducted at the Field Trial Station (FTS), Department of Agriculture, Govt of Assam, Moikoibhanga, Badarpur, Assam, India during Sali (Kharif) season in 2007. The experiment was laid out in Randomized Block Design (RBD) with three replications for each genotype. The size of each plot was 8 x 1m with a spacing 20 × 20 cm and pH of 5.8 (acidic). One seedling was grown per hill in each plot. The data on eight quantitative traits were recorded after 60 days of transplanting.

Laboratory experiment

Out of 39 genotypes only 5 genotypes were selected on the basis of morphometric traits affecting drought resistance, namely George Sail, Karmi Sail, Baodum,Halodhar, Kapili Dhan. These 5 genotypes were tested with 2 recommended yield checks namely Ranjit and Monohar Sail, of this region.

100 seeds of each of the selected 7 genotypes were surface sterilized with 0.1% $HgCl_2$ for 5 min and after washing with distilled water, the seeds were soaked in water for 12 h and were placed in Petri dishes of size (100 × 17 mm) on a single layer Whatman No.1 filter paper moistened with distilled water. The Petri dishes were observed daily. The germinated seeds were then transferred to earthen pots (20 x 30 x 40 cm) filled with soil mixture containing garden soil, sand and cow dung in the ratio 1:1:1. Fifteen day old

seedlings were cut just above the soil surface and were subjected to water stress by dipping them in 50 ml of Polyethylene glycol (PEG) – 6000 solutions to study the effect of low moisture stress. Solution was prepared according to Mansour and Al-Mutawa, (2000) as given in Table 1.

Seedlings dipped in distilled water without PEG-6000 served as control. Seedlings were exposed to stress treatment for 4 h. Leaves from stressed and control seedlings were harvested immediately after stress treatment and used for biochemical analysis.

Table 1. Preparation of polyethylene glycol (PEG)-6000 solution (Mansour and Al-Mutawa, 2000).

Treatment	Amount of PEG-6000	Water stress (MPa)
Control	0 g/l	0.0
T_1	200 g/l	-0.4
T_2	295 g/l	-0.8

Proline determination

Proline accumulation was determined by the method as described by Sadasivam and Manickam (1996). Fresh leaves (0.5 g) were ground in mortar and pestle with 10 ml of 3% sulphosalicyclic acid and the homogenate was centrifuged at 18000 g. The homogenate was filtered. 2 ml of filtrate was added to 2 ml of glacial acetic acid and 2 ml of acid ninhydrin and test tubes were kept for 1 h at 100°C in water bath, followed by ice bath. The reaction mixture was vortexed with 4 ml of toluene. Toluene layer was separated and absorbance was read at 520 nm. A standard curve of proline was used for calibration.

Protein estimation

Total Soluble Protein (TSP) was estimated spectrophotometrically using folin-phenol reagent method of Lowry et al. described by Sadasivam and Manickam (1996).

Estimation of catalase activity

The method as described by Sadasivam and Manickam (1996) was used for the assay of catalase activity. 1 g of freshly collected leaf sample was cut into small pieces and homogenized in 10 ml of 0.067 M phosphate buffer (pH 7.0) with a pre-cooled mortar and pestle. The homogenate was centrifuged at 18000 g for 15 min. The sediment was stirred with cold phosphate buffer, allowed to stand in the cold with occasional shaking and then repeated the extraction once. The supernatants were combined and used for the assay of catalase activity.

The CAT activity was determined in the homogenates by measuring the decrease in absorption at 240 nm in a 3 ml of reaction mixture containing (0.16 ml of 10% W/V H_2O_2 diluted to 100 ml with 0.067 M phosphate buffer) and 0.1 ml of enzyme extract.

Statistical analysis

The analysis of variance for each quantitative trait was carried out as per Panse and Sukhatme (1995). Correlation coefficients were calculated between two quantitative traits as per Gupta (1991) and were tested by‘t’ test.

RESULTS AND DISCUSSION

Mean performance of the rice genotypes

The mean performance of the 39 rice genotypes for various root and shoot traits conferring drought resistance are presented in Table 1. Shoot length ranged from 55.10 - 95.60 cm. Kali Makuri (95.60 cm) recorded highest shoot length followed by Soulpona (92 cm) and Methi Chikon (91 cm). For drought tolerance a semi-dwarf genotype is usually preferred. Root length ranged from 9.30 - 29.30 cm, shoot weight varied from 2.97 - 35.62 g but root weight varied from1.26 - 30.76 g. A genotype with high root length and root weight is expected to be drought tolerant. In the present study, five genotypes namely George Sail, Karmi Sail, Baodum, Halodhar and Kapili Dhan showed high root length (cm). Number of primary roots ranged from 55 - 138 but number of tillers per plant ranged from 3 - 9.30. The root:shoot ratio by length and by weight varied from 0.15 - 0.36 and 0.21 - 1.57, respectively.

Analysis of variance

The analyses of variance carried out on thirty nine (39) genotypes for eight quantitative traits, that is, shoot length (cm), root length (cm), shoot weight (g), root weight (g), number of roots, shoot weight (g), number of tillers per plant, root:shoot ratio by length and root:shoot ratio by weight, revealed that the thirty nine (39) genotypes differed significantly for all the eight traits at P=0.01. This revealed that there was significant genetic variation among the genotypes for eight traits that conferred drought resistance.

Estimates of genetic parameters

In the present study, genetic parameters namely GCV (genotypic co-efficient of variation), PCV (phenotypic co-efficient of variation), h^2_{bs} (heritability in broad sense) and GA (genetic Advance as percent of mean) at 5% selection intensity were estimated for all the eight quantitative traits (Table 2). GCV ranged from 12.63 to 50.31% among the traits and the PCV also ranged from 19.11-94.50%. For all the traits the estimate of PCV was higher than corresponding GCV indicating the role of environment in the expression of each trait. Relatively small magnitude of difference between GCV and PCV was recorded for characteristics like - shoot length, root length, number of primary roots, shoot weight, no. of tillers/plant and root:shoot ratio by length. This indicated that these traits were little influenced by environment in comparison to other traits. Large difference between

Table 2. Mean performance of the rice genotypes for various root and shoot traits.

S/N	Genotype	Shoot length (cm)	Root length (cm)	Shoot wt (g)	Root wt (g)	No. of primary roots	No of tillers/ plant	Root/ shoot ratio by length	Root/shoot ratio by wt.
1	Afha Sail	74.10	21.50	31.30	27.30	111	8.30	0.28	0.83
2	Agani Sail	81.30	26.60	27.48	30.76	104	6.00	0.32	1.49
3	Baodum	81.30	24.00	5.91	3.95	106	8.00	0.32	0.63
4	Bar Madhava	72.60	20.90	35.20	19.30	114	5.30	0.36	0.70
5	Batasail	72.60	19.10	35.20	27.50	114	5.30	0.15	0.70
6	Betguti Dhan	85.60	18.30	13.21	4.86	102	4.00	0.19	0.39
7	Chafa Sail	69.00	18.30	4.46	1.69	88	6.30	0.21	0.36
8	Chatri Sail	74.00	24.30	35.62	19.40	107	8.00	0.32	0.71
9	Chingra Sail	71.00	26.50	24.60	14.42	100	6.30	0.36	0.57
10	Chutomula	77.30	21.00	5.21	4.58	118	4.66	0.26	0.81
11	Dhudhowla	77.70	20.60	14.70	6.10	65	8.60	0.26	0.42
12	Dolamula	81.30	18.60	4.33	2.21	81	5.00	0.22	0.54
13	Dome Sail	81.30	25.60	22.51	8.70	101	8.60	0.31	0.38
14	Douva Sail	81.60	23.00	23	5.17	99	3.00	0.28	0.21
15	George Sail	26.60	26.30	5.61	2.43	101	8.30	0.32	0.43
16	Guarchor	87.00	21.00	19.40	5.04	55	5.60	0.25	0.25
17	Hacha Lath	55.10	9.30	23.37	8.78	102	7.60	0.27	0.39
18	Halodhar Sail	75.60	26.00	31.03	17.83	118	7.60	0.35	0.53
19	Hathi Sail	66.80	17.50	28.89	20.72	104	7.00	0.25	0.70
20	Hera Powa	77.60	22.00	7.80	2.54	128	13.00	0.27	0.31
21	Kali Makuri	95.60	29.00	28.40	13.64	118	6.30	0.30	0.44
22	Kamal Bhog	86.60	19.90	17.11	8.68	87	7.30	0.22	0.50
23	Kapili Dhan	84.00	23.30	5.97	3.40	91	11.00	0.27	0.69
24	Karmi Sail	70.00	22.30	4.05	7.30	98	6.00	0.31	1.57
25	Kartik kolma	72.00	16.30	3.66	1.26	85	6.30	0.22	0.34
26	Kashi	78.60	22.00	34.70	20.72	100	7.00	0.27	0.71
27	Kashi Dhan	88.80	21.30	28.80	6.81	111	6.30	0.23	0.24
28	Kuiari Sail	70.30	18.50	2.97	1.45	92	8.60	026	0.48
29	Latha Sail	71.00	20.00	27.95	17.95	105	8.30	0.27	0.67
30	Maghi Sail	76.30	25.30	29.54	13.80	109	6.00	0.35	0.49
31	Mala	70.00	22.60	26.11	18.7	106	7.60	0.31	0.69
32	Malati	85.30	29.30	25.11	13.70	138	6.00	0.34	0.53
33	Methi Chikon	91.00	20.00	4.85	2.26	99	9.30	0.21	0.44
34	Monohar sail	73.30	26.60	23.90	20.90	101	5.30	0.36	0.93
35	Ranjit	57.30	19.30	30.21	9.93	111	5.60	0.34	0.50
36	Samras	.85.60	25.00	20.60	6.19	111	4.00	0.28	0.28
37	Shem Sail	72.60	20.60	16.90	7.10	111	5.00	0.28	0.38
38	Soulpona	92.00	20.60	5.19	3.52	102	8.30	0.22	0.64
39	Zoti	88.80	17.11	31.40	18.89	125	8.00	0.19	0.59
Mean		76.37	21.78	19.65	11.01	103.02	6.89	0.28	0.57
$SE_m \pm$		1.90	0.61	1.77	1.30	2.44	0.30	0.01	0.04

GCV and PCV was observed for root weight and root:shoot ratio by weight, which revealed significant influence of environment on their expression (Table 2 and 3). High GCV and PCV for any character, in general, indicate the possibility of improvement of the character through selection breeding provided that the difference between GCV and PCV is small. Heritability of a trait is the ratio of genotypic variance to phenotypic variance. In other words, it is the proportion of phenotypic variance that is attributed to genes. Genetic advance is the

Table 3. Estimates of genetic parameters for traits affecting drought resistance.

Character	Range		GCV (%)	PCV (%)	h^2_{bs}	GA (% of mean)
	Max^m	Min^m				
1. Shoot length (cm)	96	55	19.11	20.36	88.06	36.94
2. Root length (cm)	29	16	13.12	20.50	40.95	17.08
3. Shoot weight (g)	35.20	3.66	50.31	67.22	56.05	77.59
4. Root weight (g)	27.40	1.26	23.96	94.50	44.77	87.11
5. No. of primary roots	138	81	12.63	19.11	43.24	17.11
6. No. of tillers/plant	11	4	22.55	33.34	45.74	31.36
7. Root:shoot ratio by length	0.27	0.19	13.35	24.56	29.54	14.96
8. Root:shoot ratio by weight	1.57	0.21	39.23	65.64	35.71	48.29

GCV = Genotypic coefficient of variation; PCV = phenotypic coefficient of variation; h^2_{bs} = heritability in broad sense.

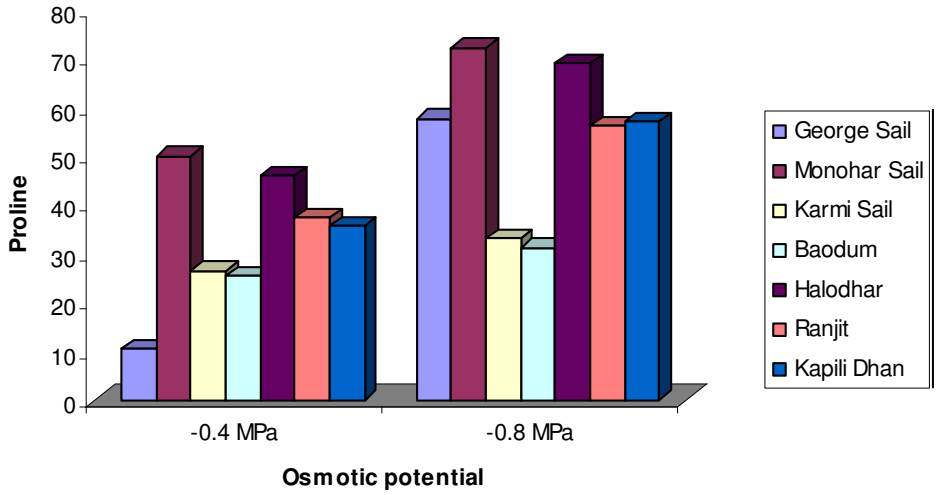

Figure 1. Effect of moisture stress imposed by PEG-6000 at -0.4 and -0.08 MPa on proline content (%) of leaf sample.

magnitude genetic improvement that could be brought about through selection breeding on a trait. In the present study, high heritability (above 60%) or moderate heritability (30 – 60%) associated with high (above 20%) or moderate (10 - 20%) GA was observed for shoot length, shoot weight, no. of tillers/plant, root weight, no. of roots, root/shoot ratio by weight and root length. This indicated that these traits were predominantly governed by additive gene action and hence would respond to artificial selection. But low heritability with moderate GA was observed for root/shoot ratio by length indicating the major role of nonadditive gene action in its expression.

Proline content and drought tolerance

High proline content is a good index for moisture resistance in genotypes. Under moisture stress condition the protein degrades and consequently the proline content increases. In the present study out of seven genotypes, Monohar Sail recorded the highest increase in proline content (50%) over control at -0.4 MPa moisture stress imposed by PEG – 6000 followed by Halodhar (46.1%) and Ranjit (37.50%). But at -0.8 MPa water stress Monohar Sail recorded the highest increase in leaf proline content (72.2%) followed by Halodhar (69.2%), George Sail (57.8%) and Kapili Dhan (57.1%). So, the rice genotypes Halodhar, George Sail and Kapili Dhan would be considered as potential genotypes for drought resistance (Figure 1).

Protein content and drought tolerance

Under moisture stress condition the leaf protein content gradually decreases. The genotypes showing highest decrease in leaf protein content could be considered as drought resistant. In the present study Monohar Sail

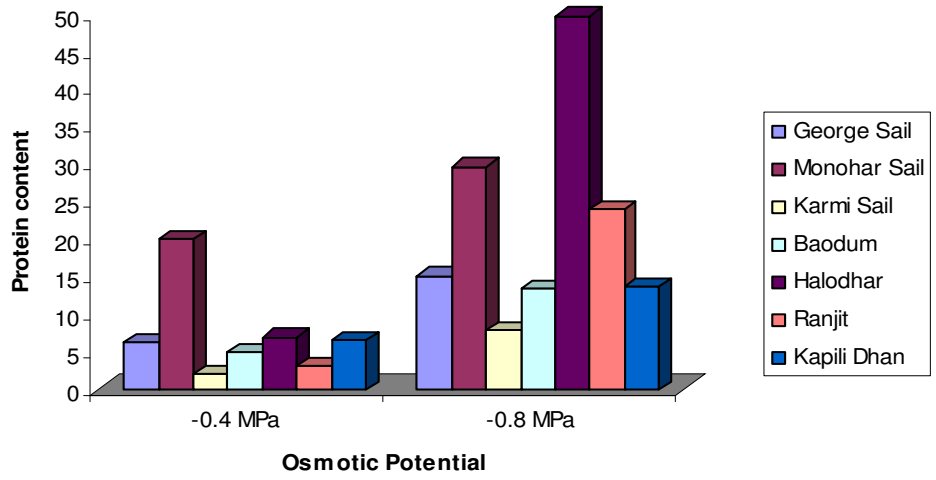

Figure 2. Effect of moisture stress imposed by PEG-6000 at -0.4 and -0.08 MPa on protein content (%) of leaf sample.

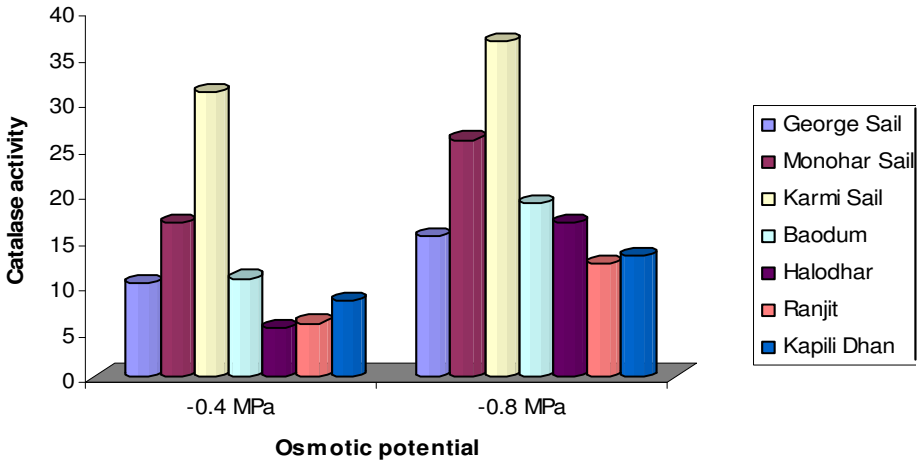

Figure 3. Catalase activity (units/g of leaf tissue) of rice genotypes under -0.4 MPa and -0.8 MPa moisture stress imposed by PEG-6000.

recorded the highest decrease in protein content (20.01%) at -0.4 MPa followed by Halodhar (6.90%) and Kapili Dhan (6.62%). But at -0.8 MPa Halodhar recorded the highest decrease in leaf protein content (49.67%) followed by Monohar Sail (29.62%) and Ranjit (24.00%). So, the genotypes Halodhar and Kapili Dhan could be considered as more drought resistant along with two checks Ranjit and Monohar Sail (Figure 2).

Catalase activity and drought tolerance

Catalase detoxifies H_2O_2, formed under moisture stress regime, to form water and oxygen. Increased catalase (CAT) activity is related to increased level of drought resistance in genotypes. Out of seven genotypes, Karmi Sail recorded the highest CAT activity (31.14%) followed by Monohar Sail (10.82%) and Baodum (10.17%) at -0.4 MPa but at -0.8 MPa Karmi Sail recorded the highest increase in catalase activity (36.62%) followed by Monohar Sail (25.87%) and Baodum (18.89%). Therefore, on the basis of catalase activity, Karmi Sail and Baodum would be considered as drought resistant genotypes along with Monohar Sail (Figure 3).

Correlation analysis

Correlation analyses were performed among leaf proline content, protein content and catalase activity of seven rice genotypes under normal (control) and drought stress

Table 4. Estimates of correlation coefficients among leaf proline content, protein content and catalase activity under normal and drought stress.

Correlation between	Drought stress condition		
	Control (normal)	-0.4 MPa	-0.8 Mpa
Proline and protein content	- 0.05	- 0.32 **	0.01
Proline and CAT activity	0.19 **	- 0.17 **	0.24 **
Protein content and CAT activity	- 0.06	0.19 **	0.25 **

** Significant at P = 0.01.

Table 5. Correlation coefficients between different conditions.

Correlation between conditions	Drought resistance parameter		
	Proline	Protein content	CAT activity
Control & -0.4 MPa stress	0.65 **	0.89 **	0.88 **
Control & -0.8 MPa stress	0.83 **	0.53 **	0.91 **
-0.4 Mpa & - 0.8 MPa stress	0.81 **	0.95 **	0.98 **

** Highly Significant at P = 0.01.

conditions, that is, -0.4 and -0.8 MPa imposed by PEG–6000 (Table 3). The analysis revealed that proline content showed highly significant negative correlation (-0.32) with protein content under -0.4 MPa stress. But proline content showed highly significant positive correlation with CAT activity under normal and -0.8 MPa stress. Protein content showed highly significant positive correlation with CAT activity under both -0.4 and -0.8 MPa drought stress conditions. A genotype with high proline, high CAT activity but low protein content under drought stress would be more adaptable than other genotypes.

The correlation study for proline content of seven genotypes among three conditions (control, -0.4 MPa stress and -0.8 MPa stress) revealed highly significant (P = 0.01) positive correlation (0.65, 0.83 and 0.81) amongst the conditions (Table 4). This indicated that all the genotypes exhibited similar response for proline content under stress environment. Similar results of correlation were also found for protein content and CAT activity individually. This suggested that all the genotypes responded in the same manner under stress environments, which in turn, could be attributed to the same physiological mechanism operating in all the genotypes in combating drought stress (Tables 4 and 5).

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