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EVALUATION OF RICE HYBRIDS AND THEIR PARENTS FOR DROUGHT TOLERANCE

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SUMMARY

A study was carried out to assess the drought tolerance ability of 39 rice genotypes (12 parents and 27 hybrids) using PEG-6000 induced moisture stress, on the basis of morphological, physiological, biochemical and histological parameters such as germination percentage, shoot length, root length, seedling dry weight, promptness index (PI), germination stress index (GSI), protein fraction and root anatomy. *In vitro* screening revealed that morphological and physiological characters were reduced significantly at -0.75MPa compared to control (0.00MPa). Among the genotypes, Nootripathu, Norungan, PMK 2, Norungan x PMK 2 and Nootripathu x PMK 2 performed better under stress in terms of morphological and physiological characters. SDS-PAGE analysis revealed prominent expression of 65 kDa and 78 kDa proteins in stressed parents, *viz.* Norungan, and Nootripathu and hybrids Norungan x PMK 2 and Nootripathu x PMK2. The expression was totally absent in PMK 2 stressed plants. These proteins could possibly be responsible for the development of water stress tolerance. Root anatomy of Norungan x PMK 2 and Nootripathu x PMK 2 grown under control and stress situation was studied. Increase in the number of xylem vessels of varying diameter, wider pith and compact cell arrangement was noticed in roots under stress.

Key words: **Drought tolerance, PEG-6000, rice**

INTRODUCTION

Rice is grown in diverse ecosystems under wide ranging temperature and water regimes. Of the cultivated area in India, 60 per cent is under irrigated and the rest is under rainfed conditions. The irrigated ecosystem has been mostly exploited by introducing high yielding varieties coupled with management practices. Chances of further increase in the rice irrigated ecosystem are not bright. In upland areas, drought is an important environmental factor adversely affecting rice production, wherein the rainfall distribution is erratic and uncertain. Drought is both a meteorological and hydrological event involving precipitation, evaporation and soil water storage (McWilliam 1986). Impact of drought is a function of duration, crop growth stage, type of crop species or cultivar within species, type of soil and adopted management practices. A well defined research programme in crop stress physiology is needed for the improvement of rainfed rice because increase in rice production from rainfed rice ecosystem is necessary to achieve the required target as the irrigation water availability is becoming scarce and large areas are under rainfed cultivation.

Among the various traits which help assess drought tolerance, root traits are more reliable on account of their high correlation with drought tolerance mechanism (Chang *et al*. 1982) and considerable genotypic variations for root traits existing in rice. So understanding the changes that

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take place in rice roots under water stress would help in developing cultivars better suited to the rainfed ecosystem. Therefore, the present study was undertaken to assess the effect of PEG-6000 induced short term moisture stress on drought tolerance of genotypes on the basis of changes in some important morphological parameters.

MATERIALS AND METHODS

Twelve genotypes, i.e. nine lines Varappukudanchan (L_1), Poongar (L_2), Vellaichitraikar (L_3), Kuliyadichan (L_4), Nootripathu (L_5), Norungan (L_6), Sivappuchitraikar (L_7), Mattaikar (L_8), Kavuni (L_9), and three testers, PMK 2 (T_1), PM 9106 (T_2) and TGR 75 (T_3) obtained from the rice germplasm collection maintained in Agricultural Research Station, Paramakudi, Tamilnadu were used for the present study. Among the parents nine drought resistant local landraces were used as lines and three short duration rice cultivars were used as testers. Most of the parents included in the present investigation are recommended for rainfed and semi-dry cultivation. Each of the nine lines was crossed with three testers and the crossed seeds from the 27 cross combinations were collected at maturity. The selfed seeds from individual parents were also collected at maturity.

Screening of genotypes was done at water potential of –0.75 MPa (240 g of PEG – 6000 in 1 litre of distilled water gives a water potential of –0.75 MPa) and 0.00 MPa. An external water potential of –0.75 MPa was selected based on a preliminary standardization study (Maibangsa 1998) with 39 rice genotypes (9 lines, 3 testers and 27 hybrids) were screened against control. PEG was used as an osmolyte because it is an inert, non-toxic and non-penetrating solute in plant research, unlike other osmolytes such as mannitol, sodium chloride and sugar (Kramer and Boyer 1995).

Two layers of filter paper in each Petri dish were moistened with the PEG-6000 solution and 25 surface sterilized seeds were placed. For initial wetting of the filter papers, 5 ml of the PEG-6000 solutions were required and 2 ml every alternate day. The same procedure was followed for control (0.00 MPa) using distilled water. Each treatment was replicated thrice. Emergence of 2 mm radicle from the seed coat was taken as the criterion for germination (Hadas 1976, Goswami and Baruah 1994). Seed germination was recorded on 2^{nd}, 4^{th}, 6^{th}, 8^{th} and 14^{th} day after sowing and expressed in per cent. Ten seedlings from each Petri dish were randomly selected on the 14^{th} day after sowing and the root and shoot lengths were measured. The average values were expressed in centimeters. Ten randomly selected seedlings were removed and dried in an oven at 80°C for 48 hours. The average seedling dry weight was expressed in milligrams. Promptness index under each treatment was computed as described by George (1967). GSI was computed for each variety by using the relationship described by Dhopte and Livera (1989). Five hundred milligram of freshly collected leaf samples were cut into small pieces and homogenized in 2 ml of 50 mM potassium phosphate buffer in a prechilled pestle and mortar. The homogenate was centrifuged in a refrigerated centrifuge at 15,000 rpm for 15 minutes and the supernatant was used for analysis. Electrophoretic separation of protein was achieved with 12.5% SDS – PAGE as described by Laemmli (1970).

Root samples were collected from ten day-old seedlings grown under control (distilled water) and water stress conditions (PEG-6000 treated). Roots were cut into bits one cm long along their midlength and placed in a fixative made up of 95% ethyl alcohol (50 ml), glacial acetic acid (5 ml), formalin (10 ml) and water (25 ml) for 24 hr. Roots were transferred to a series of dehydrating solutions containing increasing order (70, 80, 90 and 100%) of the dehydrant, tertiary butyl alcohol for 12 hr each. Roots were equilibrated in a solution containing 1:1 liquid paraffin and tertiary butyl alcohol for one hr and then transferred and embedded in pure melted paraffin wax and allowed to solidify. The specimen was then sectioned using a microtome to obtain 20 – 30 µm thin sections. The slides were subjected to deparaffinising in xylene for 2 minutes, stained with safranin and observed through microscope for anatomical features (Umayal 2001). All the above observations recorded were subjected to factorial completely randomized design (FCRD) with treatments replicated thrice.

RESULTS AND DISCUSSION

A preliminary standardization study revealed that an external water potential of –0.75 MPa was appropriate for screening rice genotypes. On this basis, 39 rice genotypes (12 parents and 27 hybrids) were subjected to two different water potentials, *viz*. 0.00 MPa (control) and –0.75 MPa (stress). Statistical analysis exhibited significant difference in genotypes, treatment and their interactions. Germination percentage of rice genotypes under control ranged between 80.67 (L_9 x T_3) and 100 per cent (T_1) with a mean of 92.55 per cent (Table 1). The germination percentage of all genotypes was significantly reduced under stress with a mean germination percentage of 62.99 with a range from 39.33 (L_1 x T_3) to 83.33 per cent (L_6 x T_1). Shoot length of rice seedlings was significantly reduced under stress (Table 1), when compared to control, ranging from 0.61 (L_8 x T_3) to 3.13 cm (L_6 x T_1) with a mean of 1.47 cm, whereas the control had a range of 3.01 (L_8 x T_2) to 7.00 cm (L_6 x T_1) with a mean of 5.38 cm. Significant variations in the root length of genotypes were recorded both under control and stress conditions (Table 1). Root length ranged between 2.24 (L_3 x T_3) and 5.15 cm (L_5 x T_1) under control with a mean of 3.96 cm while under stress, it ranged from 0.75 (L_3 x T_2) to 5.05 cm (L_6 x T_1) with a mean of 2.43 cm.

Dry weight of seedling under control varied from 3.00 (L_3 x T_3) to 7.53 mg (L_6) with a mean of 5.61 mg (Table 2). Seedling dry weight was reduced significantly at lower water potential (-0.75 MPa) as compared to control. Under stress, dry weight per seedling ranged from 0.47 (L_3 x T_3) to 2.40 mg (L_6) with a mean of 1.31 mg. Promptness index (PI) under control varied significantly from 131.56 (L_3 x T_3) to 205.50 (L_6 x T_1) with a mean of 174.30 (Table 2). PI was reduced significantly under stress and it ranged from 25.59 (L_8 x T_3) to 62.76 (L_6 x T_1) with a mean of 39.97. Germination stress index ranged from 16.27 (L_3 x T_1) to 30.45 (L_6 x T_1) with a mean of 22.72 (Table 2).

Genotypic variations in germination and seedling growth under lower water potential were critical for quick establishment ability under water stress condition (Redona and Mackill 1996). Results showed that germination was reduced at –0.75 MPa and genotypes varied significantly in their germination capability under reduced water potential. Decreased germination might be due to the additive effect of both water potential and osmotic potential on the inhibition of seed germination as reported by Bernstein (1961). Seedling growth in terms of seedling dry weight, shoot and root length was also reduced in most of the genotypes at –0.75 MPa. Among the parents, Norungan (L_6), Nootripathu (L_5), Kavuni (L_9) and PMK 2 (T_1) and the hybrids Norungan x PMK 2 (L_6 x T_1), Nootripathu x PMK2 (L_5 x T_1), Norungan x PM 9106 (L_6 x T_2), Nootripathu x PM 9106 (L_5 x T_2) and Kavuni x PM 9106 (L_9 x T_2) recorded high germination percentage, shoot and root length and seedling dry weight under reduced water potential indicating their ability to tolerate drought. This was in confirmation with study of Goswami and Baruah (1994) and Jha and Singh (1997).

Promptness index indicates the speed of germination. Tolerant genotypes, *viz*. Norungan (L_6), Nootripathu (L_5), Kavuni (L_9), PMK 2 (T_1), Norungan x PMK 2 (L_6 x T_1), Nootripathu x PMK 2 (L_5 x T_1), Norungan x PM 9106 (L_6 x T_2), Nootripathu x PM 9106 (L_5 x T_2) and Kavuni x PM 9106 (L_9 x T_2) recorded higher promptness index and germination stress index than other genotypes. This was in confirmation with findings of Maibangsa (1998). So these genotypes are noteworthy for inclusion in a breeding programme to develop drought tolerant rice.

The SDS–PAGE protein profile (Fig. 1) revealed the qualitative and quantitative differences in protein profile under stress. Prominent expression of 65 kDa and 78 kDa proteins in stressed parents Norungan (L_6), and Nootripathu (L_5) and hybrids Norungan x PMK 2 (L_6 x T_1) and Nootripathu x PMK 2 (L_5 x T_1) was observed but it was totally absent in PMK 2 (T_1) stressed plants. These proteins may be responsible for providing osmotic adjustment to the cells either by facilitating the accumulation of solutes or certain metabolic changes in the cell, which may be helpful in osmotic adjustment and thus might be responsible for the development of water stress tolerance. Similar results were reported by Maibangsa (1998) and Rajkumar (2001) in rice. Dure

Table 1. Effect of water stress on germination, root length, shoot length, seedling dry weight, promptness index and germination stress index of rice genotypes

Genotypes	Germination (%)		Root length (cm)		Shoot length (cm)		Seedling dry weight (mg)		Promptness index		Germination stress index
	0.00 MPa	-0.75 MPa	0.00 MPa	-0.75 MPa	0.00 MPa	-0.75 MPa	0.00 MPa	-0.75 MPa	0.00 MPa	-0.75 MPa	
L_1	96.67 (79.60)	65.33 (53.93)	4.66	2.14	5.72	1.03	6.70	1.30	178.18	38.61	21.67
L_2	97.33 (80.74)	68.67 (55.96)	3.43	2.36	5.95	1.46	5.23	0.97	171.50	40.54	23.64
L_3	97.33 (80.74)	53.33 (46.91)	4.33	2.36	5.36	1.24	4.70	0.80	165.57	34.65	20.93
L_4	98.67 (84.52)	55.33 (48.06)	3.42	3.02	6.03	1.73	4.53	1.43	180.51	35.54	19.69
L_5	98.00 (81.87)	74.67 (59.79)	4.72	3.54	6.29	2.26	7.20	2.23	192.33	54.56	28.36
L_6	99.33 (87.16)	80.67 (63.92)	3.51	4.95	5.04	2.51	7.53	2.40	198.75	58.49	29.45
L_7	96.67 (79.60)	64.67 (53.53)	3.80	1.46	4.85	1.60	6.40	1.53	183.56	42.24	23.01
L_8	97.33 (80.74)	58.67 (49.99)	4.53	3.15	5.26	1.45	6.13	1.17	160.59	32.59	20.30
L_9	98.67 (84.52)	76.67 (61.12)	5.01	3.34	5.95	1.77	6.90	2.07	188.31	48.47	25.54
T_1	100.00 (89.82)	70.67 (57.21)	4.00	4.06	6.95	3.03	6.80	1.80	190.47	42.48	22.30
T_2	98.67 (84.52)	63.33 (52.74)	3.95	3.08	6.55	1.20	6.23	1.43	182.56	38.64	21.65
T_3	99.33 (87.17)	61.33 (51.55)	3.25	2.84	5.16	1.03	4.80	1.03	167.35	32.65	19.51
$L_1 \times T_1$	92.67 (74.32)	48.67 (44.24)	4.79	2.04	6.04	0.88	6.40	1.43	181.56	38.50	21.24
$L_1 \times T_2$	90.67 (72.24)	40.67 (39.62)	2.52	1.27	5.57	1.27	6.03	1.20	175.34	34.45	19.48
$L_1 \times T_3$	89.33 (70.96)	39.33 (38.84)	4.71	1.47	4.25	1.04	5.50	0.83	160.46	27.55	16.76
$L_2 \times T_1$	90.67 (72.24)	60.67 (51.16)	4.14	2.06	6.16	1.16	5.80	1.07	176.66	32.76	18.55
$L_2 \times T_2$	82.67 (65.41)	62.67 (52.34)	3.45	1.68	6.02	0.92	4.97	0.93	180.52	40.86	22.64
$L_2 \times T_3$	94.67 (76.70)	56.67 (48.83)	4.86	2.15	4.12	0.72	4.70	0.60	131.57	28.24	21.46
$L_3 \times T_1$	93.33 (75.07)	55.33 (48.83)	4.77	0.91	5.45	1.44	4.50	1.17	157.51	25.61	16.27
$L_3 \times T_2$	90.67 (72.24)	45.33 (42.23)	3.60	0.75	3.11	1.06	3.70	0.97	162.52	34.23	21.06
$L_3 \times T_3$	85.33 (67.49)	41.33 (40.01)	2.24	1.56	4.07	0.83	3.00	0.47	131.56	30.34	23.07
$L_4 \times T_1$	93.33 (75.07)	63.33 (52.74)	4.82	2.50	6.17	1.22	6.17	1.53	190.46	44.66	23.45
$L_4 \times T_2$	92.00 (73.57)	69.33 (56.38)	4.73	2.64	4.80	0.73	5.73	1.17	165.36	40.33	24.38
$L_4 \times T_3$	85.33 (67.49)	60.67 (51.16)	2.47	1.84	3.25	1.03	3.50	1.00	143.59	33.65	23.44
$L_5 \times T_1$	94.67 (76.70)	81.33 (64.41)	5.15	3.64	6.62	2.37	6.83	1.83	196.54	55.42	28.20
$L_5 \times T_2$	95.33 (77.58)	73.33 (58.91)	4.93	3.04	6.05	2.04	6.13	1.53	192.47	52.42	27.23
$L_5 \times T_3$	83.33 (65.92)	69.33 (56.38)	4.04	2.76	5.66	1.67	5.47	1.23	180.71	41.75	23.10
$L_6 \times T_1$	95.33 (77.58)	83.33 (65.92)	4.52	5.05	7.00	3.13	7.00	2.10	205.50	62.76	30.45
$L_6 \times T_2$	94.67 (76.70)	82.67 (65.41)	4.85	3.52	6.83	2.23	6.67	1.77	196.59	54.72	27.83
$L_6 \times T_3$	90.67 (72.24)	71.33 (57.63)	3.35	2.07	6.17	1.64	6.10	1.47	187.54	48.62	25.92
$L_7 \times T_1$	89.33 (70.96)	59.33 (50.38)	2.78	1.23	5.48	0.95	5.67	1.57	189.59	36.52	19.26
$L_7 \times T_2$	81.33 (64.41)	66.67 (54.74)	2.43	0.96	3.75	1.65	4.73	1.33	179.23	30.58	17.06
$L_7 \times T_3$	89.33 (70.96)	51.33 (45.77)	3.72	1.03	4.06	1.28	3.83	1.03	162.52	37.31	22.96
$L_8 \times T_1$	90.67 (72.23)	61.33 (51.55)	2.42	2.52	6.24	1.08	5.17	0.80	158.75	33.43	21.06
$L_8 \times T_2$	88.67 (70.35)	54.67 (47.68)	3.56	1.84	3.01	0.86	5.77	0.77	152.33	28.45	18.06
$L_8 \times T_3$	89.33 (70.96)	49.33 (44.62)	4.01	0.84	5.57	0.61	4.83	0.57	148.44	25.59	17.24
$L_9 \times T_1$	92.67 (74.32)	71.33 (57.63)	4.65	2.95	4.01	1.83	6.50	1.53	180.56	45.58	25.25
$L_9 \times T_2$	94.67 (76.70)	80.67 (63.92)	5.02	3.32	6.55	2.14	6.90	1.83	190.51	54.52	28.43
$L_9 \times T_3$	80.67 (63.92)	63.33 (52.74)	3.32	2.75	4.51	1.24	4.00	1.07	159.49	40.67	25.50
Mean	92.55 (75.52)	62.99 (52.79)	3.96	2.43	5.38	1.47	5.61	1.31	174.30	39.97	22.72
	G	**T**	**G**	**T**	**G**	**T**	**G**	**T**	**G**	**T**	
SED	0.88	0.20	0.02	0.004	0.03	0.006	0.05	0.01	0.59	0.13	0.50
CD (5%)	1.74	0.39	0.03	0.007	0.05	0.01	0.10	0.02	1.16	0.26	0.99

Values in parentheses indicate the arc sine transformation.

et al. (1989) and Dure (1993) suggested that these proteins might play a role in reducing cellular damage during water stress.

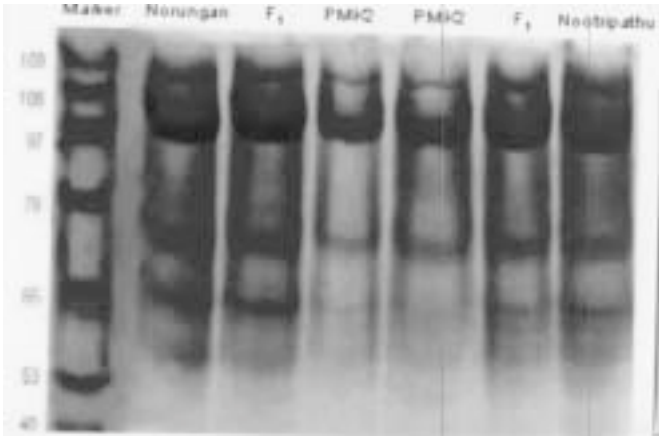

Fig. 1. Protein profile: SDS – PAGE: Norungan x PMK 2 and Nootripathu x PMK 2

Root anatomy was studied under control and stress situations in Norungan x PMK 2 (L_6 x T_1). Striking differences were observed between control and water stressed roots (Fig.2a, b). More number of xylem vessels with different diameters was noticed in stressed roots. Besides, the central portion of the root under stress was wider and xylem vessels were broader compared to control. Pith was wider in stressed root cells while the cell arrangement was compact in the control. Yambao *et al*. (1992) and Salih *et al*. (1999) reported that the broader xylem vessels had resistance to water flux and hence greater capacity for water uptake.

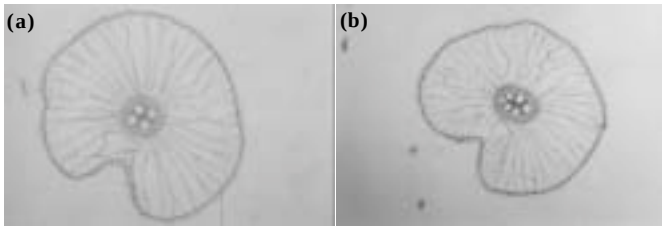

Fig. 2. Root anatomy of Norungan x PMK 2: (a) under control (b) under stress

The study revealed that germination and seedling growth in terms of seedling dry weight, shoot and root length were reduced significantly at -0.75MPa. Genotypes such as Nootripathu, Norungan, PMK2, Norungan x PMK2, and Nootripathu x PMK2 had recorded high germination percentage, shoot and root length and seedling dry weight under reduced water potential indicating their drought tolerance behaviour. SDS-PAGE analysis revealed prominent expression of 65 kDa and 78 kDa proteins in stressed parent of Norungan, Nootripathu and hybrids Norungan x PMK 2 and Nootripathu x PMK2 and was totally absent in PMK 2 stressed plants. These proteins might be responsible for the development of water stress tolerance. Increase in the number of xylem vessels of varying diameter, wider pith and compact cell arrangement was also noticed in roots under stress. Some of these genotypes are therefore, noteworthy for inclusion in breeding programme to develop drought tolerant rice.

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