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EFFECT OF PEG STRESS ON GROWTH AND ASSOCIATED PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN SELECTED AND NON SELECTED UPLAND RICE

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SUMMARY

Calli of two rice genotypes, viz. Rasi amd Valya were selected under PEG stress. Both varieties exhibited significant differences in growth, turgor potential, solute accumulation and lipid peroxidation parameters. There was better growth, increased better turgor potential values, greater accumulation of reducing sugars, amino acids, proline and superoxide dismutase (SOD) activity in the selected tissues of the varieties. However, between the two genotypes, tissues of Rasi exhibited higher values in terms of growth, water potential, solute accumulation and SOD than cv Valya which exhibited lowest values. In this study, it was concluded that the recurrent selection has improved the PEG tolerance in Rasi than Valya yet normal growth was not restored as that of control genotypes.

Key words: **PEG stress, pressure potential, solute potential, superoxide dismutase, water potential.**

INTRODUCTION

Cellular mechanism of stress tolerance are important in developing drought tolerance among rainfed crops. Somaclonal selection technique is one of the recent methods applied to develop tolerant crops. (Warne and Hickok 1987). *In vitro* selection to develop stress tolerance rainfed rice is of utmost importance (Kavikishor and Reddy 1984, Chandrasekhara Reddy *et al.* 1994). Accumulation of different solutes in rice varied under water stress; composition of solute concentration in stressed cells accumulated more sucrose and reducing sugars and exhibited greater osmotic potential, than control (Chandrasekhara Reddy *et al.* 1994).

Growth of non adapted or adapted calli in stress media is shown to have different mechanisms of tolerance, developed during adaptation and selection. Generally, the phenomenon of growth is accompanied by changing water relations and osmotic adjustments leading to build up of turgor pressure, reaction of more positive turgor to the continued extension of cell wall and growth (Hasegawa *et al.* 1984).

Drought/ stress affects physiological processes both at whole plant and cellular levels (Morgan 1984). Stress affects the membrane integrity (Dhindsa and Matowe 1981). The activity of some of the enzymes leads to a greater production of free radicals. These free radicals cause lipid peroxidation and membrane deterioration in plants (Chandrasekhara Reddy and Vajranabhaiah 1993). These lipid peroxidation products inhibit the protein synthesis by hydrolysis of m-RNA (Corcuera *et al.* 1989). The mechanism of prevention of lipid peroxidation consists of free radical scavenging enzymes, including superoxide dismutase and catalse which scavenge the free radicals,

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thus lead to drought tolerance (Spychalla and Desborough 1990). Hence, in the present study, cell lines selected under PEG stress are compared with the non selected ones to understand the cellular mechanisms of drought endurance as a step towards development of improved rice genotypes.

MATERIALS AND METHODS

Seed (embryo) calli from two rice genotypes, viz. Rasi and Valya initiated on Murashige and Skoog's (1962) modified medium supplemented with 2, 4-D (2 mg l^{-1}) and kinetin (0.25 mg l^{-1}) were subcultured and maintained on the same medium, where NAA (2.0 mg l^{-1}) replaced 2, 4-D.

Polyethylene glycol (PEG 6000) was incorporated in the media to create -0.4, -0.6, -0.8 and -1.0 Mpa of stress. Callus initiated in these were cultured repeatedly in PEG free and PEG MS media with growth regulators (NAA 2.0 mg l^{-1} and kinetin 0.25 mg l^{-1}) for nine generations (R9), were used for this experiment. Calli grown on PEG free and PEG medium from R10 generation, the fresh (FW) and dry (DW) weights and cell number per culture were determined on 30th day after inoculation (DAI). Water potential (-Ψw) was determined as described earlier (Barr's 1968), solute potential (-Ψs) was determined by using vapour pressure osmometer (Wescor, HP-115) and pressure potential (+Ψp) was calculated as difference between -Ψw and -Ψs (+Ψp=-Ψw-(-Ψs). Solutes, reducing sugars (Nelson 1994), proline (Bates *et al.* 1973), amino acids (Spices 1957) and melon dialdehyde (MDA) (Chandrashekara Reddy and Vajranabhaiah 1993), superoxide dismutase (SOD) (Beauchamp and Fridowich 1971) and soluble proteins (Lowry *et al.* 1951) were also determined.

RESULTS AND DISCUSSION

Fresh (FW) and dry (DW) weights reduced by PEG stress more at higher levels of stress. This decline was common in both the genotypes. Selected tissues (ST) maintained higher growth, as indicated in FW and DW recorded on 30th day at all stress levels, than in

Table 1. Effect of PEG stress on selected and non selected rice calli

Treatments genotypes	FW (mg)		DW (mg)		Cell no (x 10^7)	
	ST*	NST**	ST	NST	ST	NST
CONTROL						
Rasi	647.0		75.8		2.30	
Valya	569.0		58.3		1.50	
STRESS (-Mpa)						
Rasi						
0.4	361	186	67.4	22.4	1.66	1.16
0.6	262	162	31.3	19.9	1.50	1.05
0.8	202	131	24.0	16.1	1.29	0.83
1.0	152	91	16.4	11.6	1.04	0.58
Valya						
0.4	193	158	20.9	16.0	1.10	1.00
0.6	149	135	16.4	14.1	0.90	0.70
0.8	125	81	14.0	8.7	0.50	0.30
1	118	Dead	13.0	Dead	0.50	Dead
CD 0.01 Var	36	30	3.00	2.20	0.10	0.10

*Selected tissue - tissues continuously grown under PEG stress media for 10 generations

** Non-selected tissues - tissues continuously grown in control medium and transferred to stress medium on 10th generation.

corresponding treatments given to non-selected (NST) calli of same age group. The same observations were made for cell numbers also, where decrease in cell number due to increase in PEG stress led to more reduction in NST than ST (Table 1). Water (-Ψw), solute (-Ψs) and pressure (+Ψp) potentials (Table 2) examined during the course of 30 DAI in culture revealed that the -Ψw and -Ψs were more negative in ST in general at all stress level and ST of rice genotypes exhibited greater reduction in -Ψw and -Ψs than NST at all stress levels(Table 2). Pressure potential values were also generally more positive in ST of all the genotypes than NST. A decline in +Ψp with

Table 2. Effect of PEG stress on water relation (-Mpa) in selected and non selected rice calli

Treatments genotypes	-Ψw		-Ψs		Ψp	
	ST	NST	ST	NST	ST	NST
CONTROL						
Rasi	0.80		1.79		0.99	
Valya	0.80		1.29		0.49	
STRESS (-Mpa)						
Rasi						
0.4	2.07	1.47	2.96	2.23	0.89	0.76
0.6	2.67	1.90	3.40	2.51	0.73	0.61
0.8	3.07	2.30	3.90	2.67	0.83	0.37
1.0	3.37	2.73	4.14	2.97	0.77	0.23
Valya						
0.4	1.83	1.57	2.27	1.60	0.44	0.03
0.6	2.50	1.83	2.55	1.88	0.05	0.05
0.8	2.50	1.40	2.59	1.33	0.09	0.07
1.0	2.90	Dead	2.90	Dead	0.0	Dead
CD 0.01 Var	0.15	0.11	0.16	0.13	0.09	0.08

Table 3. Effect of PEG stress on solute accumulation in selected and non slected rice calli

Treatments genotypes	Reducing sugars (mM)		Amino Acid (mM)		Proline (mM)	
	ST	NST	ST	NST	ST	NST
CONTROL						
Rasi	177		170		54	
Valya	116		128		42	
STRESS (-Mpa)						
Rasi						
0.4	204	110	185	179	91	66
0.6	245	189	196	183	115	78
0.8	276	200	202	184	166	86
1.0	250	210	213	180	187	92
Valya						
0.4	119	129	130	116	69	47
0.6	130	115	141	100	98	35
0.8	121	77	131	26	97	41
1.0	107	Dead	118	Dead	94	Dead
CD 0.01	17	16	8.0	6.0	5.0	4.0

increase in PEG concentration was noticed among the NST calli (Table 2). Solutes, reducing sugar (RS), aminoacid (AA) and proline accumulation in general are greater in the ST than in NST at all the stress levels (Table 3).

Two genotypes, Rasi and Valya, differed significantly in their growth, solute accumulation and also water relations. In general Rasi performed far better in terms of its growth, solute accumulation and osmotic adjustment than Valya (Table 1, 2 and 3) in all the stress levels.

Selected (S-S and S-C) tissues which maintained higher growth contained lower MDA levels under stress (Table 4). There was a general increase in MDA contents of both ST (S-S) and NST (C-S) tissues of all the rice varieties when subjected to stress. But this enhancement was relatively low in ST (S-S). SOD activity in the control tissues grown in normal medium was low. The activity of SOD increased, when the control tissues were subjected to stress. ST in PEG and PEG free media exhibited higher values of SOD activity. Highest activity was found in ST grown in stress medium. ST in stress medium maintained relatively higher protein than NST subjected to stress. Protein contents further increased in ST (S-S) when transferred to normal medium (S-C).

Rasi and Valya were on two extremes in their accumulation of MDA, SOD and soluble proteins. Rasi exhibited lower MDA and higher SOD activity and protein content as compared to Valya in all situations (Table 4).

Continued decline in FW and DW with increasing stress is common to both ST and NST tissues. Better growth, in terms of FW and DW of the ST than NST at 10^{th} generation (R10) shows that capacity to tolerate stress is more in ST rather than NST. NST of Valya became senescent at-1.0 Mpa of stress because of highly susceptible nature of stress. Lower percentage reduction in growth among the ST calli at different stress levels over the NST, clearly suggests that selection enhanced the tolerance and growth. Similar observations were reported earlier (Bressan *et al.* 1981). However in ST the growth is not fully recovered when they were transferred to control medium. This pattern of response is in conformity with that of tomato (Handa *et al.* 1983). Effect of stress has reduced proportionately the cell number per culture, suggesting a retardation effect of stress on cell division too. Similar observation made by Chandrasekhara Reddy and Vajranabhaiah (1996) in rice and this cell division is less affected in ST than NST at all stress level. This suggests that the selected tissues are able to survive and grow better under stress. Cell number was greatly affected in Valya as compared to Rasi at all stress levels this

Table 4. Effect of PEG stress on MDA content, SOD activity and soluble proteins in ST and NST of rice calli

Treatments	Fw (mg)	Dw (mg)	MDA (OD/g dw)	SOD (Units/mg dw)	Sol-proteins (mg/g dw)
Rasi					
C	657	77	1.52	145	50
C-S	161	22	2.61	278	43
S-C	459	68	1.66	261	48
S-S	201	28	2.04	373	47
Valya					
C	569	58	3.00	266	60
C-S	118	13	5.14	250	37
S-C	301	31	4.00	261	50
S-S	128	15	5.00	252.0	43
CD 0.01	10	2	0.1	10.0	2.0

C-Control
C-S - Control to stress medium (non-selected)
S-C - Stress medium to control medium (adapted cells in control medium)
S-S - Stress to stress medium (selected)

indicated that Rasi had more osmotic adjustment than Valya which led to better growth under severe stress.

Continued selection under stress from R_1 through R_{10} has led to the amplification of responses by way of greater reduction in -Ψw and -Ψs leading to more positive turgor at R_{10} in tissues of all gentotypes and at different stress levels. This osmotic adjustment leading to greater growth and turgor in ST than NST grown at different concentration of PEG are also reported earlier (Handa et al. 1986). There are identical reports on growth and osmotic adjustments in ST and NST of brassica (Chandler and Thorpe 1987, Paek *et al.* 1988). Selected tissues showed the degree of inhibition of growth to be lesser than NST under decreasing -Ψw. Similar observation made earlier (Fallon and Philipis 1989). Where the decline in FW and DW continued in un-adapted cells with decrese in -Ψw. This was greater than adapted cells.

A comparison of the ST and NST revealed that the degree of response is greater in ST calli which accumulated more of all the three solutes. Similar response was reported earlier (Fallon and Philips 1989), where, greater accumulation of the solute was of higher order than present observation in ST. Accumulation of these solutes (RS, AA and proline) in the ST and NST cell lines under PEG stress indicates that this may be the part of osmotic adjustment mechanism operating to maintain the turgor.

Since, stress is also known to cause membrane damage and leakage of solutes, attempts were also made to study the extent of lipid peroxidation and defense mechanisms as an indication of drought tolerance. The MDA content may indicate extent of resistance to membrane lipid peroxidation, which is also important in stress tolerance. Desiccation tolerance in the ST have more active free radical scavenging enzyme systems which retard the peroxidation of membrane lipids and accumulation of MDA. The free radicals quenching system is represented by SOD enzyme, enhanced activity of this enzyme in all the ST suggests prevention of damage caused to cell membrane by scavenging super oxide radicals. The increase in specific activity of this enzymes in the light of the reduced soluble protein contents in the ST and NST subjected to stress and ST in normal medium suggests more of biosynthesis of this class of proteins. This means the switching on and off of defensive mechanism against enhanced lipid peroxidation caused by stress. Such similar defensive mechanisms have been reported earlier (Spychalla and Desborough 1990).

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