Madras Agric. J., 102 (4-6): 144-150, June 2015
https://doi.org/10.29321/MAJ.10.001087

In vitro Studies on Drought Tolerance in Rice through Polyethylene Glycol (PEG) Induced Osmotic stress

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Selection by tissue culture through an osmoticum agent polyethylene glycol is precise for drought assessment compared to actual field condition. Mature seeds of fourteen parental lines (four lines and ten testers) and forty hybrids were used for developing callus on Murashige and Skoog (MS) medium supplemented with two treatments *viz.,* MS + 2, 4 –D 2 mg/l and MS + 2, 4-D 2 mg/l + CH 1 mg/l. One hundred mg of embryogenic callus was exposed to basal medium supplemented with different concentrations *viz*., 0, 0.5, 1, 1.5 and 2 per cent of polyethylene glycol (PEG) - 6000 as chemical drought inducer. Callus induction duration, effect of PEG on callus mass, proline content and plantlet regeneration were studied. Callus volume decreased and total proline content was found to be increased significantly with increased in PEG concentration. In five parents and twelve hybrids, plantlets regeneration was observed up to the highest concentration of PEG. On over all comparison, three parents namely, BR 2655, MAS 26, Vandana and three hybrids namely, TNAU CMS 2A x MAS 26, IR 58025 A x BR 2655, and IR 68897 A x MAS 26 exhibited positive response for all the four characters acting as drought governing indices.

Key words: Rice, PEG, Drought tolerance, Callus growth, Proline content, Plant regeneration

Drought, the most serious threat to world agriculture demands breeding for drought prone areas. Classical plant breeding for stressful environment is time consuming and inefficient because of the low heritability of yield under stress and low inherent variation in the field. (Ribaut *et al*., 1997). Development of rice hybrids with high yield potential for limited source of water would be one of the exciting research to be carried out to overcome the existing water crisis in India.

Therefore, identification of drought tolerant rice hybrids through *in vitro* screening using callus growth and proline accumulation in response to polyethylene glycol through induced osmatic stress is one of the promising approach. Plant cell and tissue culture had been known as an useful tool to study stress tolerance mechanisms under *in vitro* (Bajji *et al*., 2000). To stimulate the effect of water stress *in vitro,* researchers have incorporated polyethylene glycol (PEG) in the culture medium (Handa *et al.,* 1982). This compound is a high molecular weight, non-ionic and non plasmolysing substance that simulates drought stress in cultured cells almost in a similar sense to that observed in the cells of intact plants, subjected to drought condition (Attree *et al.,* 1991). Hence, this experiment was carried out to screen the parents and their hybrids for their inherent tolerance against drought stress.

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Materials and Methods

The study was conducted during 2012 in the plant tissue culture laboratory, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, India. Experimental materials consist of four A lines, ten testers and their resultant hybrids. Seeds were surface sterilized in 0.1% mercuric chloride for 8 to 10 min. Sterilized seeds were cultured in Petri dishes containing semisolid medium (Murashige and Skoog, 1962) supplemented with two different compositions *viz.,*MS + 2 mg / l, 2,4 – D + 0.5 mg / l kinetin and MS + 2 mg / lit 2,4 – D + 0.5 mg / l kinetin + 1 g / lit casein hydrolyte. After four weeks of incubation, the induced calli were sub-cultured under the same growth conditions, in the same MS medium to which various concentrations of PEG - 6000 (0, 0.5, 1.0, 1.5 and 2.0 %) were added. Resulting calli were excised and transferred into test tubes containing MS basal salts medium supplemented with 2.0 mg l^{-1} benzylaminopurine + 0.5 mg l^{-1} kinetin + 0.5 mg l^{-1} naphthalene acetic acid + 30 g l^{-1} sucrose + 8 g l^{-1} agar 30 g l^{-1} for shoot initiation, for a period of four weeks. Rooting was initiated on half strength MS medium.

Regenerating calli, showing shoot and root formations were transferred to MS basal medium with no phytohormones and placed in a chamber to sustain growth of regenerated plantlets. The pH of media was adjusted to 5.8 with 0.1 N NaOH prior to

autoclaving. The culture medium was autoclaved at 121ºC for 30 min. The following observations *viz.,* callus induction duration (days taken for callus induction), callus fresh weight, proline content (µg) at two extreme concentrations *viz.,* 0.5 and 2.0 per cent and plantlet regeneration(%). Data recorded were subjected to statistical scrutiny through factorial completely randomized design with two replications.

Results and Discussion

The overall aim of the experiment was to screen the rice hybrids and parents for drought tolerance

Table 1. Response of parents and hybrids in different media composition for callus duration

Genotypes	Media composition		Mean
	MS+ 2,4 –D 2 mg/l	MS + 2,4-D 2 mg/l + CH 1 g/l	
Lines IR 58025A	10.20*	9.75*	9.98
IR 68897A	12.82	11.52	12.17
TNAU CMS 2A	12.49	11.25	11.87
IR 68888 A	10.92*	10.25*	10.59
Mean	11.61	10.69*	11.15
Testers	9.75*	9.52*	9.64*
IR 60199 R	10.33*	9.75*	10.04*
IR 65912 R	12.50	11.25	11.88
IR 21567 R	15.25	14.69	14.98
CB 87 R	16.00	15.28	15.64
MAS 946-1	11.20*	10.90*	11.05*
MAS 26	14.75	13.27	14.01
BI 33	10.50*	9.78*	10.14*
KMP 148	9.75*	9.28*	9.52*
BR 2655	9.52*	8.85*	9.19*
Vandana	9.02*	8.57*	8.80*
MeanHybrids	11.88	11.16*	11.52
IR 68897 A x KMD 148	10.92*	10.27*	10.60*
IR 68897 A x BR 2655	10.85*	10.89*	10.87*
IR 68897 A x Vandana	9.82*	9.52*	9.67*
TNAU CMS 2A x IR 60199 R	11.22	11.12	11.17
TNAU CMS 2A x IR 65912 R	9.19*	9.01*	9.10*
TNAU CMS 2A x IR 21567 R	13.22	12.88	13.05
TNAU CMS 2A x CB 87 R	12.52	11.48	12.00
TNAU CMS 2A x MAS 946 -1	11.74	11.28	11.51
TNAU CMS 2A x MAS 26	10.24*	10.11*	10.18*
TNAU CMS 2A x BI 33	11.52	10.28*	10.90*
TNAU CMS 2A x KMP 148	11.17	10.99*	11.08
TNAU CMS 2A x BR 2655	10.72*	10.25*	10.49*
TNAU CMS 2A x Vandana	11.29	11.05	11.17
IR 68888A x IR 60199 R	13.28	13.05	13.17
IR 68888A x IR 65912 R	13.69	13.09	13.39
IR 68888A x IR 21567 R	9.77*	10.05*	9.91*
IR 68888A x CB 87 R	12.78	11.99	12.39
IR 68888A x MAS 946 -1	13.85	13.27	13.56
IR 68888A x MAS 26	13.37	13.72	13.55
IR 68888A x BI 33	7.25*	8.25*	7.75*
IR 68888A x KMD 148	13.75	12.75	13.25
IR 68888A x BR 2655	11.20	10.75*	10.98
IR 68888A x VandanaMEAN	13.5011.88	12.5511.16*	13.0311.52

	Lines		Testers			Hybrids	
	SEd	CD (0.50)	SEd	CD (0.50)		SEd	CD (0.50)
Genotypes (g)	0.06	0.15	0.06	0.12	Hybrids (h)	0.04	0.09
Media (m)	0.04	0.10	0.03	0.05	Media (m)	0.01	0.02
g x m	0.09	0.21	0.08	0.17	h x m	0.06	0.13

under *in vitro* conditions (Fig 1). Callus growth under stress was used as an index of stress tolerance and continued for stepwise selection, as a method to develop stress tolerant tissues and plants (Kavikishor and Reddy, 1985). PEG 6000 in different concentrations is commonly used as osmoticum to reduce the water potential and create osmotic stress in culture medium. The cell lines that survive under higher concentrations of PEG can be selected as drought tolerant types(Purohit *et al.,* 1998).

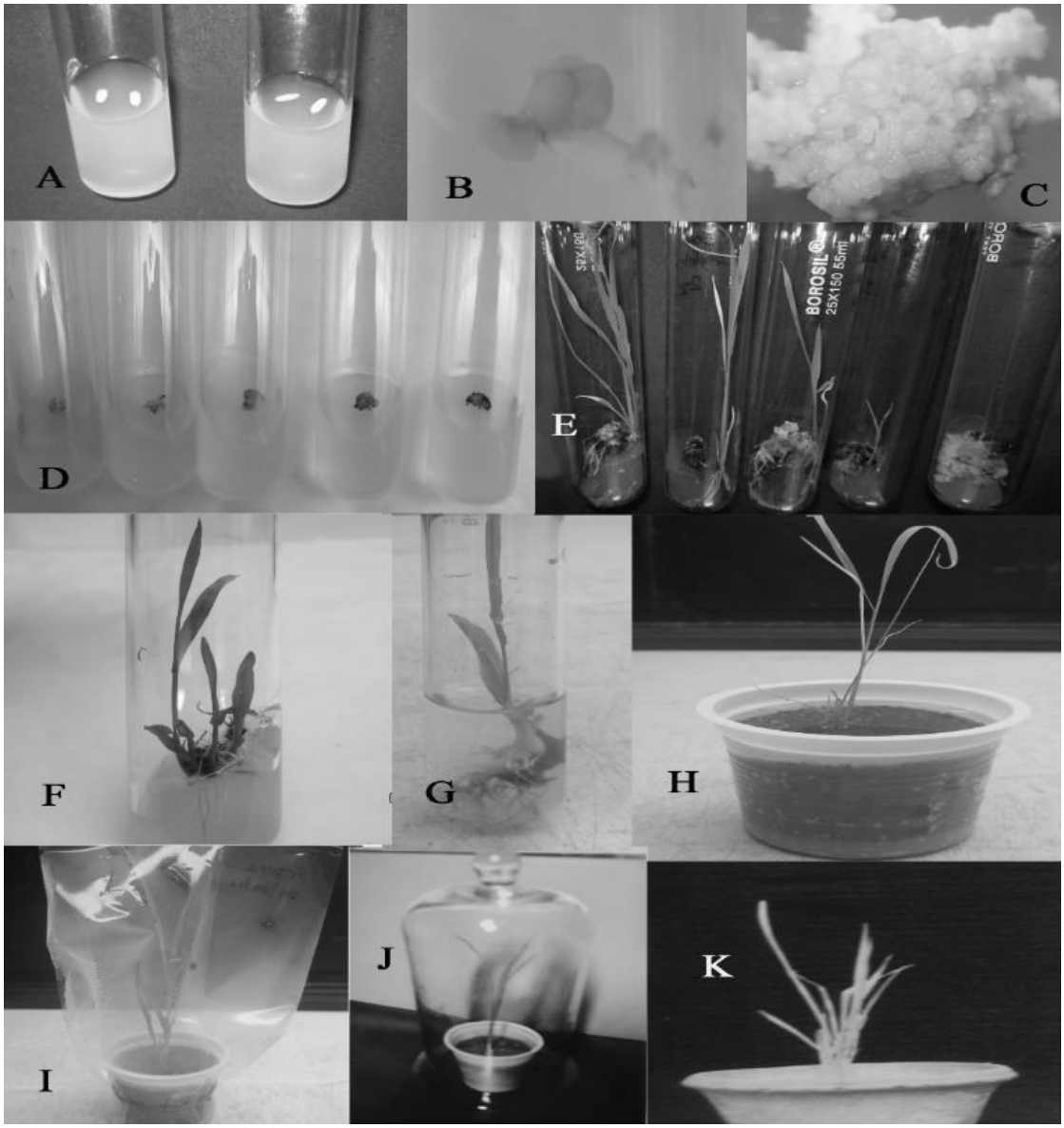

Fig 1. ***In vitroinductionof rice callus for drought toleranc e*** **a.** Seed inoculation **(**MS + 2, 4 ±D 2 mg/1 and MS + 2, 4-D 2 mg/1 + CH 1 mg / 1), **b.** Callus induction, **c.** proliferated callus, **d&e.** Callus effect on different concentration of PEG -6000(0. 0.5,1,1.5), **f &g**. Plantlets on root and shoot medium, **h &i**. Hardening in plastic cup**, j.** hardening in plastic mist chamber**, k.** hardened plantlets

Genotypic callus induction capacity

Callus induction from mature seeds was assessed; further, the response of calli to elevated levels of PEG (6000) was recorded as fresh weight. The response of the regenerating callus to PEG (6000) stress was also observed. Among the parents, Vandana was earlier (8.80 days) and CB 87 R was late (15.64 days) in callus induction. The treatment MS + 2, 4 – D 2mgl^{-1} + CH 1gl^{-1} was good for callus induction than MS + 2, 4-D 2 mgl$^{-1.}$ The days to callus induction ranged from 8.57 (Vandana in MS + 2, 4 – D 2mgl^{-1} + CH 1gl$^{-1)}$ to 16.00 (CB 87 R in MS + 2, 4 – D 2mgl^{-1} + CH 1gl$^{-1)}$ in interaction effects of parents with media. Eight interaction effects showed early callus induction (Table 1).

Table 2. Effect of PEG on callus growth (g) and proline content (µg) in parents

Parents	Fresh weight (g) PEG concentrations (%)						Proline content (µg) PEG concentrations (%)		Mean
	0.0	0.5	1.0	1.5	2.0	Mean	0.5%	2.0%	
Lines									
IR 58025A	0.41*	0.34*	0.22	0.17	0.10	0.25	5.19	10.33	7.76
IR 68897A	0.51*	0.40*	0.30	0.23	0.15	0.32*	10.72	29.65*	20.18*
TNAU CMS 2A	0.43*	0.32*	0.24	0.27	0.12	0.28	5.06	16.46*	10.76
IR 68888 A	0.33*	0.23	0.19	0.22	0.05	0.18	5.43	16.47*	10.95
Mean	0.42*	0.32*	0.24	0.20	0.10	0.26	**6.60**	**18.23***	12.42
Testers									
IR 60199 R	0.44*	0.36	0.33	0.27	0.19	0.32	16.35	66.40	41.37
IR 65912 R	0.53*	0.45*	0.36	0.27	0.12	0.35	29.08	119.60*	74.34
IR 21567 R	0.44*	0.35	0.26	0.16	0.12	0.27	16.44	66.47	41.46
CB 87 R	0.50*	0.44*	0.37	0.32	0.12	0.35	29.59	119.38*	74.49
MAS 946-1	0.46*	0.34	0.37	0.22	0.07	0.28	16.50	119.79*	68.14
MAS 26	0.57*	0.51*	0.39	0.33	0.29	0.42*	40.51	186.46*	113.49*
BI 33	0.45*	0.40	0.37	0.27	0.15	0.33	29.15	140.63*	84.89
KMP 148	0.56*	0.49*	0.39	0.27	0.14	0.37	52.43	240.06*	146.25*
BR 2655	0.61*	0.49*	0.47*	0.42*	0.30	0.46*	52.47	166.69*	109.58*
Vandana	0.59*	0.49*	0.40	0.31	0.22	0.40*	66.46	299.93*	183.20*
Mean	**0.51***	**0.43***	**0.37**	**0.28**	**0.17**	**0.35**	**34.90**	**152.54***	93.72

	Fresh weight (g)					Proline content (µg)			
	Lines		Testers			Lines		Testers	
	SEd	CD (0.50)	SEd	CD (0.50)		SEd	CD (0.50)	SEd	CD (0.50)
Parents (p)	0.01	0.02	0.01	0.02	Genotypes (g)	0.04	0.08	0.31	0.65
Media (m)	0.01	0.02	0.01	0.02	Media (m) 0.018	0.04	0.14	0.29	
p x m	0.02	0.05	0.03	0.05	g x m 0.07	0.14	0.44	0.91	

Among hybrids, callus induction ranged from 7.75 (IR 68888 A x BI 33) to 13.56 per cent (IR 68888A x MAS 946 -1). By interaction, it ranged from 10.91 (MS + 2,4 – D 2mgl^{-1} + CH 1gl^{-1}) to 11.21 (MS + 2,4-D 2 mgl^{-1}) among the treatments, and from 7.25 (IR 68888 A x BI 33 in MS + 2,4 – D 2mgl^{-1} to 13.85 (IR 68888 A x MAS 946 -1 in MS + 2,4-D 2 mgl^{-1}) among hybrid treatment interaction were found to be earlier in days to callus induction. Among the treatments, MS + 2, 4 – D 2mgl^{-1} + CH 1gl^{-1} was found to induce callus earlier (Table 1).This indicated differential genotypic ability for callus induction. The hybrids and parents were more responsive to media composition MS + 2, 4-D 2 mgl^{-1} and MS + 2, 4 – D 2mgl^{-1} + CH 1gl^{-1}, which appeared to be the best suited for *in vitro* tissue culture. This agrees with previous reports in which a significant difference in callus induction was found among different genotypes of indica rice (Seraj *et al.,* 1997). Among the two media compositions MS + 2,4-D 2 mg / 1 + CH 1 mg /1 was superior in inducing callus quicker with high percentage of induction both in parents and hybrids. This indicates that organic supplements like casein hydrolysate could enhance the callus induction in rice.

Effect of PEG on fresh weight (g) of callus

Both parents and hybrids showed a similar response under PEG stress. Approximately, 100 mg of one month old embryogenic callus was exposed to different PEG (6000) concentrations. As the PEG (6000) concentration in the medium increased, there was a decrease in callus fresh weight. Lower (0%) and higher (2.0%) concentrations of PEG recorded the maximum (0.42 g in lines and 0.51 g in testers) and minimum (0.10 g in lines and 0.17g in testers) fresh weight of callus. Lower concentrations *viz.,* 0 and 0.5 % showed significantly higher callus weight. Seven interaction effects between two factors *viz.,* parental genotype x media and eighteen interaction effects between three factors *viz.,* parental genotypes x media x PEG concentrations recorded significantly higher callus weight (Table 2). Among the hybrids, the fresh weight of callus varied between 0.21 9 g (IR 68888A x BI 33) and 0.71 g (TNAU CMS 2A x MAS 26). Among the two factors interaction effects, TNAU CMS 2A x KMP 148 in 2.0% (0.06 g) and TNAU CMS 2A x MAS 946-1 in 0% (0.93 g) registered the minimum and maximum fresh weight of callus. Thirteen hybrids, two PEG concentrations and 51 interaction effects of three factors exhibited significantly more fresh weight of callus (Table 3). A decreasing trend in fresh weight was noticed in parents and hybrids with increasing concentrations of PEG which was in line with the findings of Wani *et al.,* (2010) and Rohit Joshi *et al.,* (2011).

Table 3. Effect of PEG on callus growth (g) and proline content (µg) in hybrids

Hybrids	Fresh weight (g)						Proline content (µg)		
	PEG concentrations (%)						PEG concentrations (%)		
	0.0	0.5	1.0	1.5	2.0	Mean	0.5%	2.0%	Mean
IR 58025 A x IR 60199 R	0.56*	0.41	0.28	0.19	0.16	0.32	29.17	186.60	107.89
IR 58025 A x IR 65912 R	0.47	0.36	0.27	0.21	0.19	0.29	165.50	525.51*	362.51*
IR 58025 A x IR 21567 R	0.51	0.43	0.37	0.21	0.14	0.33	52.50	225.00	138.75
IR 58025 A x CB 87 R	0.43	0.37	0.27	0.20	0.19	0.29	140.00	440.25*	290.13
IR 58025 A x MAS 946 -1	0.47	0.34	0.25	0.23	0.17	0.29	40.00	186.67	113.34
IR 58025 A x MAS 26	0.77*	0.68*	0.27*	0.46	0.39	0.57*	32.50	240.20	161.35
IR 58025 A x BI 33	0.69*	0.59*	0.55	0.46	0.44	0.54*	75.00	269.17	172.32
IR 58025 A x KMP 148	0.69*	0.58*	0.44	0.36	0.28	0.47	300.01*	652.51*	476.26*
IR 58025 A x BR 2655	0.81*	0.71*	0.70*	0.51	0.51	0.65*	253.34	1400.36*	826.85*
IR 58025 A 2 x Vandana	0.70*	0.65*	0.62*	0.44	0.40	0.56*	225.00	933.35*	579.18*
IR 68897 A x IR 60199 R	0.54	0.51	0.48	0.43	0.33	0.46	52.50	162.51	107.50
IR 68897 A x IR 65912 R	0.54	0.48	0.47	0.39	0.36	0.45	53.15	166.50	109.83
IR 68897 A x IR 21567 R	0.46	0.33	0.21	0.26	0.22	0.29	100.25	440.20*	270.23
IR 68897 A x CB 87 R	0.68*	0.43	0.40	0.27	0.23	0.40	62.67	269.67	166.17
IR 68897 A x MAS 946 -1	0.72*	0.62*	0.60*	0.53	0.43	0.58*	64.63	352.51*	208.57
IR 68897 A x MAS 26	0.91*	0.80*	0.92*	0.61*	0.51	0.69*	166.67	1166.40*	666.54*
IR 68897 A x BI 33	0.59*	0.43	0.41	0.39	0.33	0.43	40.20	162.50	101.35
IR 68897 A x KMD 148	0.57*	0.48	0.37	0.32	0.27	0.40	100.50	479.17*	289.84
IR 68897 A x BR 2655	0.63*	0.52	0.49	0.45	0.44	0.51*	82.50	402.51*	242.50
IR 68897 A x Vandana	0.68*	0.63*	0.53	0.52	0.42	0.55*	100.00	562.51*	331.26*
TNAU CMS 2A x IR 60199 R	0.43	0.38	0.28	0.17	0.12	0.28	140.00	700.00*	422.50*
TNAU CMS 2A x IR 65912 R	0.73*	0.49	0.40	0.23	0.21	0.41	66.69	100.00	83.35
TNAU CMS 2A x IR 21567 R	0.62*	0.50	0.43	0.34	0.27	0.43	52.50	186.30	119.40
TNAU CMS 2A x CB 87 R	0.51	0.44	0.32	0.31	0.22	0.36	55.50	240.60	148.05
TNAU CMS 2A xMAS 946 -1	0.93*	0.72*	0.62*	0.44	0.37	0.62*	54.50	241.50	148.00
TNAU CMS 2A x MAS 26	0.92*	0.72*	0.71*	0.63*	0.56*	0.71*	253.34	1460.58*	856.96*
TNAU CMS 2A x BI 33	0.74*	0.63*	0.60*	0.42	0.31	0.54*	75.00	300.01*	187.51
TNAU CMS 2A x KMP 148	0.44	0.21	0.20	0.18	0.06	0.22	140.25	606.68*	373.22*
TNAU CMS 2A x BR 2655	0.77*	0.53	0.42	0.41	0.33	0.49*	75.00	366.50*	220.95
TNAU CMS 2A x Vandana	0.58*	0.39	0.28	0.20	0.14	0.32	52.50	186.90	119.70
IR 68888A x IR 60199 R	0.62*	0.48	0.43	0.39	0.29	0.44	140.00	606.70*	373.35*
IR 68888A x IR 65912 R	0.40	0.27	0.22	0.17	0.07	0.22	100.00	402.15*	251.08
IR 68888A x IR 21567 R	0.58*	0.43	0.41	0.37	0.32	0.42	119.17	562.51*	340.84*
IR 68888A x CB 87 R	0.61*	0.41	0.39	0.31	0.29	0.40	75.52	332.60*	203.90
IR 68888A x MAS 946 -1	0.49	0.33	0.32	0.26	0.21	0.32	52.50	166.70	109.60
IR 68888A x MAS 26	0.57*	0.28	0.23	0.18	0.16	0.28	186.67	520.01*	353.34*
IR 68888A x BI 33	0.41	0.21	0.20	0.13	0.09	0.21	58.50	332.80*	195.65
IR 68888A x KMD 148	0.74*	0.69*	0.58*	0.55	0.46	0.60*	75.23	402.50*	238.87
IR 68888A x BR 2655	0.57*	0.42	0.41	0.39	0.29	0.41	225.36	1108.40*	666.88*
IR 68888A x Vandana	0.63*	0.43	0.41	0.35	0.26	0.42	186.58	1048.40*	617.49*
Mean	0.61	0.48*	0.44	0.34	0.29	0.43	109.21	478.44*	293.82

	Fresh weight (g)			Proline content (µg)	
	SEd	CD (0.50)		SEd	CD (0.50)
Hybrids (h)	0.03	0.05	Hybrids (h)	0.58	1.15
Media (m)	0.01	0.02	Media (m)	0.13	0.26
h x m	0.06	0.12	h x m	0.82	1.62

Effect of PEG on proline content of callus

Proline accumulation

Accumulation of proline in plant tissues exposed to osmotic stress and thereby, maintain both turgor and the driving gradient for water uptake under osmatic stress has been well established in cell and callus culture (Al. Bahrany, 2002). In the present study, IR 58025 A (7.76 ìg) and IR 68897 A (20.18 ìg) in lines, IR 60199 R (41.37 ìg) and Vandana (183.20 ìg) in testers recorded the minimum and maximum proline content, respectively. The PEG concentration of 2.0 % recorded the significant and maximum proline content. One line, four testers, three lines x media interactions and eight testers x media interaction effects showed significantly higher

Table 4. Effect of PEG on regeneration percentage in promising parents and hybrids

Parents	PEG concentrations (%)				Mean
	0.0	0.5	1.0	1.5	
VANDANA	90.25*	85.20*	79.50*	70.90	82.71*
	(73.84)	(68.19)	(63.79)	(57.99)	(65.95)
KMP 148	86.44*	80.19*	74.08	62.54	78.06
	(70.13)	(65.04)	(60.72)	(54.06)	(62.49)
BR 2655	92.50*	89.55*	85.66*	78.48*	88.01*
	(77.78)	(72.10)	(68.62)	(63.06)	(70.39)
MAS 26	98.99*	90.06*	84.61*	75.78*	89.61*
	(89.60)	(73.64)	(68.54)	(63.28)	(73.76)
MAS 946 -1	85.35*	83.84*	65.64	69.18	78.50*
	(69.16)	(67.90)	(55.94)	(58.17)	(62.79)
IR 58025 A x KMP 148	81.50*	76.80	50.69	38.49	63.87
	(64.69)	(61.20)	(50.00)	(38.35)	53.56)
IR 58025 A x BR 2655	92.25*	86.20*	80.50*	71.90	82.71*
	(73.84)	(68.19)	(63.79)	(57.99)	(65.95)
IR 58025 A x VANDANA	88.44*	82.19*	76.08	65.54	78.06
	(70.13)	(65.04)	(60.72)	(54.06)	(62.49)
IR 68897 A x IR 21567 R	95.50*	90.55*	86.66*	79.48*	88.01*
	(77.78)	(72.10)	(68.62)	(63.06)	(70.39)
IR 68897 A x MAS 26	90.66*	89.41*	72.90	66.40	79.84*
	(72.20)	(71.01)	(58.63)	(54.57)	(64.10)
IR 68897 A x BR 2655	88.76*	80.91*	69.66	65.12	76.11
	(70.41)	(64.09)	(56.58)	(54.57)	(61.22)
IR 68897 A x VANDANA	86.24*	74.77	72.35	70.23	75.90
	(68.24)	(59.85)	(58.27)	(56.93)	(60.82)
TNAU CMS 2A x MAS 946 -1	85.15*	78.73	58.64	32.61	63.78
	(67.33)	(62.54)	(49.98)	(34.82)	(53.67)
IR 68888 A x KMP 148	89.59*	86.54*	65.44	52.49	73.51
	(71.17)	(68.48)	(53.99)	(46.43)	(60.02)
TNAU CMS 2A x MAS 26	99.99*	92.06*	86.61*	79.78*	89.61*
	(89.60)	(73.64)	(68.54)	(63.28)	(73.76)
IR 68888 A x BR 2655	87.35*	85.84*	68.64	72.18	78.50*
	(69.16)	(67.90)	(55.94)	(58.17)	(62.79)
IR 68888 A x VANDANA	91.06*	80.92*	67.15	68.29	76.85
	(72.60)	(64.10)	(55.03)	(55.73)	(61.86)
MEAN	89.70*	83.74*	71.94	63.54	77.23
	(72.26)	(66.51)	(58.34)	(53.10)	(62.55)

Note: Figures in parentheses are arc sine transformed values

	SEd	CD (0.50)		SEd	CD (0.50)
Parents (p)	0.44	0.90	Hybrids (h)	0.46	0.92
Media (m)	0.24	0.53	Media (m)	0.26	0.53
p x m	0.91	1.80	h x m	0.92	1.84

proline content (Table 2). In hybrids, minimum and maximum proline content was registered with IR 58025 A x IR 60199 R (29.17 ìg) and TNAU CMS 2 A x MAS 26 in 2.0% (1460.58 ìg), respectively. Twenty six hybrids x media interactions, 14 hybrids and one PEG concentration (2.0%) exhibited significantly higher proline content (Table 3). Hence, pronounced accumulation of proline is an indication of stress tolerance in plants. Accordingly, in the present investigation higher proline content in tissues was noticed in higher concentration of PEG both in parents and hybrids. Such results were earlier reported by Chandrasekara Reddy *et al.* (1994) in rice.

Effect of PEG on regeneration

Among the Parents, studied Vandana, MAS 26, BR 2655 and KMP 148 registered high regeneration percentage irrespective of all concentrations of PEG except in the highest concentration 1.5 % of PEG. Promising parents are depicted in Table 4. The lower and higher regeneration percentage exhibited by hybrids were 63.78 % (TNAU CMS 2A x MAS 946-1) and 86.96 % (TNAU CMS 2 A x MAS 26), respectively**.** Five hybrids, two PEG concentrations and 26 hybrids x media interaction effects showed significantly higher regeneration percentage of 96.72 % (Table 4). Among the hybrids studied, TNAU CMS 2 A x

MAS 26 registered the maximum regeneration percentage irrespective of all concentrations of PEG except in the higher concentrations of 1.5% of PEG. The plantlet regeneration of the selected hybrids was found to be in decreasing order with increased PEG concentrations supplemented in the callus induction medium, which showed the adverse effect of PEG on plant regeneration. Similar results were earlier reported by Wani *et al.* (2010) in rice.

Conclusion

Combining callus weight, proline accumulation and regeneration under media containing PEG for selecting toleranct lines; BR 2655, MAS 26 and Vandana in parents;TNAU CMS 2A x MAS 26, IR 58025 A x BR 2655 and IR 68897 A x MAS 26 in hybrids could be adjudged as most drought tolerant genotypes. Hence, these highly drought tolerant parental genotypes could be exploited in hybridization programme while evolving drought resistant rice varieties.

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Received after revision: February 23, 2015; Accepted: June 6, 2015