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In vitro and field screening of rice hybrids for drought tolerance

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Abstract
Drought stress caused by water deficit is the most impacting adverse condition for rice production. **S**election under field condition for drought tolerance is slow in attaining progress due to the inherent variation in the field. *In vitro* selection of tolerant cell lines is one of the promising areas of plant tissue culture which eliminates the environmental discrepancies by maintaining controlled condition. The present investigation was framed to select superior genotypes for drought tolerance by screening forty rice hybrids along with their parental lines under *in vitro* and *in vivo* conditions. Matured seeds of above genotypes were inoculated in the callus induction media MS + 2mg / lit 2,4 - D + 0.5mg / lit kinetin + 1g / lit CH and the developed callus were screened in the media containing PEG in varied concentration *viz*., 0.0%, 0.5%, 1.0%, 1.5% and 2.0%. Callus morphology, callus fresh weight and proline content of callus were studied. Callus morphology scores and fresh weight of callus tend to reduce with increasing concentrations of PEG in both parents and hybrids thus indicating the adverse effect of PEG on the growth of tissues. Proline content increased with the increasing level of PEG concentration in the tolerant genotypes. Based on callus morphology, callus weight and proline content, the genotypes Norungan and Varappukudanchan in parents and GD 99033/PMK 2, GD 98049/Norungan and GD 99017/PMK 2 in hybrids could be adjudged as most tolerant genotypes. Among the above three hybrids, GD 99033 / PMK 2 and GD 98049 / Norungan showed better drought tolerance coupled with yield when evaluated under rainfed environment demonstrating that the cell lines surviving under *in vitro* drought stress also have adjustments in their hostile environment.

Keywords: Rice, drought stress, *in vitro*, rainfed, proline

Introduction
Rice (*Oryza sativa* L.) is the major food crop of more than half of the world's population. Drought in rice is a real concern and a serious yield limiting factor. (O' Toole and Chang, 1978) [12]. Evaluation of the genotypes in their target environment is crucial in identifying the suitable genotypes for drought tolerance. Such selection programs are slow in attaining progress because of the low heritability of yield under stress. Screening for drought tolerance under field requires suitable environmental conditions for the effective and repeatable phenotypic expression of drought tolerance. It is therefore necessary to use simple but effective screening methods that relate to the field screening (M. A. El Siddig *et al*., 2013) [8]. The similarities in the effects induced by the stress both under *in vivo* and *in vitro* conditions offers *in vitro* screening as an alternate for field evaluation for studying the effect of water stress on plant growth and development.

Resistant to abiotic stresses such as drought generally implies mechanisms operating at cell level. The development of numerous plant cell and tissue culture techniques have opened new research avenues for screening and identifying the tolerant genotypes. *In vitro* culture is also used to obtain information concerning the physiological and biochemical parameters which are operating in the cellular component of stress resistance. Hence, characterizing the physiological and biochemical effects of drought through tissue culture and obtaining well defined phenotypes of drought tolerant genotypes under *in vitro* condition is very essential for selecting and isolating new variants having drought tolerance.

PEG induced *in vitro* culturing has been used as a tool for identifying drought tolerant plants under laboratory condition. Stress sensitivity of callus was positively correlated with stress sensitivity of intact plants (Dolgykh *et al*., 2001) [2]. Stimulated drought conditions have been achieved *in vitro* through incorporation of osmotic solutes such as PEG in the media and it was utilized by several authors to screen various genotypes for drought tolerance (Rao and Jabeen, 2013; Kacem *et al*., 2017) [14, 6].

The selection precision for drought tolerance could be enhanced by exercising *in vitro* selection coupled with evaluation in the field condition. Hence, the present investigation is framed to select superior genotypes for drought tolerance through screening of callus on media stressed with different levels of polyethylene glycol (PEG) and also to find the relative efficiency under field condition for drought tolerance and yield.

Materials and Methods

In vitro Screening

Fourteen parents and forty hybrids developed from those parents were subjected to *in vitro* screening for drought resistance by employing polyethylene glycol (PEG) in tissue culture laboratory, Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai. The nutrient media used for the study was MS (Murashige and Skoog, 1962) [11] for callus induction. Matured seeds of parents and hybrids were used as explants for callus induction. Seeds were dehulled and surface sterilized using 70 per cent ethanol for one minute followed by 0.1 per cent mercury chloride for ten to fifteen minutes. Then, the seeds were thoroughly washed three times with sterilized distilled water and inoculated at the rate of three seeds per tube in callus induction media *viz.*, MS + 2 mg / lit 2,4 - D + 0.5mg / lit kinetin + 1g / lit CH. Cultures were incubated at 25 ± 2ºC under 16 hrs of lighting and 8 hrs of darkness for callus induction. Sub culturing was done into fresh medium with same hormone level after 21 days of culturing for proliferation of callus.

Varied concentration of PEG *viz*., 0.0%, 0.5%, 1.0%, 1.5% and 2.0% were utilized for the artificial induction of drought stress under *in vitro* condition. From the sub culturing callus, callus with known weight was taken and cultured in the medium MS + 2mg / lit 2, 4 - D + 0.5mg / lit kinetin with different concentrations of PEG and each concentration served as a treatment while the control was maintained without PEG. The calli were left for 21 days to proliferate on the medium supplemented with PEG at 25 ± 2^0C. After proliferation, the following observations were made.

Fresh weight of callus

Increased growth of callus in the medium with PEG was recorded as fresh weight and expressed in grams.

Proline content

The proline content of the callus was estimated only in two concentrations of PEG *viz*., 0.5 and 2.0%.and expressed in µg/g of callus.

Callus morphology

At the end of callus proliferation period, the morphology of the callus was recorded and scored according to the rating scale given by Pushpam and Sree Rangasamy, 2000.

Core	Description of callus
1	Completely turned black or dark brown, dead
3	Watery / sticky appearance, more than 75 per cent of callus turned brown
5	Yellow to brown in colour, water soaked with slimy surface
7	Yellow to pale yellow, watersoaked areas interspersed with pale yellow friable callus
9	Pale yellow to white in colour, healthy, nodular and friable

The effect of different treatment combinations on fresh weight of callus, callus morphology score and proline content were studied by subjecting the mean values into factorial completely randomized design.

In vivo screening

The 40 F_1 hybrids along with the parents were evaluated in three different environments *viz*., Managed stress (E_1), Rainfed (E_2) and Irrigated (E_3) condition in RBD with three replications adopting the spacing of 20 x 10 cm to assess their stability over environments. Among the three environments, Rainfed (E_2) trial was taken up at Agricultural Research Station, Paramakudi and the remaining trials were raised at Agricultural College and Research Institute, Madurai. The yield performance of the hybrids under field condition was assessed based on Stability analysis proposed by Eberhart and Russell (1966) [3] and Zobel *et al.,* (1988) [19].

Results and Discussion

Evaluation *under in vitro* condition

In vitro screening of plant cell or tissue culture is a powerful tool for dissecting the physiological, biochemical, molecular mechanisms of stress and stress response phenomena. The utility of rice drought-tolerant callus is to improve the selection efficiency and accuracy in terms of drought tolerance in genotypes due to the condition of callus growth, which is not affected by environmental factors (Rahim *et al*. 2020) [13].

In vitro selection for tolerance to abiotic stress depends on the development of efficient and reliable callus induction and plant regeneration systems (Zahidul Haque *et al.,* 2013). The culture medium comprising growth hormones like 2, 4- D and organic supplements was found to highly influence the callus induction. The role of 2,4-D is to increase the rate of cell division thus enhancing callus induction. In the present study, MS + 2,4-D 2mg/ l + CH 1mg/ l was used for callus induction.

Screening of callus for drought tolerance

Polyethylene glycol (PEG) of higher molecular weight has proved its role as an osmotic agent (Muhammad *et al*., 2010) [10] and its application has been found to lower water potential (Khodarahmpour, 2011; Saepudin, 2017; Wani *et al*., 2018; Islam *et al*., 2018) [7, 15, 17, 4]. Mishra *et al*., 2021 developed drought-tolerant soma clones from two drought-susceptible soybean genotypes through *in vitro* selection using PEG as a selection agent. Polyethylene glycol (PEG 6000) in different concentrations was used as osmoticum to reduce the water potential and create osmotic stress in the culture medium in the present study. The cell lines that survive under higher concentrations of PEG were selected based on callus morphology, fresh weight of callus and proline content.

The morphology of callus was rated into 1-9 scores, score 1 being low and indicating apparent death of tissues and 9 being high and indicating healthy nature of tissues. The morphology of callus has already been used as a criterion for selection of tolerant cell lines (Pushpam and Sree Rangasamy (2000); Sharma *et al*., 2016) [16]. In the present study, high scores for callus morphology were recorded in no and low concentrations of PEG *viz*., 0 and 0.5%. The morphology scores tend to reduce with increasing concentrations of PEG in both parents and hybrids thus indicating the adverse effect of PEG on the growth of tissues. The range of callus morphology was between 1.50 and 9.00 in both lines (GD 99036 in 2.0% PEG and GD 98049, GD 99017 and GD 99033 in 0% PEG respectively) and testers (TM 97017 in 2.0mg/l PEG and all except TM 97017 in 0mg/l PEG respectively). Among the genotypes, the testers TM 97056, PMK 2, IR 36, Norungan and Varappukudanchan and the hybrids GD 98049/MDU 5, GD 98049/ Norungan, GD 99017/ PMK 2, GD 99017/ Norungan, GD 99033/ PMK 2 and GD 99036/ Varappukudanchan exhibited significantly high callus morphology scores. Norungan and Varappukudanchan recorded comparatively high scores even in higher concentrations of PEG *viz.*, 1 and 1.5% and could be adjudged as best genotypes with tolerance to drought stress. Among the hybrids, GD 99033/ PMK 2 showed no difference in callus morphology and recorded the high score of 9 in all the concentrations of PEG (Table 1; Fig.1). This indicates that the particular hybrid is not at all affected by the osmotic stress induced and is highly drought tolerant.

Fresh weight of callus varied between 0.18 (GD 99036) and 0.32g (GD 99017) in lines, between 0.27 (TM 97017) and 0.46g (Norungan) in testers. Lower (0%) and higher (2.0%) concentrations of PEG recorded the maximum (0.42g in lines and 0.51g in testers) and minimum (0.10g in lines and 0.17g in testers) fresh weight of callus. Minimum and maximum fresh weight among the parents was recorded by GD 99036 in 2.0% (0.05g) and GD 99017 in 0% (0.51g) in lines and by MDU 5 in 2.0% (0.07g) and Norungan in 0% (0.61g) in testers respectively. 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 Al-Bahrany, 2002 and Wani *et al*., 2010 [1, 17]. The decrease in water content resulted in a decrease in cell turgor pressure and consequently reduced callus growth and callus volume (Joshi *et al*., 2011) [5]. Resistant genotypes were found to show lesser reduction in fresh weight even at higher concentrations of PEG. Such of those include PMK 2, Norungan and Varappukudanchan in parents and GD 99033 / PMK2, GD 99017 / PMK 2, GD 98049 / Norungan, GD 99017 / MDU 5, GD 99036 / IR 36 and GD 99033 / MDU 5 in hybrids inferring the resistant nature of these genotypes to osmotic stress (Table 1; Fig.2).

The accumulation of free proline in response to osmotic stress is an adaptive reaction in plant. The important function of this amino acid maintenance of cell membrane structure preventing electrolyte breakage from the cell. Hayat *et al.*, 2012 reported that a stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance and stabilizing membranes thereby preventing electrolyte leakage thus preventing oxidative burst in plants. Hence pronounced accumulation of proline is an indicator of stress tolerance in plant. Higher proline content in tissues were noticed in higher concentration of PEG in both parents and hybrids. GD 98049 (7.76µg) and GD 99017 (20. 18 µg) in lines and PM 01010 (41.37µg) and Varappukudanchan (183.20µg) in testers recorded the minimum and maximum proline content respectively. The PEG concentration of 2.0% recorded the significant and maximum proline content. Rahim *et al*., 2020 [13] also reported that proline levels increase in proportion to polyethylene glycol (PEG) concentrations. Varappukudanchan, IR 36, PMK 2, Norungan, PMK 3, PM 01011, TM 97056 and MDU 5 in parents and GD 99033/PMK 2, GD 99049/Norungan, GD 99017/PMK2, GD 99036/Norungan, GD 99036/Varappukudanchan, GD 98049/IR 36, GD 98049/Varappukudanchan, GD 99033/PM 01010, GD 99033/IR 36 and GD 99036/PM 01010 in hybrids exhibited higher proline accumulation in high concentrations of PEG and found to adjust osmotically to drought stress (Table 1; Fig.3). Proline content increased with the increasing level of PEG concentration in case of the varieties that showed significant growth under high level of drought stress and the varieties having very low amount of proline showed retarded growth.

Based on callus morphology, callus weight and proline accumulation under media containing PEG for selecting drought tolerant lines, Norungan and Varappukudanchan in parents and GD 99033/PMK 2, GD 98049/Norungan and GD 99017/PMK 2 in hybrids could be adjudged as most tolerant among the genotypes studied.

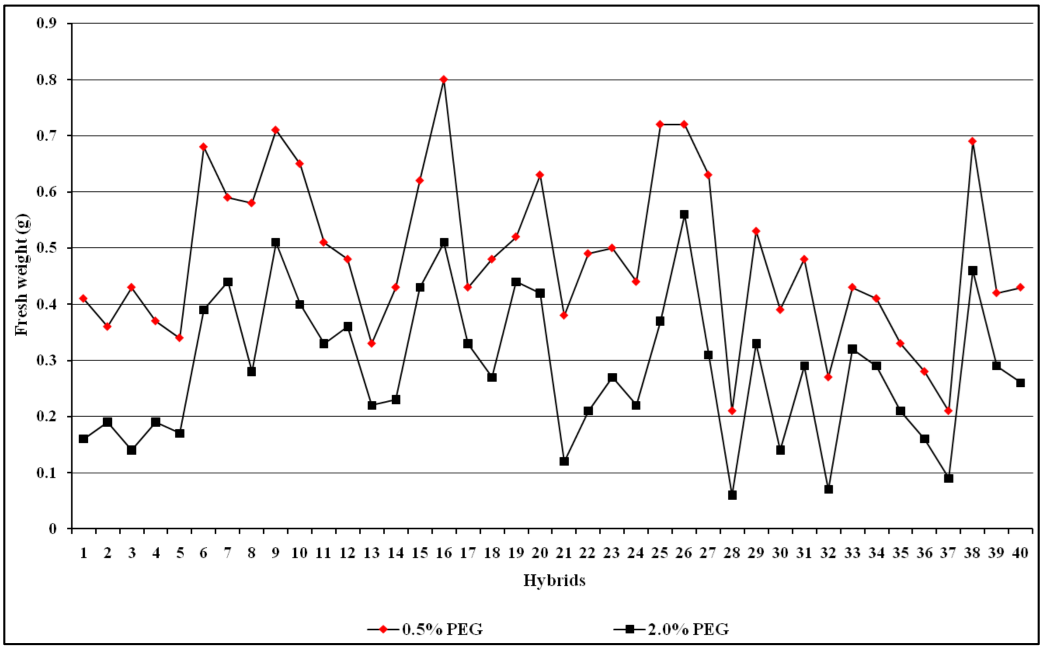

Fig 1: Effect of PEG on hybrids callus morphology

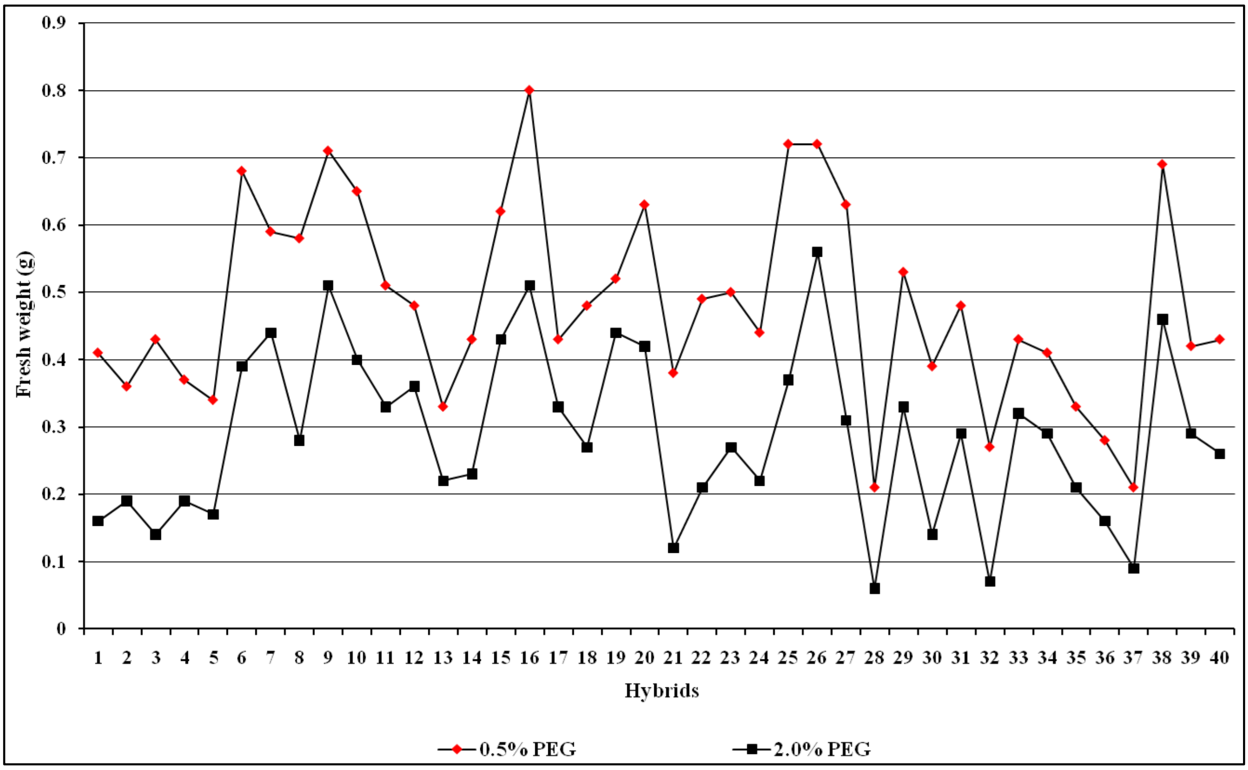

Fig 2: Effect of PEG on hybrids callus fresh weight

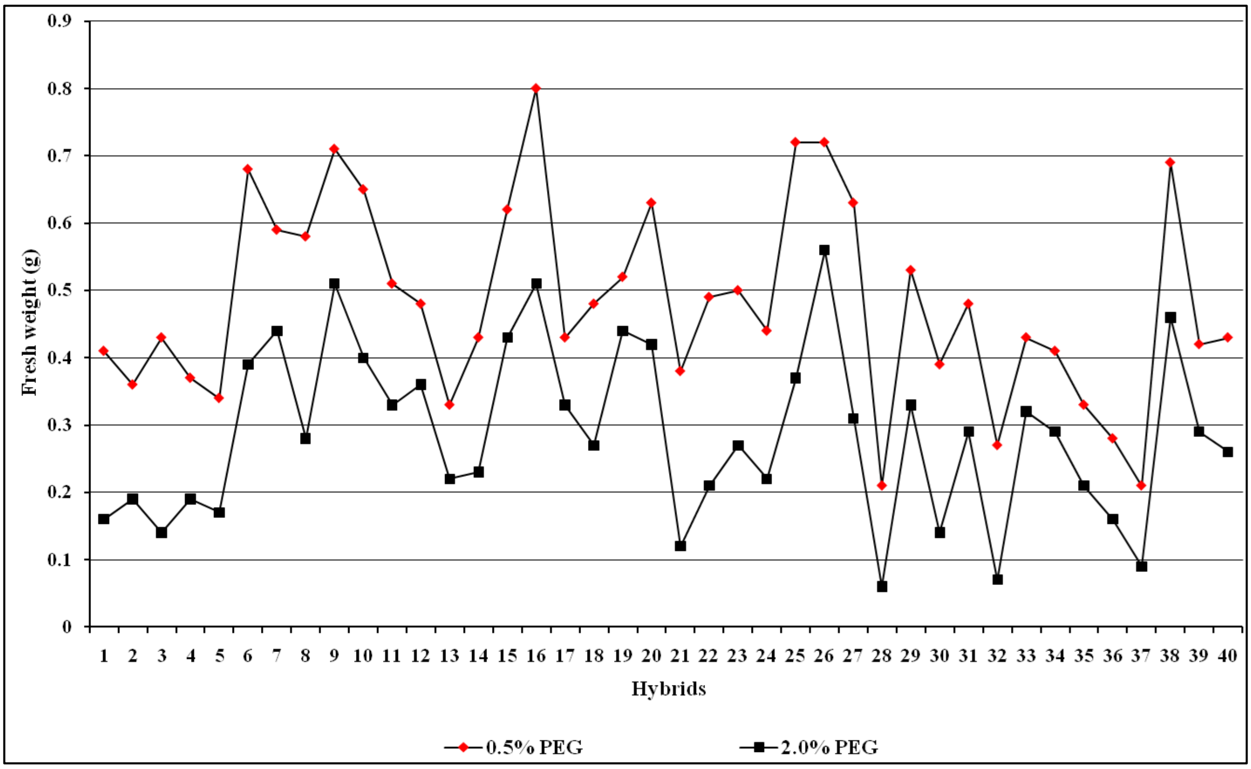

Fig 3: Effect of PEG on hybrids proline content

Table 1: Promising genotypes based on the effect of peg on callus fresh weight, callus morphology and proline content

	Callus Fresh weight					**Callus morphology score**					**Proline content**	
	0.0	**0.5**	**1.0**	**1.5**	**2.0**	**0.0**	**0.5**	**1.0**	**1.5**	**2.0**	**0.5**	**2.0**
PM 01011	0.53*	0.45*	0.36	0.27	0.12	9.00	6.50	6.34	4.00	2.50	29.08	119.60*
TM 97056	0.50*	0.44*	0.37	0.32	0.12	9.00	8.00*	7.67*	5.67	2.34	29.59	119.38*
MDU 5	0.46*	0.34	0.37	0.22	0.07	9.00	6.50*	5.50	3.80	1.60	16.50	119.79*
PMK 2	0.57*	0.51*	0.39	0.33	0.29	9.00	8.84*	6.17	5.00	3.45	40.51	186.46*
PMK 3	0.45*	0.40	0.37	0.27	0.15	9.00	5.67	5.50	3.50	2.00	29.15	140.63*
IR 36	0.56*	0.49*	0.39	0.27	0.14	9.00	7.66*	6.34	5.50	3.00	52.43	240.06*

Norungan	0.61*	0.49*	0.47*	0.42*	0.30	9.00	8.00*	7.67*	6.50	3.55	52.47	166.69*
Varappukudanchan	0.50*	0.49*	0.40	0.31	0.22	9.00	8.84*	7.50*	5.50	2.20	66.46	299.93*
GD 98049/MDU 5	0.47	0.34	0.25	0.23	0.17	9.00	9.00*	8.33*	7.67*	5.00	40.00	186.67
GD 98049/IR 36	0.69*	0.58*	0.44	0.36	0.28	9.00	9.00*	9.00*	6.33	4.33	300.01*	652.51*
GD 98049/Norungan	0.81*	0.71*	0.70*	0.51	0.51	9.00	9.00*	9.00*	8.33*	7.00	253.34	1400.36*
GD 98049/Varappukudanchan	0.70*	0.65*	0.62*	0.44	0.40	9.00	9.00*	8.33*	6.33	5.67	225.00	933.35*
GD 99017/PMK 2	0.91*	0.80*	0.92*	0.61*	0.51	9.00	9.00*	9.00*	7.33*	7.00	166.67	1166.40*
GD 99017/Norungan	0.63*	0.52	0.49	0.45	0.44	9.00	7.67*	7.67*	7.33*	7.00	82.50	402.51*
GD 99033/PM 01010	0.43	0.38	0.28	0.17	0.12	9.00	7.00	5.00	3.00	3.00	140.00	700.00*
GD 99033/MDU 5	0.93*	0.72*	0.62*	0.44	0.37	9.00	7.00	7.00	5.00	5.00	54.50	241.50*
GD 99033/PMK 2	0.92*	0.72*	0.71*	0.63*	0.56*	9.00	9.00*	9.00*	9.00*	9.00*	253.34	1460.58*
GD 99033/IR 36	0.44	0.21	0.20	0.18	0.06	9.00	7.00	6.00	6.00	4.67	140.25	606.68*
GD 99036/PM 01010	0.62*	0.48	0.43	0.39	0.29	9.00	7.00	5.00	5.00	5.00	140.00	606.70*
GD 99036/IR 36	0.74*	0.69*	0.58*	0.55	0.46	9.00	9.00*	9.00*	9.00*	7.00	75.23	402.50*
GD 99036/Norungan	0.57*	0.42	0.41	0.39	0.29	9.00	9.00*	9.00*	9.00*	5.00	225.36	1108.40*
GD 99036/Varappukudanchan	0.63*	0.43	0.41	0.35	0.26	9.00	8.33*	7.67*	7.67*	5.68	186.58	1048.40*

Evaluation *under in vivo* condition

The hybrids and parents were evaluated based on their yield performance under three different environments and rainfed environment was taken in to account for the present study. In Eberhart and Russell (1966) [3] model, the genotypes with high mean, regression coefficient more than one and non-significant deviation from regression were considered as the genotypes specifically adapted to poor environment *i.e.* rain fed environment. Based on this criteria, the hybrids GD 98049 / PMK 3, GD 98049 / Norungan, GD 99017 / PMK 2, GD 99017 / Varappukudanchan, GD 99017 / PMK 3, GD 99036 / PMK 3 and GD 99036 / IR 36 were identified as best hybrids with higher yield under rain fed condition (Table 2). Similarly in AMMI model (Zobel *et al*., 1988) [19], the genotypes having high mean with negative Interactive Principal Axes (IPCAI) score were considered as the suitable genotypes for unfavorable environment. The hybrids GD 98049/PMK 3, GD 98049/Norungan, GD 99017/PMK 2, GD 99017/Norungan, GD 99017/Varappukudanchan, GD 99033/PMK 3, GD 99033/Norungan, GD 99036/PMK 3, GD 99036/IR 36 and GD 99036/ Varappukudanchan had negative IPCA 1 score with high mean effect and hence, considered as the suitable hybrids for rain fed environment based on AMMI analysis for yield (Table 3).

Table 2: Estimates of stability parameters for single plant yield

Stability parameters	Parents		PM 01010	PM 01011	TM 97017	TM 97056	MDU 5	PMK 2	PMK 3	IR 36	Norungan	Varappuku Danchan
Mean	Testers		18.49	12.80	16.95	13.88	11.73	18.44	16.85	14.72	16.67	13.98
	Lines		**Hybrids**									
	GD 98049	0.00	28.05*	22.02	38.04*	23.92	19.01	37.11*	33.48*	20.50	30.22*	26.66*
	GD 99017	0.00	25.99*	22.02	40.24*	18.33	17.72	38.71*	27.10*	17.90	28.95*	31.59*
	GD 99033	0.00	26.57*	25.86*	30.08*	19.45	21.45	36.43*	33.46*	22.56	26.58*	28.68*
	GD 99036	0.00	29.54*	26.22*	35.57*	20.98	20.43	36.76*	28.48*	27.61*	29.34*	26.98*
Regression Coefficient (b_i)	Testers		0.46	0.77*	0.42	0.73*	0.58*	0.62*	0.62*	0.81*	0.79*	0.77*
	Lines		**Hybrids**									
	GD 98049	0.00	1.99*	1.70*	1.58*	1.83*	0.83*	1.81*	0.81*	1.39*	0.57*	1.12*
	GD 99017	0.00	1.07*	1.01*	1.31*	1.10*	1.19*	0.81*	1.22*	0.95*	0.56*	0.77*
	GD 99033	0.00	1.55*	1.11*	1.70*	1.33*	1.22*	2.39*	0.76*	1.68*	0.49*	1.01*
	GD 99036	0.00	1.36*	1.09*	1.72*	1.12*	1.09*	1.10*	0.74*	0.71*	1.06*	1.54*
Deviation from Regression (δ^2_{di})	Testers		0.11	0.58	0.28	0.63	0.94	0.83	0.83	0.75	3.11*	0.81
	Lines		**Hybrids**									
	GD 98049	0.00	0.80	3.67*	8.56*	20.28*	0.03	0.13	0.17	3.24*	0.53	0.04
	GD 99017	0.00	0.10	0.37	10.79*	4.07*	0.85	0.29	1.76	0.37	4.24*	0.96
	GD 99033	0.00	9.53*	0.24	4.87*	1.06	0.88	0.20	1.93	0.04	34.26*	0.02
	GD 99036	0.00	5.69*	4.71*	1.41	0.13	0.07	12.24*	0.00	0.85	0.04	0.64

* Significant at 5% level

Table 2.1: Define Hybrids for Rain Fed Environment

Hybrids for Rain Fed Environment	Mean	b_i	δ^2_{di}
GD 98049 / PMK 3	33.48	0.81	0.17
GD 98049 / Norungan	30.22	0.57	0.53
GD 99017 / PMK 2	38.71	0.81	0.29
GD 99017 / Varappukudanchan	31.59	0.77	0.96
GD 99033 / PMK 3	33.46	0.76	1.93
GD 99036 / PMK 3	28.48	0.74	0.00
GD 99036 / IR 36	27.61	0.71	0.85
General mean	23.24 g		
SE (Mean)	1.17		
SE (b_i)	0.24		

Table 3: Estimates of stability parameters (AMMI) for Single plant yield

Stability Parameters	Parents		PM 01010	PM 01011	TM 97017	TM 97056	MDU 5	PMK 2	PMK 3	IR 36	Norungan	Varappuku Danchan
Mean	**Testers**		18.49	12.80	16.95	13.88	11.73	18.44	16.85	14.72	16.67	13.98
	Lines		**Hybrids**									
	GD 98049	0.00	28.05	22.02	38.04	23.92	19.01	37.11	33.48	20.50	30.22	26.66
	GD 99017	0.00	25.99	22.02	40.24	18.33	17.72	38.71	27.10	17.90	28.95	31.59
	GD 99033	0.00	26.57	25.86	30.08	19.45	21.45	36.43	33.46	22.56	26.58	28.68
	GD 99036	0.00	29.54	26.22	35.57	20.98	20.43	36.76	28.48	27.61	29.34	26.98
	Environment		E_1	22.94		E_2	18.41		E_3	28.37		
IPCA 1	Testers		-0.74	-0.32	-0.79	-0.35	-0.55	-0.50	-0.50	-0.28	-0.25	-0.29
	Lines		**Hybrids**									
	GD 98049	-1.36	1.36	0.91	0.73	1.24	-0.23	1.09	-0.26	-0.49	-5.98	0.16
	GD 99017	-1.36	0.09	0.004	0.50	0.18	0.28	-0.25	0.26	-0.06	-0.64	-0.33
	GD 99033	-1.36	0.82	0.14	1.00	0.42	0.28	1.90	-0.30	0.02	-0.82	0.01
	GD 99036	-1.36	0.55	0.08	1.01	0.17	0.13	0.06	-0.35	-0.41	0.08	-0.60
	Environment		E_1	-0.70		E_2	-3.25		E_3	3.95		

Table 3.1: Define Hybrids selected for rain fed condition

Hybrids selected for rain fed condition	Mean	IPCA 1 score
GD 98049 / PMK 3	33.48	-0.26
GD 98049 / Norungan	30.22	-5.98
GD 99017 / PMK 2	38.71	-0.25
GD 99017 / Norungan	28.95	-0.64
GD 99017 / Varappukudanchan	31.59	-0.33
GD 99033 / PMK 3	33.46	-0.30
GD 99033 / Norungan	26.58	0.82
GD 99036 / PMK 3	28.48	-0.35
GD 99036 / IR 36	27.61	-0.41
GD 99036 / Varappukudanchan	29.34	0.08

General mean – 23.24g

Conclusion

PEG induced moisture stress in the culture medium offered rapid and precise screening for the identification of drought-tolerant hybrids and parental lines. Combining callus morphology, callus weight and proline accumulation under media containing PEG for selecting tolerance lines, Norungan and Varappukudanchan in parents and GD 99033/PMK 2, GD 98049/Norungan and GD 99017 / PMK 2 in hybrids could be adjudged as most tolerant among the genotypes studied. While considering the performance of hybrids both under *in vivo* and *in vitro* condition, the two hybrids GD 98049 / Norungan and GD 99017 / PMK 2 were identified as the best hybrids for drought stress environment. This finding also demonstrates the fact that stress sensitivity of callus was positively correlated with the stress sensitivity of intact plants under field condition and the cell lines surviving under the cell lines surviving under *in vitro* drought stress also have adjustments in their hostile environment.

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