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Research Article

In-vitro screening and field validation of rice (*Oryza sativa* L.) genotypes for drought tolerance

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Abstract
In vitro screening using polyethylene glycol (PEG) was performed with matured seed-derived callus from six elite rice varieties and eight advanced cultures to understand the response to drought stress. Murashige and Skoog medium supplemented with 2 mg l^{-1} of 1-napthaleneacetic acid and 5, 10, 15 and 20 g l^{-1} of PEG 6000 were used to induce drought stress in callus. Significant differences were observed among the genotypes, treatments and their interactions for callus morphology and fresh weight. The genotypes PMK3, RMD(R)1, ARB6 , ARB7 and ARB8 were identified as the most tolerant to drought stress using principal component analysis (PCA). A total of 48 crosses made from these genotypes showed significant yield increase over their respective parents. The hybrids RMD(R)1/ARB7, PMK3/ARB7 and PMK3/ARB8 were identified as drought tolerant using PCA analysis. From the study it is proved that *in vitro* screening method can be used to identify drought tolerant rice genotypes.

Key words: Rice, polyethylene glycol, drought tolerance

Introduction:
Rice (*Oryza sativa* L.) is an important food cereal crop in the World. Genetic improvement of rice largely focuses on breeding varieties suitable for water-limited environments; since increasing rice production in the future will rely on rainfed ecosystems. However, progress in this direction was slow and more limited because of lack of knowledge on the mechanism, inheritance, low heritability of yield and lack of efficient techniques for screening breeding materials for drought tolerance (Boopathi *et al.*, 2013). Tolerance to drought stress operates at cellular level through osmotic adjustment and cell membrane stability. Improved tissue water status may be achieved by accumulation of compatible solutes like glycine betaine, sugars and proline through osmotic adjustment mechanism. Osmotic adjustment allows the cell to decrease osmotic potential and, as a consequence, increases the gradient for water influx and maintenance of turgor potential. This is critical for maintaining physiological function during drought stress. It is known that during drought stress plants produces reactive oxygen species; which can react with various biomolecules, causing oxidative damage to cell membranes and impairing the normal functions of cells (Farooq *et al*., 2009). The cell lines surviving under *in vitro* drought conditions have adjustment at their cellular level to survive under field level drought stress (Bajji *et al*., 2000).

Selection procedure at the plant cellular level followed by genetic improvement constitutes a necessary tool for drought stress breeding. *In vitro* selection of drought tolerant cell lines is one of the promising areas of plant tissue culture. Callus growth under drought condition is used as an index of stress tolerance (Handa *et al*., 1983 and Sabbah and Tal, 1990) because sensitivity of callus to drought stress was positively correlated with whole plant tolerance in field (Dolgykh *et al*., 2001). Stimulated drought conditions under *in vitro* have been achieved through incorporation of osmotic solutes such as PEG in the media. Earlier work by Nabors *et al*. (1980) showed that rice tissue derived from salinity tolerant plantlets exhibited salinity tolerance under *in vitro* condition. This finding opened a new door for *in vitro* screening using PEG as drought stress inducing agent. However, little information is available on screening rice varieties using this protocol.

Biswas *et al*. (2002) performed *in vitro* screening in rice varieties of diverse geographical origin used matured seeds derived callus and found that lines showing tolerance at cellular level had drought tolerance at field evaluation. All genotypes displayed callus induction percentage and plantlet regeneration in decreasing order with increased PEG concentrations (Biswas *et al.*, 2002). Hence the present investigation was undertaken to select superior rice variants from fourteen genotypes by studying the callus growth and morphology under stimulated drought stress using PEG and to validate their hybrids for drought tolerance under field condition.

Material and Method

In vitro screening: Six high yielding rice varieties and eight drought tolerant advanced cultures were subjected to *in vitro* screening for drought tolerance by employing polyethylene glycol (PEG 6000). Mature seeds were used as explants and the study was conducted at the Tissue Culture Laboratory, Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai, India. Seeds were dehulled and surface sterilized using 70% ethanol for one minute followed by 0.1% mercuric chloride for fifteen minutes. Then the seeds were thoroughly with sterilized distilled water for three times and inoculated at the rate of three seeds per tube in MS medium (Murashige and Skoog, 1962) supplemented with 2.5 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin (standardization of the concentrations of auxin and cytokinin was done separately; data not shown). Cultures were incubated at 25 ± 2ºC under darkroom for callus induction. Sub culturing was done using a fresh MS medium with same concentration of the hormones after 21 days of culturing to effect more callus growth. PEG with different concentrations: (0.5, 1.0, 1.5 and 2.0 mg L^{-1}) were used to induce the drought stress. A known callus quantity of 0.1gm was taken from the sub culture and cultured again in the MS medium containing different concentrations of PEG and each concentration was 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°C. After proliferation, fresh weight of callus and callus morphology was recorded. The callus morphology was rated into 1 to 9, score 1 indicating apparent death of tissues and 9 indicating healthy tissues (Nabors *et al*., 1980; Pushpam and Sree Rangasamy, 2000).

Field validation: Field experiment was conducted with 62 genotypes incuding 48 hybrids and 14 parents in a randomized block design (RBD) with two replications at Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai. Hybrids and parents were directly sown in non-puddled and non-flooded soil. Each genotype was accommodated in two rows of 1.5 m length with a spacing of 20 cm row to row and 15 cm plant to plant distance in each replication. A uniform population of 20 hills per genotypes with single seedling was maintained in each replication. The plants were fertilized with 7.5 kg of urea (N), 15 kg of single super phosphate (SSP) and 3 kg of muriate of potash (MOP) during field preparation (150:50:60 NPK ha^{-1}). First split application of 7.5 kg of urea (N) and 3 kg of MOP (K) was broadcasted 25 days after sowing. Second split dose of 7.5 kg of urea (N) and 3 kg of MOP (K) was broadcasted 20 days after first split application. The pest and diseases were below the threshold level; hence the plants were not sprayed with any chemicals. Alternate wetting and drying once in 7 days interval was followed for irrigating the field noticed as hair line cracks. At flowering stage irrigation was skipped and drought imposed for 15 days. After the stress period the plants were irrigated as mentioned earlier. Data on chlorophyll stability index (CSI), and days to 50% flowering were recorded during flowering stage on five tagged plants in each genotype. The top second leaf was used to estimate the chlorophyll stability index (Koloyereas 1958). The plant height, number of productive tillers $plant^{-1}$, length of the panicle, spikelet fertility, number of grains per panicle, 100 grain weight, harvest index and grain yield per plant were recorded at harvest as per standard evaluation system for rice (IRRI, 1996).

Statistical Analysis: The analysis of variance and principal component analysis (PCA) were performed using PROCGLM and PROCFACTOR (SAS, 2009). The combining ability analysis was done by using Line x Tester mating design as described by Kempthorne (1957). The performance of F_1 hybrids was evaluated on the basis of heterosis estimates (Fonseca and Patterson 1968) and standard heterosis against the best high yielding drought tolerant variety PMK3 by Virmani *et al.* (1982).

Results and Discussion

Drought tolerance in rice is an important trait, as water availability is the most limiting factor for rice production. Besides, drought tolerance is a complex trait and the expression is under the control of polygenes and highly influenced by environment. Hence, drought tolerance needs to be evaluated under multi-location/environment testing which is time and labor intensive process. In the past, drought tolerance has been assessed in field trials to measure drought related physiological traits and final yield that are predictive for yield under stress. Yield is the most important economic trait but with low heritability due to other related polygenic component traits. In this regard, in vitro cellular studies compliment and help to accelerate the conventional breeding procedures and the selection of genotypes for stress tolerance would be more reliable fast and accurate. The ability of genotypes to maintain high ratings of callus morphology and fresh weight under *in vitro* condition enhances plant survival at field level under drought stress. We used PEG as the *in vitro* drought stressing agent to quantify drought tolerance.

The morphology scores and fresh weight of callus tend to reduce with increasing concentrations of PEG in genotypes, thus indicating the adverse effect of PEG on the callus development as evinced from the findings of Bressan *et al*. (1982), Kavikishor and Reddy (1985) and Chandrasekhara Reddy *et al*. (1994). Significant (P>0.05) effects

of genotype, concentration of PEG and its interaction on callus morphology and fresh weight was observed. High callus morphology scores (8.96) were recorded in 0 (no PEG) and 0.5%. The highest PEG concentration of 2 mg L^{-1} recorded the lowest score for callus morphology (1.39). The callus morphology score for the genotypes ranged from 4.71 to 6.76. Genotypes ARB6, ARB7, ARB8, PMK3 and RMD(R)1 recorded comparatively higher scores with higher concentrations of PEG. Fresh weight of callus ranged from 0.11 to 0.70g with the mean ranged between 0.24 and 0.56g among the genotypes. Genotypes ARB7, ARB8, RMD(R)1, PMK3 and IR 74371-70-1-1 had increased callus fresh weight in 2 mg L^{-1} concentration of PEG (Table 2). Tolerant genotypes as revealed by PCA analysis were found to show lesser reduction in callus morphology and fresh weight even at higher concentrations of PEG showing the resistant nature of these genotypes to drought stress. Combining callus morphology and callus fresh weight data showed that genotypes like PMK3, RMD(R)1, ARB7, ARB8 and ARB6 was the most drought tolerant among the genotypes studied (Fig. 2). These parental lines and their crosses selected from *in vitro* screening performed better in the field under drought stress condition.

The genotypes PMK3, RMD(R)1, ARB6, ARB8, ARB7, ADT43, ADT48 and MDU5 recorded high *per se* and *gca* effects for most of the traits. These genotypes were identified as good combiners to exploit high yield under drought stress condition and similar results were earlier reported by Sharma *et al*. (2005). In heterosis breeding, best hybrids are selected based on high *per se*, significant *sca* effects and magnitude of standard heterosis. In this study, the following hybrids were selected based on these criteria: RMD(R)1/ARB7, PMK3/ARB7, PMK3/ARB8, MDU5/ARB6, PMK3/ARB6, ADT48/ARB6, ADT43/IR77080-B-34-3, MDU5/Anjali, PMK3/IR74371-70-1-1, RMD(R)1/ARB6, ADT48/ARB7 and PMK3/Anjali (Table 5). Most of these crosses involved PMK3, RMD(R)1, ARB7, ARB8 and ARB6 as male or female parents and were selected as drought tolerant from the *in vitro* studies. It clearly indicated that these genotypes not affected by the osmotic stress induced by PEG proved their drought tolerance in their hybrid field evaluation as well. The PCA analysis also showed the same indicating that whole plant response is correlated with callus morphology score and fresh weight (Fig. 1 and 2). Crosses involving both *in vitro* drought tolerant and good combining genotypes RMD(R)1/ARB7, PMK3/ARB7, PMK3/ARB8, PMK3/ARB6 and RMD(R)1/ARB8 had significantly high *per se*, standard heterosis and *sca* effect for most of the traits (Table 5). Selection following pedigree breeding from these hybrids will result high yielding drought tolerant lines in the advanced generations (Manonmani and Fazlullah Khan, 2003). This finding demonstrates that callus stress sensitivity was positively correlated with sensitivity of whole plants under field condition (Dolgykh *et al*., 2001). The hybrids RMD(R)1/ARB7, PMK3/ARB7 and PMK3/ARB8 was suitable for heterosis breeding under drought stress, since it exhibited desirable *per se*, *sca* effects and standard heterosis for most of the traits with grain yield $plant^{-1}$(Kshirsagar *et al*., 2005 and Muthuramu *et al*., 2010). In conclusion, *in vitro* screening with the induction of drought stress using PEG would serve as an appropriate complimentary method to develop drought-tolerant lines in rice under water limited conditions.

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Table 1. Origin and parentage of rice genotypes used in this study

Genotype	Pedigree	Source
Local high yielding varieties		
ADT36	Tiruveni/IR20	Aduthurai, India
ADT43	IR50/Improved white ponni	Aduthurai, India
ADT48	IET11412/IR64	Aduthurai, India
MDU5	*O.glaberimma*/Pokkali	Madurai , India
PMK3	UPLRI7/CO43	Paramakudi, India
RMD(R)1	Selection from TGR75	Ramanathapuram, India
Aerobic rice Cultures		
ARB6	IR64/Buddha	UAS, Bangalore, India
ARB7	IR64/Buddha	UAS, Bangalore, India
ARB8	IR64/Buddha	UAS, Bangalore, India
Anjali	Sneha/RR149-1129	Paramakudi, India
IR74371-70-1-1	IR55419-42/Way Rarem	IRRI, Philippines
IR77080-B-34-3	IR68077-82-2-2-23/IR59548-122-1-4-1	IRRI, Philippines
R-1216-6-1	R671/R371-1	Coimbatore , India
RR-286-1	RR165-1160/RR145-22	Coimbatore , India

Table 2. Effect of PEG on Callus morphology and Fresh weight

Parents	Callus Morphology						Fresh Weight (gm)					
	PEG concentration (%)						PEG concentration (%)					
	Control	0.5	1.00	1.5	2.0	Mean	Control	0.5	1.00	1.5	2.0	Mean (gm)
ADT36	8.96	6.67	5.54	3.60	1.66	5.28	0.35	0.29	0.25	0.20	0.12	0.24
ADT43	8.96	5.57	4.63	3.01	1.39	4.71	0.47	0.42	0.37	0.29	0.19	0.35
ADT48	8.96	6.67	5.54	3.60	1.66	5.28	0.58	0.51	0.46	0.40	0.24	0.43
MDU5	8.96	6.47	5.48	3.56	1.64	5.22	0.48	0.35	0.28	0.22	0.11	0.29
PMK3	8.96	6.47	5.48	3.49	2.09	5.30	0.55	0.50	0.44	0.39	0.29	0.44
RMD(R)1	8.96	7.27	6.39	4.16	1.91	5.74	0.60	0.56	0.48	0.45	0.32	0.48
ARB6	8.96	8.56	7.62	5.94	2.74	6.76	0.45	0.42	0.38	0.35	0.27	0.37
ARB7	8.96	8.06	7.18	5.60	2.58	6.47	0.62	0.59	0.52	0.48	0.40	0.52
ARB8	8.86	7.86	7.00	6.15	2.45	6.46	0.70	0.64	0.57	0.50	0.39	0.56
ANJALI	8.96	6.37	5.29	3.44	1.58	5.13	0.55	0.44	0.37	0.28	0.12	0.35
IR74371-70-1-1	8.91	7.56	6.28	4.08	1.88	5.74	0.58	0.51	0.44	0.41	0.27	0.43
IR77080-B-34-3	8.96	7.17	5.95	3.87	1.78	5.54	0.44	0.38	0.31	0.22	0.14	0.29
R1216-6-1	8.91	6.87	5.70	3.71	1.71	5.38	0.50	0.42	0.33	0.23	0.11	0.32
RR286-1	8.96	6.27	5.21	3.38	1.56	5.07	0.55	0.46	0.37	0.22	0.14	0.35
Mean	8.94	6.99	5.95	4.11	1.90	5.58	0.53	0.46	0.40	0.33	0.22	0.39

	Callus morphology		Fresh weight	
	SEd	**CD (0.05)**	**SEd**	**CD (0.05)**
Parents (p)	0.019	0.038	0.006	0.011
Media (m)	0.012	0.023	0.003	0.007
p x m	0.043	0.086	0.013	0.026

Table 3. *Per se* performance of parents

Parents	Days to 50% flowering	Plant height (cm)	Productive tillers	Panicle length (cm)	Grains per panicle	Spikelet fertility (%)	Hundred grain weight (g)	Harvest index (%)	Chlorophyll stability index	Yield plant^{-1} (g)
ADT36	87.50	57.45	8.80	21.05	121.05	93.33	1.62	0.38	68.36	17.54
ADT43	93.50	56.70	10.20	19.95	118.25	97.53	1.25	0.32	64.31	15.08
ADT48	85.50	59.90	13.00	21.02	110.90	90.13	1.87	0.41	73.65	26.90
MDU5	84.00	57.55	11.50	20.56	99.25	93.64	1.78	0.35	65.06	20.32
PMK3	90.00	77.10	11.00	20.45	100.90	97.68	2.33	0.33	63.39	26.65
RMD(R)1	81.00	71.20	13.30	19.92	61.45	93.47	1.92	0.36	68.08	15.69
ARB6	81.50	72.10	10.40	19.65	93.05	91.59	2.14	0.33	63.83	21.60
ARB7	82.00	71.60	11.80	20.75	71.65	89.34	2.08	0.38	68.85	17.62
ARB8	82.50	65.35	11.00	18.55	58.75	92.39	2.01	0.27	56.00	12.99
Anjali	77.50	68.10	7.70	20.45	69.85	96.17	1.69	0.21	50.80	8.06
IR74371-70-1-1	80.50	66.50	13.40	19.55	79.85	95.47	1.73	0.36	66.71	18.51
IR77080-B-34-3	84.00	64.05	12.60	20.50	66.90	96.54	1.94	0.30	59.51	16.35
R1216-6-1	89.50	69.60	7.90	21.80	98.70	92.22	2.55	0.41	71.11	20.60
RR-286-1	72.50	58.70	8.80	17.05	43.95	87.51	1.49	0.16	43.38	5.76
Mean	83.68	65.42	10.81	20.09	85.32	93.36	1.89	0.33	63.07	17.41
CD (0.01)	4.17	1.87	1.18	1.44	3.33	4.72	0.11	0.03	2.57	2.79
CD(0.05)	3.14	1.41	0.89	1.09	2.50	3.56	0.08	0.02	1.94	2.10

Table 4. General combining ability of parents

Parents	Days to 50% flowering	Plant height (cm)	Productive tillers	Panicle length (cm)	Grains per panicle	Spikelet fertility (%)	Hundred grain weight (g)	Harvest index (%)	Chlorophyll stability index	Yield plant^{-1} (g)
ADT36	0.97 **	-2.07 **	-0.63 **	-0.45 **	-1.04 **	-2.69 **	-0.15 **	-0.01 *	-1.11 **	-4.13 **
ADT43	6.03 **	-1.97 **	-0.36 **	0.41 **	5.15 **	-2.21 **	-0.20 **	0.02 **	1.28 **	-2.07 **
ADT48	-3.59 **	-4.51 **	0.16 ns	-0.43 **	-20.96 **	-1.34 **	0.13 **	-0.01 **	-2.29 **	-2.44 **
MDU5	-1.34 **	-3.96 **	1.60 **	-1.41 **	-27.24 **	-0.42 ns	0.06 **	0.01 *	1.22 **	-1.86 **
PMK3	1.59 **	9.84 **	-0.42 **	2.07 **	25.76 **	4.28 **	0.19 **	0.00 ns	0.37 ns	8.06 **
RMD(R)1	-3.66 **	2.66 **	-0.35 **	-0.21 ns	18.33 **	2.38 **	-0.03 **	-0.01 ns	0.54 *	2.44 **
ARB6	-3.82 **	3.08 **	2.50 **	0.25 ns	6.23 **	2.63 **	0.02 ns	0.03 **	3.20 **	4.74 **
ARB7	0.18 ns	3.06 **	1.35 **	0.28 ns	15.43 **	-0.87 ns	0.04 **	0.00 ns	0.14 ns	8.39 **
ARB8	-1.91 **	0.35 ns	0.37 **	-0.17 ns	7.83 **	-1.39 **	-0.02 ns	0.04 **	6.01 **	2.34 **
Anjali	-2.32 **	2.30 **	0.75 **	0.38 *	9.32 **	0.26 ns	-0.11 **	0.01 *	-0.10 ns	-0.61 ns
IR74371-70-1-1	1.01 *	-0.90 **	-0.51 **	-0.04 ns	-7.65 **	0.27 ns	-0.11 **	0.00 ns	0.48 ns	-3.01 **
IR77080-B-34-3	5.76 **	-4.66 **	-1.04 **	-0.48 **	-9.38 **	1.34 *	0.01 ns	-0.04 **	-3.65 **	-3.19 **
R1216-6-1	2.18 **	-4.53 **	-1.95 **	-0.02 ns	-13.09 **	-0.24 ns	0.22 **	-0.03 **	-2.83 **	-4.04 **
RR-286-1	-1.07 **	1.29 **	-1.48 **	-0.21 ns	-8.69 **	-2.00 **	-0.05 **	-0.02 **	-3.26 **	-4.61 **

** Significant at 1% level * Significant at 5% level ns – Non significant

Table 5. Specific combining ability, heterosis and *per se* performance of hybrids and general combining ability of parents involved in crosses based on grain yield per plant (g)

Crosses	Mean	*sca* effect	Standard heterosis	gca of parents
RMD(R)1/ARB7	56.05	23.38 **	175.84 **	G/G
PMK3/ARB7	48.86	10.58 **	140.48 **	G/G
PMK3/ARB8	43.39	11.15 **	113.51 **	G/G
MDU5/ARB6	33.17	8.45 **	63.26 **	A/G
PMK3/ARB6	32.32	-2.32 **	59.06 **	G/G
ADT48/ARB6	31.57	7.44 **	55.39 **	P/G
ADT43/IR77080-B-34-3	30.29	13.71 **	49.06 **	A/P
MDU5/Anjali	29.68	10.31 **	46.06 **	A/A
PMK3/IR74371-70-1-1	27.77	0.88 ns	36.66 **	G/P
RMD(R)1/ARB6	27.27	-1.75 *	34.20 **	G/G
ADT48/ARB7	25.05	-2.73 **	23.30 **	P/G
PMK3/Anjali	24.37	-4.92 **	19.91 **	G/A
RMD(R)1/ARB8	23.61	-3.01 **	16.19 **	G/G
MDU5/ARB8	23.47	1.15 ns	15.48 **	A/G
PMK3/RR-286-1	23.19	-2.09 *	14.15 **	G/P
RMD(R)1/IR74371-70-1-1	23.18	1.90 *	14.05 **	G/P
ADT43/R1216-6-1	22.49	6.77 **	10.70 *	A/P
PMK3/IR77080-B-34-3	20.73	-5.98 **	2.02 ns	G/P
ADT36/Anjali	20.42	3.32 **	0.49 ns	P/A
ADT36/IR77080-B-34-3	20.13	5.61 **	-0.94 ns	P/P
ADT43/RR-286-1	19.65	4.49 **	-3.32 ns	A/P
ADT48/R1216-6-1	19.61	4.25 **	-3.52 ns	P/P
ADT48/Anjali	19.38	0.60 ns	-4.63 ns	P/A
ADT43/ARB6	19.1	-5.42 **	-6.03 ns	A/G
ADT36/ARB8	18.99	-1.07 ns	-6.57 ns	P/G
MDU5/ARB7	18.75	-9.62 **	-7.75 ns	A/G
ADT48/ARB8	18.63	-3.11 **	-8.34 ns	P/G
PMK3/R1216-6-1	18.56	-7.30 **	-8.66 ns	G/P
RMD(R)1/Anjali	17.47	-6.20 **	-14.05 **	G/A
ADT36/RR-286-1	17.35	4.25 **	-14.62 **	P/P
RMD(R)1/IR77080-B-34-3	17.2	-3.89 **	-15.35 **	G/P
ADT43/ARB7	17.09	-11.07 **	-15.92 **	A/G
ADT43/ARB8	16.99	-5.11 **	-16.39 **	A/G
ADT36/R1216-6-1	16.95	3.28 **	-16.56 **	P/P
ADT43/IR74371-70-1-1	16.49	-0.26 ns	-18.82 **	A/P
ADT36/IR74371-70-1-1	16.27	1.56 ns	-19.93 **	P/P
ADT36/ARB6	16.06	-6.40 **	-20.96 **	P/G
ADT43/Anjali	16.05	-3.10 **	-20.99 **	A/P
MDU5/IR74371-70-1-1	15.74	-1.23 ns	-22.51 **	A/P
RMD(R)1/RR-286-1	15.57	-4.10 **	-23.40 **	G/P
ADT36/ARB7	15.55	-10.55 **	-23.45 **	P/G
MDU5/R1216-6-1	15.29	-0.65 ns	-24.75 **	A/P
ADT48/RR-286-1	14.31	-0.48 ns	-29.58 **	P/P
RMD(R)1/R1216-6-1	13.9	-6.34 **	-31.57 **	G/P
ADT48/IR74371-70-1-1	13.53	-2.85 **	-33.39 **	P/P
MDU5/RR-286-1	13.31	-2.06 *	-34.47 **	A/P
ADT48/IR77080-B-34-3	13.09	-3.11 **	-35.56 **	P/P
MDU5/IR77080-B-34-3	10.45	-6.34 **	-48.55 **	A/P

** Significant at 1% level * Significant at 5% level ns – Non significant
G: Good combiner; A: Average combiner; P: Poor combiner

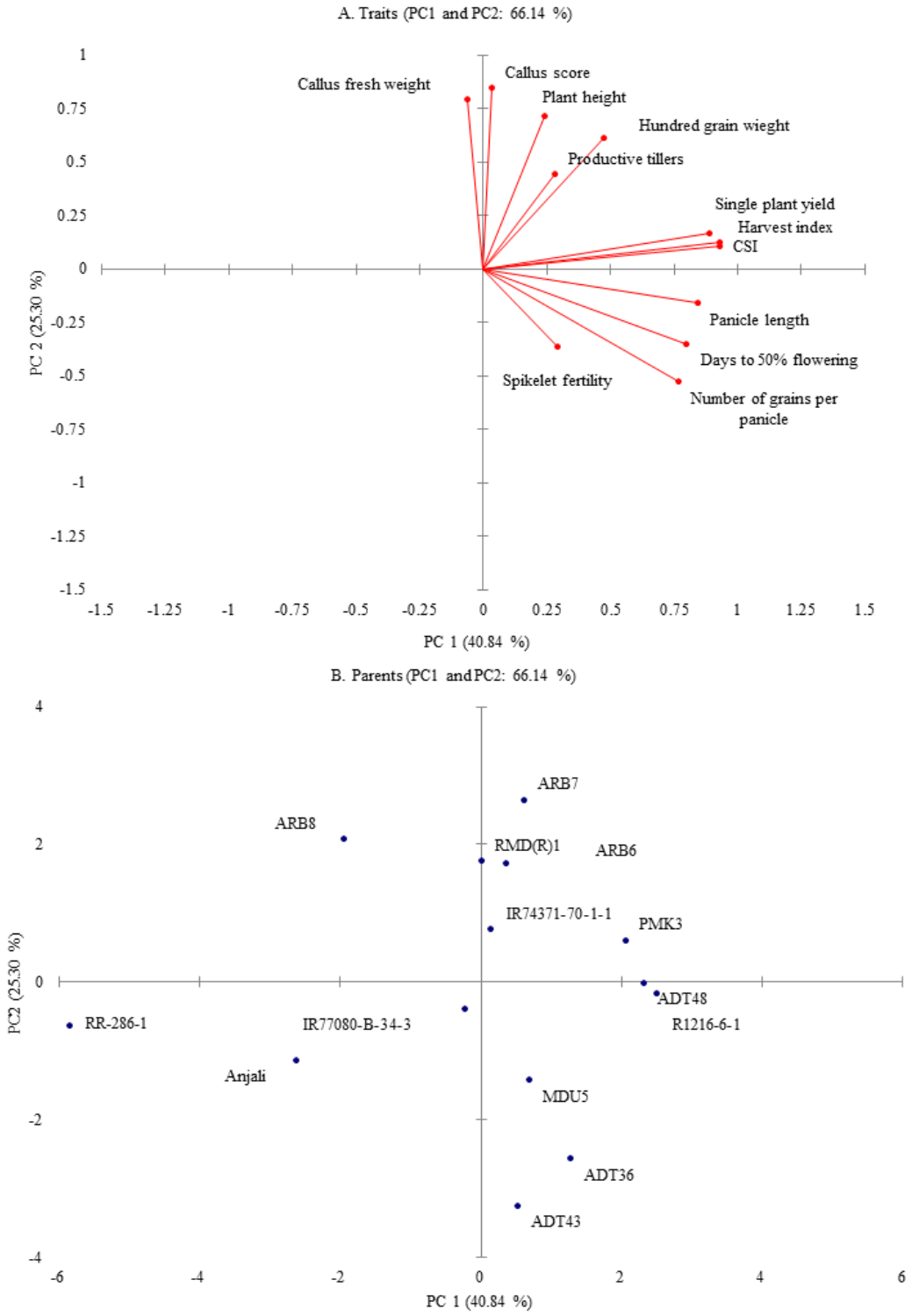

Figure 1. Principal component analysis (PCA) separated traits by *in vitro* and field observed traits (A) and genotypes as drought tolerant and susceptible genotypes (B).

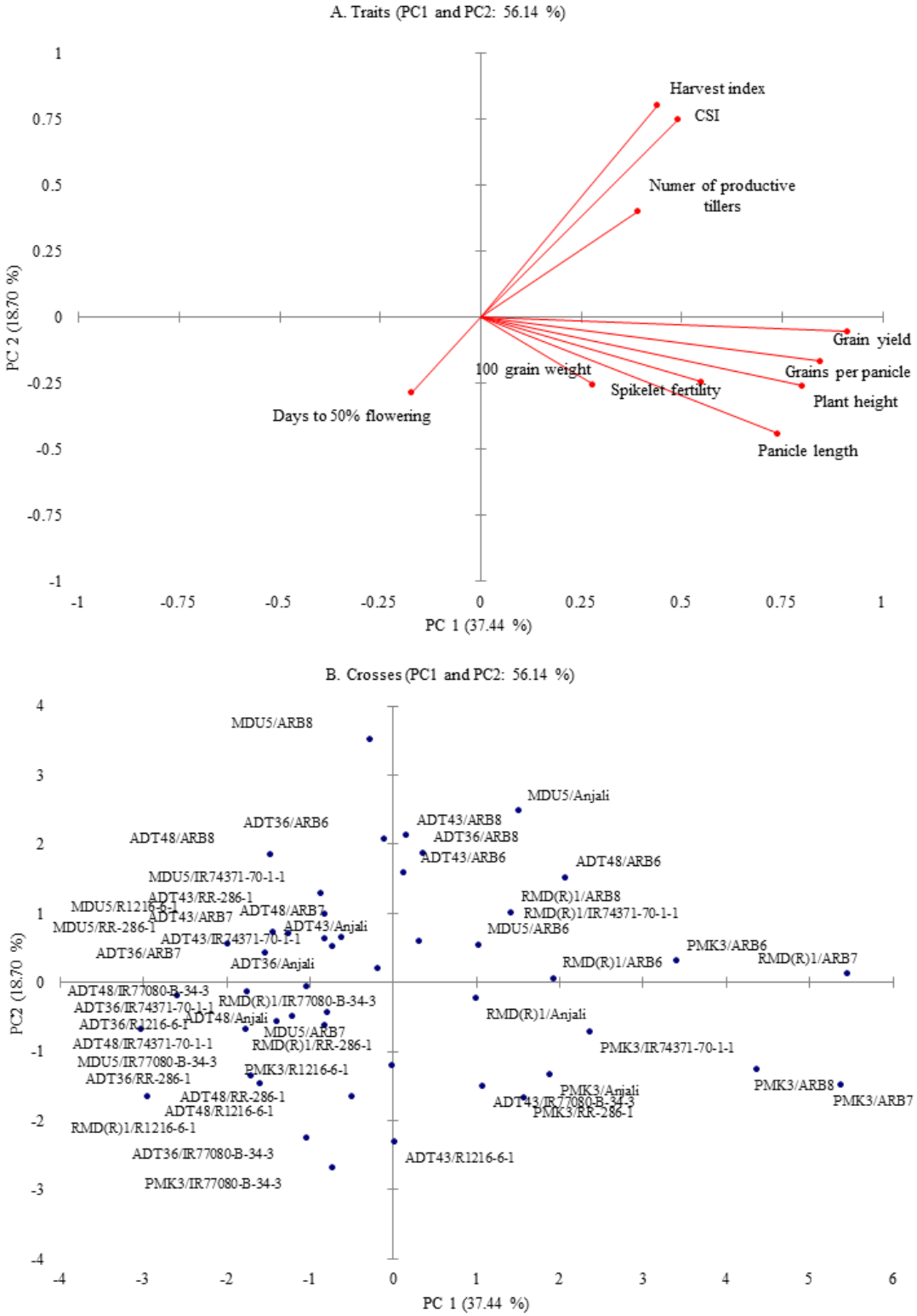

Figure 2. Principal component analysis (PCA) separated traits by flowering and yield component traits (A) and hybrids as drought tolerant and susceptible (B).