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Characterization of some Indian native land race rice accessions for drought tolerance at seedling stage

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Abstract

In rice, a major cereal, drought stress is one of the major constraints for production and yield stability in rainfed ecosystems. In an effort to identify promising rice accessions having tolerance against drought, one hundred and thirty four land races that represent different geographic regions of India and a few from Indonesia and Philippines were examined at the vegetative stage under both field and laboratory conditions. Thirty-day-old seedlings were subjected to moisture stress and the experimental field conditions include a ground water table at a depth of > 90 cm, low soil moisture content (10-12%) and high soil moisture tension (up to -50 kPa) at 30 cm soil depth during the stress period. With an SES (Standard Evaluation System) score of 0-3 in the 0-9 scale, seventy eight accessions were scored as tolerant with twelve having '0' score, eighteen with '1' and forty eight with the score ‘3’. Of the seventy eight genotypes, thirteen had recorded yield over 1.0 t ha^{-1} while the tolerant (CR 143-2-2) and susceptible controls (IR 20) recorded 2.70 t ha^{-1} and zero yield respectively. The tolerant genotypes were also evaluated further against poly ethylene glycol-6000 induced osmotic stress at both germination (-6 bar) and seedling stages (-8 bar and -10 bar). From the pooled data, six genotypes *i.e.* IRGC 12263, IRGC 636, IRGC 45699, IRGC 40275, IRGC 53989 and IRGC 51231 were identified as drought tolerant with good yield potential (0.7-1.95 tha^{-1}) under stress conditions.

Keywords: Rice, Drought tolerance, Poly ethylene glycol, Seedling stage
Abbreviations: PEG_Polyethylene glycol; SES_Standard Evaluation System; HI_Harvest Index; LSD_Least significant difference; EVV_Early vegetative vigor; BY_Biomass yield; SMC_Soil moisture content.

Introduction

Existence of genetic diversity for resistance/tolerance against different biotic and abiotic stresses has special significance for the maintenance and enhancement of productivity in agricultural crops most specifically for rice (*Oryza sativa* L.), a crop grown in varied agro-climates. Drought stress is one of the major constraints to rice production and yield stability in rainfed upland ecology and estimates indicate that 70% of the yield losses can be attributed to abiotic stresses, especially drought (Bray et al., 2000). Drought is a multifaceted stress condition with respect to timing and severity, ranging from long drought seasons where rainfall is much lower than demand, to short periods without rain where plants depend completely on available soil water (Lafitte et al., 2006). Incorporation of drought tolerance has always been a challenge to plant breeders, because of the complexity of the trait that involve several physiological and molecular mechanisms and different mechanisms often combine to confer drought tolerance (Wang et al., 2001; Parida and Das, 2005). Drought at the vegetative stage can cause a moderate reduction in yield but the reproductive stage (from panicle initiation to flowering) is recognized as the most critical stage at which drought stress can cause serious damage to the crop and can even entirely eliminate yield (O'Toole, 1982; Zhang, 2007). Evaluation of genotypes under field conditions in the dry season was found to be ideal for identification of drought tolerant genotypes that are able to retain a large proportion of green living tissues under soil water deficit both at vegetative and reproductive stages (Chang et al., 1974; De Datta et al., 1988). The major criteria to evaluate the performance of genotypes against drought under field conditions are drought score, grain yield and spikelet fertility. Delayed leaf rolling under water stress for dehydration avoidance is also an important selection criterion as the genotypes that have the capacity to maintain high leaf water potential are known shows less leaf rolling (Blum, 1988). Screening under stimulated water stress conditions induced by osmotic substances having high molecular weight like polyethylene glycol (PEG) for identification of tolerant genotypes against drought is one of the popular approaches (Turkan et al., 2005; Landjeva et al., 2008). Polyethylene glycol is a non-penetrating inert osmoticum that can lower the water potential of nutrient solutions without being taken up or being phytotoxic (Lawlor, 1970). It has been reported that an increase in drought stress by PEG was accompanied by a sudden decline in moisture content of tissues (El-Tayeb and Hassanein, 2000) as PEG mimics in a way similar to soil drying. This approach has been used to simulate drought stress in plants and selection of tolerant genotypes in different crops (Nepomuceno et al., 1998; Cherian and Reddy 2003; Badiane et al., 2004) and it was reported to be an effective strategy for selection at the early growth stages of rice (Jing and Chang, 2003).

In addition to seedling screening, the other commonly used approach to determine the tolerance of genotypes against drought is the assessment of the germination ability of the

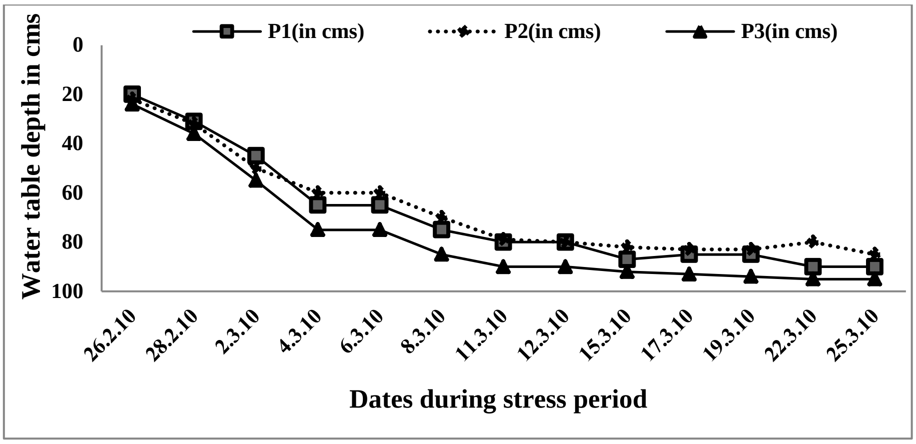

Fig 1. The status of water table depth during the stress period (P1-Peizometer 1, P2-Peizometer 2 and P3-Peizometer 3).

seeds under induced water stress conditions. Screening with aqueous solutions of poly ethylene glycol-6000 and mannitol (Costa et al., 2004; Fanti and Perez, 2004) aided the identification of cultivars having higher levels of tolerance to drought in rice (Pirdashti et al., 2003).
The present study was an effort to identify genotypes having tolerance to drought at vegetative stage through field evaluation followed by assessment of the selected genotypes against induced stress under controlled conditions.

Results

Phenotyping at seedling stage against drought stress under field conditions

The major criteria chosen for identification of drought tolerance are drought score, retention of a major proportion of green living tissue under water deficit condition, spikelet fertility and grain yield. During the stress period, ground water table was below 90 cm for a major portion of the drought stress period (Fig. 1) and the soil moisture content at 30 cm soil depth was 10-12%. With progressive decrease in moisture content in the soil, an increase in soil moisture tension (up to -50 kPa) was recorded (Fig. 2).

After recovery from the water stress, the crop was grown till maturity and analysis of the data on the six important agronomic traits including yield indicated wide variation for all the traits and differences between genotypes are significant (Table 1).The variation observed for different traits was in the range of 44-120 days for days to fifty percent flowering (DFF), 1.70 -7.09 tha^{-1} for total biomass, 0.0 to 2.70 tha^{-1} for grain yield and 0.01 to 0.23% for harvest index (HI). The grain filling percentage varied from 0.8% (IR 20) to 74.3% (IRGC 67720) and highest yield was recorded in CR 143-2-2 (2.70 tha^{-1}), the tolerant control, followed by IRGC 44975 (2.24 tha^{-1}) and IRGC 12263 (1.95 tha^{-1}). Out of 78 accessions, six genotypes recorded more than 1.5 tha^{-1} yield, seven genotypes between 1.0 to 1.5 tha^{-1}, and 20 genotypes between 0.5 to 1.0 tha^{-1} while the remaining 45 genotypes recorded less than 0.5 tha^{-1} while IR 20, the susceptible control, recorded zero yield. Biomass accumulation was > 6.0 tha^{-1} in 11 genotypes, > 5.0 tha^{-1} in six genotypes, > 4.0 tha^{-1} in 21 genotypes while in the rest of the genotypes, the value varied between 0 to 3.0 tha^{-1}.

Phenotyping at germination and seedling stages under chemically induced water stress

The PEG induced water stress inhibited germination, affected both shoot and root growth of the genotypes. At '-6 bar' moisture stress, only nine genotypes showed more than 40% germination while 43 genotypes including the susceptible control did not germinate at all, 11 genotypes had a value < 20% and for 12 genotypes, the frequency varied between 20 - 39% (Table 2). Three accessions *i.e*. IRGC 44975, IRGC 45699 and IRGC 53989 showed more than 80% germination and in comparison, the two tolerant controls showed > 60% germination only. However, when the stress was released after 10 days, the germination has gone up to 80-90% in most of the genotypes.

The second set of experiments was conducted with five days old seedlings employing two levels of water stress. At '-8 bar' water stress, only 15 genotypes showed 100% survival while 15 genotypes did not survive the stress resulting in complete death of the seedlings. The other genotypes showed varied levels of survival with 16 genotypes showing 90-100%, 2 genotypes 80-90%, 12 genotypes 50-80% and the others showing < 50% survival. When the water stress was at '-10 bar', only 5 genotypes showed 100% survival followed by 10 genotypes with 90-100%, 21 genotypes with 80-90%, 20 genotypes with 50-80%, 11 genotypes with less than 50% survival while eight genotypes did not survive the stress indicating a positive relationship between the level of osmotic stress and the mortality rates. From the combined data of the two experiments conducted with seedlings, eighteen genotypes having 80-100% seedling survival were identified as drought tolerant (Table 3). From the pooled data obtained in three experiments (T_1, T_2, T_3) conducted with seeds and seedlings under induced osmotic stress, four genotypes, IRGC 9175, IRGC 44975, IRGC 45699 and IRGC 45992 were found to be highly promising.

Discussion

Rice crop is highly susceptible to water stress (Tao et al., 2006; Yang et al., 2008) and the magnitude of yield losses depends on the duration of water stress and stage of the crop growth. One of the techniques employed for identification of promising genotypes is screening of seeds and seedlings against drought stress under simulated conditions using

Table 1. Morphological traits including yield and yield attributes of the genotypes.

S.NO	Genotypes	EVV	DFF	Fertility(%)	BY (tha^{-1})	Grain Yield (tha^{-1})	HI (%)
1	IRGC 55173	3	67	41.1	2.32	0.76	0.22
2	IRGC 63113	3	89	19.4	6.164	0.48	0.05
3	IRGC 74762	3	92	16.4	2.517	0.19	0.05
4	IRGC 67706	3	60	42.9	4.142	0.69	0.11
5	IRGC 74775	7	83	34.1	1.953	0.56	0.19
6	IRGC 74773	5	83	52.1	2.543	0.84	0.22
7	IRGC 67698	3	87	19.0	3.411	0.26	0.05
8	IRGC 67730	7	60	33.3	2.089	0.54	0.17
9	IRGC 74734	3	60	67.5	3.261	0.93	0.19
10	IRGC 74779	5	83	31.3	7.328	0.82	0.07
11	IRGC 74777	5	71	65.7	4.315	0.88	0.13
12	IRGC 52785	7	60	16.5	6.791	0.22	0.02
13	IRGC 67720	1	74	74.3	4.998	1.50	0.2
14	IRGC 52734	3	76	1.2	4.679	0.14	0.02
15	IRGC 67699	3	74	15.6	4.61	0.17	0.02
16	IRGC 74774	5	73	26.9	2.758	0.55	0.13
17	IRGC 54656	3	74	16.7	4.188	0.23	0.04
18	IRGC 61127	5	83	4.1	2.268	0.13	0.04
19	IRGC 61133	3	74	10.1	2.29	0.15	0.04
20	IRGC 12485	5	73	55.1	4.67	0.84	0.12
21	IRGC 12254	5	112	4.3	1.927	0.15	0.05
22	IRGC 12685	1	73	50.5	3.107	1.94	0.41
23	IRGC 12166	1	60	1.1	5.682	0.12	0.01
24	IRGC 12331	3	83	21.9	2.565	0.47	0.12
25	IRGC 12254	1	70	25.6	4.775	0.38	0.05
26	IRGC 12485	3	60	13.2	4.462	0.17	0.03
27	IRGC 40972	3	83	17.8	4.996	0.46	0.06
28	IRGC 6663	1	60	72.1	6.593	1.37	0.14
29	IRGC 11099	5	115	16.3	2.293	0.14	0.04
30	IRGC 12380	3	120	0.4	1.729	0.09	0.03
31	IRGC 12603	1	60	5.6	4.177	0.13	0.02
32	IRGC 41216	1	77	28.5	5.363	0.36	0.04
33	IRGC 12263	1	56	53.1	6.972	1.95	0.18
34	IRGC 6294	3	73	60.9	4.658	1.21	0.17
35	IRGC 12894	1	60	34.1	3.601	0.62	0.11
36	IRGC 6264	1	47	51.5	4.855	1.20	0.16
37	IRGC 6298	5	83	2.1	3.902	0.12	0.02
38	IRGC 636	1	72	64.3	7.958	0.80	0.15
39	IRGC 9069	5	81	12.8	1.855	0.16	0.06
40	IRGC 11486	1	74	12.4	4.459	0.16	0.02
41	IRGC 13746	3	56	14.8	2.904	0.23	0.05
42	IRGC 9091	5	100	0.9	1.912	0.07	0.01
43	IRGC 3681	5	97	6.1	1.131	0.15	0.08
44	IRGC 3685	3	56	25.9	2.554	0.28	0.07
45	IRGC 3641	5	83	1.9	4.163	0.12	0.02
46	IRGC 63	5	74	16.8	6.613	0.43	0.04
47	IRGC 9175	5	83	13.3	2.248	0.13	0.04
48	IRGC 44975	3	60	72.9	6.491	2.24	0.23
49	IRGC 44976	3	60	68.5	3.066	0.96	0.21
50	IRGC 45701	3	99	20.1	2.996	0.64	0.14
51	IRGC 46047	7	74	5.8	6.345	0.13	0.01
52	IRGC 41234	3	83	1.2	2.58	0.08	0.02
53	IRGC 6274	3	83	1.8	3.847	0.13	0.02
54	IRGC 45699	3	100	30.9	5.99	1.03	0.11
55	IRGC 41019	1	80	34.3	3.443	1.00	0.19
56	IRGC 6144	3	79	7.4	1.739	0.15	0.06
57	IRGC 4819	5	74	52.2	4.709	1.22	0.17
58	IRGC 8887	3	73	26.9	2.758	0.55	0.13
59	IRGC 4895	7	83	1.8	4.503	0.14	0.01
60	IRGC 3742	3	72	58.2	4.95	1.38	0.18
61	IRGC 40275	1	72	2.8	2.777	0.11	0.03
62	IRGC 53989	1	47	57.7	3.722	0.87	0.15
63	IRGC 51932	1	60	27.8	3.305	0.35	0.07
64	IRGC 51971	5	99	6.3	2.013	0.14	0.05
65	IRGC 51923	3	44	51.9	6.775	1.70	0.17
66	IRGC 51903	3	72	2.3	4.615	0.12	0.02
67	IRGC 51774	3	83	8.8	2.18	0.15	0.05
68	IRGC 51231	1	60	38.9	4.878	0.72	0.12

69	IRGC 51869	1	83	10.8	2.673	0.21	0.05
70	IRGC 17042	7	83	10.3	1.813	0.15	0.05
71	IRGC 39735	5	97	11.9	3.443	0.21	0.04
72	IRGC 13758	1	72	1.3	2.628	0.12	0.01
73	IRGC 41001	3	89	19.3	7.087	0.29	0.03
74	IRGC 45992	3	97	13.7	4.919	0.12	0.02
75	IRGC 12469	3	72	2.1	5.715	0.12	0.01
76	CR 143-2-2	3	90	68.6	5.949	2.70	0.17
77	Vandana	1	86	63.3	5.352	1.27	0.16
78	IR-20	5	90	0.8	2.863	0.00	0.02
	.SD (5%)	2.13	4.79	8.93	0.50	0.02	0.16
		40.0	3.9	19.0	8.0	1.1	37.3

(IRGC-International Rice Genome Centre; EVV- Early vegetative vigor; DFF-Days to 50% flowering; Fertility(%)-Percentage of grain filling; BY t ha^{-1}- Biomass yield t $ha^{-1;}$ Grain Yld/ t ha^{-1}- Grain Yield t ha^{-1} ; HI-Harvest Index).

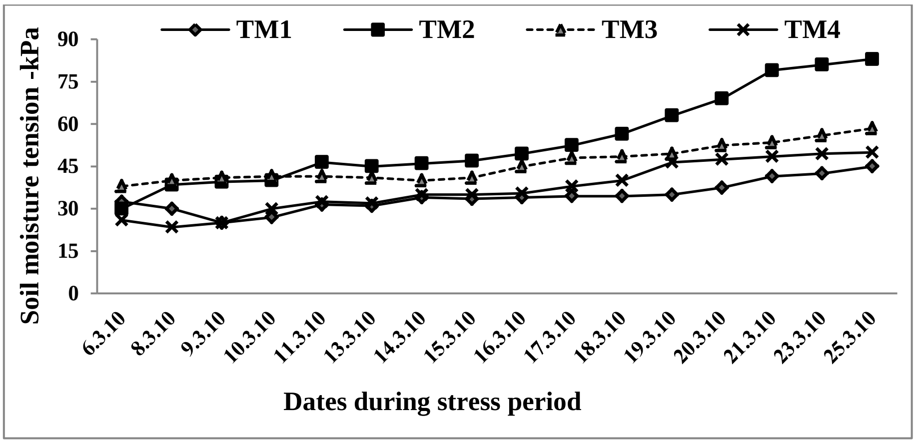

Fig 2. The status of water tension during the stress period (TM1-Tensiometer 1, TM2-Tensiometer 2, TM3-Tensiometer 3 and TM4-Tensiometer 4).

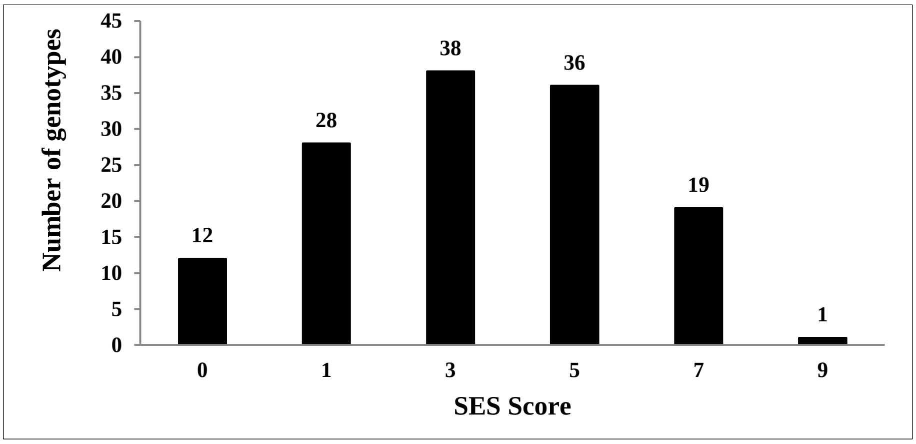

Fig 3. Frequency distribution of genotypes with different drought tolerance scores (in SES scale 0-9).

polyethylene glycol and mannitol in solution cultures (Misra et al., 2002; Costa et al., 2004; Blum, 2008). As germination is severely affected under water stress, the survival rate of genotypes has been employed as the selection criterion to identify donors (Zeid and El-Semary 2001; Kaboli and Sadeghi, 2002; Li et al., 2005; Bermingham et al., 2006; Nasirzadeh and Shookoh, 2006; Hassanpanah, 2009). In the present study also, wide variation was observed among the genotypes and three land races *i.e*. IRGC 44975, IRGC 45699 and IRGC 53989 showed higher levels of germination than the two tolerant controls.

In the experiments where the five days old seedlings were subjected to water stress at '-8 bar' and '-10 bar' treatments, the genotypes showed wide variation in their survival rates. While 15 genotypes showed 100% survival at '-8 bar' stress and only five genotypes showed 100% survival at '-10 bar' stress demonstrate the utility of the technique for identification of donors. A total of eighteen genotypes were identified as drought tolerant on the basis of their higher (80-100%) rates of survival while four genotypes, (IRGC 9175, IRGC 44975, IRGC 45699 and IRGC 45992) were identified as most promising at all levels of osmotic stress conditions. The reduction in germination and seedling survival that was observed in the two experiments (T_2, T_3) has corroborated the findings reported in several crops like rice (Jiang and Lafitte 2007), maize (Ibrahim et al., 2001), wheat (Khakwani et al., 2011), sesame (Bahrami H et al., 2012), cotton (Gadelha Meneses et al., 2011), Parkia pendula (Pereira Sousa et al., 2012) and carrot (Perez-Fernandez et al., 2006) where it was reported that an increase in the level of drought stress significantly affects the germination. The germination in drought sensitive genotypes is regulated by duration of

Table 2. The germination frequencies (%) of promising genotypes under simulated stress (- 6 bar)

More than 40% germination under stress	
Genotypes	Germination percentage under stress condition
IRGC 67720	42.9
IRGC 74774	43.8
IRGC 6298	45.5
IRGC 9091	41.2
IRGC 9175	58.8
IRGC 44975	85.0
IRGC 45699	84.6
IRGC 53989	83.3
IRGC 45992	53.3
CR 143-2-2 (Tolerant control)	75.0
Vandana (Tolerant control)	64.3

Table 3. Survival rates (%) of promising genotypes under simulated osmotic stresses (-8 bar and -10 bar).

Genotypes	Survival percentage of germinated seeds under osmotic stress condition
IRGC 74762	100.0
IRGC 12254	100.0
IRGC 12685	80.0
IRGC 12263	100.0
IRGC 636	100.0
IRGC 13746	100.0
IRGC 51869	95.7
IRGC 9175	87.0
IRGC 44975	100.0
IRGC 45699	94.4
IRGC 8887	93.8
IRGC 3742	100.0
IRGC 40275	92.0
IRGC 51971	95.7
IRGC 51231	94.7
IRGC 17042	92.9
IRGC 45992	100.0
IRGC 12469	95.8
CR 143-2-2 (Tolerant control)	70.0
Vandana (Tolerant control)	66.6
IR-20 (Susceptible control)	0.0

wetting and the amount of moisture in the growth medium (Schutz and Milberg 1997; Gill et al., 2002).

In the field experiment, data on leaf rolling scored after 28 days of stress (when the susceptible control showed permanent wilting) revealed that more than 50% genotypes had a SES score up to "3" showing delayed leaf rolling and fast recovery after re-watering on 28th day. Of the 134 accessions, 78 accessions were scored as drought tolerant on the basis of the SES score "0-3" while 56 lines with SES score '5-9' were scored as susceptible (Fig.3). However, out of them, only thirty genotypes were identified as best for vegetative stage drought (12 with score '0' and 18 with SES score '1') as they showed early recovery (Table 4). As drought severely affects the production and productivity of rice in field condition, selection for yield under water stress was considered as important criteria (Yang et al., 2001; Pantuwan et al., 2002; Kumar et al., 2008). As yield reduction in rice is 65-85% under severe drought stress situation compared to non-stress conditions (Kumar et al., 2008), development of drought tolerant genotypes that maintain good yield under drought is suggested as a priority area of rice research for sustainable rice production (Swamy et al., 2011). The yield data indicates that out of the 78 accessions, 13 genotypes recorded more than 1 tha^{-1}, 20 genotypes between 0.5 to 1 tha^{-1} while the remaining 45 genotypes recorded less than 0.5 tha^{-1} grain yield. Comparison of promising genotypes indicated that two genotypes IRGC 12263 and IRGC 45699 had more than 1 tha^{-1} and 3 genotypes IRGC 636, IRGC 53989 and IRGC 51231 had more than 0.7 tha^{-1}. The fertility percentage varied significantly among the genotypes and four genotypes (IRGC 44975, IRGC 12263, IRGC 636, IRGC 53989) and CR 143-2-2, the tolerant control showed > 50% grain filling. The yield data also indicates that some genotypes like IRGC 12263 and IRGC 45699 yielded more than 1 tha^{-1}. It was suggested that under drought stress condition, yield and yield attributes have a higher phenotypic correlation with some of the yield components like spikelet fertility, biomass and grain numbers (Lafitte et al., 2004; Hirayama et al., 2006; Venuprasad et al., 2007) and similar results were observed in the present study also. The pooled data from all the experiments indicates that six genotypes *i.e.* IRGC 12263, IRGC 636, IRGC 45699, IRGC 40275, IRGC 53989 and IRGC 51231 were found to be drought tolerant with good yield potential under stress conditions.

Materials and Methods

Field screening

One hundred and thirty four rice accessions that include 128 accessions of Indian origin, two from Indonesia and one from Philippines, obtained from International Rice Research Institute, Philippines, were evaluated for their tolerance to drought under field conditions at Central Rice Research Institute, Cuttack during the dry season of 2010 along with two tolerant (Vandana and CR 143-2-2) and one susceptible (IR 20) controls (Supplementary table 1).

The crop was direct seeded and a randomized block design with three replications was employed for the experiment. Recommended dosage of fertilizers ($N:P_2O_5:K_2O$ @40:20: 20kg/ha) were applied basally. The plot was irrigated at three-day intervals and the 30 days old seedlings were subjected to drought stress by stopping the irrigation till the susceptible control showed permanent wilting.

Ground water table was monitored on alternate days after withdrawal of irrigation with three peizometers (P1, P2 and P3) randomly installed in the plot. Soil moisture content (SMC) was measured at 15 and 30 cm soil depth at seven-day intervals after suspension of irrigation and soil moisture tension was measured at 30 cm soil depth at two day intervals using four tensiometer tubes (TM1, TM2, TM3 and TM4)

Leaf rolling, drying and drought scores were recorded in a 0 to 9 scale as per IRRI SES method (IRRI, 1996). The crop was re-irrigated after the stress and the recovered crop was raised till maturity and observations were recorded on parameters like total biomass, spikelet fertility (%), grain yield and harvest index. The statistical analysis was done using Crop Stat 7.2 software (IRRI, 2009) and Gomez and Gomez (1984).

Table 4. Drought scores of promising genotypes and controls under field condition.

Genotypes	DSR	Genotypes	DSR
IRGC 12263	0	IRGC 45699	1
IRGC 6663	0	IRGC 55173	1
IRGC 6264	0	IRGC 44976	1
IRGC 41216	0	IRGC 45701	1
IRGC 74734	0	IRGC 74777	1
IRGC 636	0	IRGC 74762	1
IRGC 41019	0	IRGC 61127	1
IRGC 53989	0	IRGC 40972	1
IRGC 51231	0	IRGC 12380	1
IRGC 12166	0	IRGC 3681	1
IRGC 12254	0	IRGC 3685	1
IRGC 51932	1	IRGC 41234	1
IRGC 51923	1	IRGC 40275	1
IRGC 6294	1	Vandana	0
IRGC 51869	1	CR 143-2-2	1

Laboratory assays

The seventy eight genotypes that were scored as tolerant (0-3 score) in the field experiment were screened under controlled osmotic stress conditions simulated by using polyethylene glycol (PEG 6000) at two stages of the crop: i) at germination stage and ii) at seedling stage.

The seeds were subjected to germination in aqueous solution of PEG 6000 at three different concentrations: T_1 (-6 bar), T_2 (-8 bar) and T_3 (-10 bar). As none of the entries germinated at both T_2 and T_3 treatments, data could be recorded only for -6 bar treatment. In the other experiment, five days old seedlings were subjected to moisture stress at two levels *i.e*. T_2 (-8 bar) and T_3 (-10 bar) as per Chandrasekhara Reddy et al. (1994) and Badiane et al. (2004). The stress was released after 72 hrs when the susceptible control showed wilting and observations were recorded on the survival rates.

Conclusions

Development of rice cultivars with tolerance to drought, a complex trait, is a major challenge and a thorough understanding of the physiological and molecular mechanisms that govern the yield of rice under water stress condition is a prerequisite. Accurate phenotyping information is a critical step that can lead to identify genes/alleles associated with drought tolerance in rice. In the present study, phenotyping at field level has led to the identification of six highly promising genotypes while the experiments conducted under controlled conditions identified four genotypes having high levels of tolerance to all levels of osmotic stress. From the pooled data, IRGC 45699 was observed to possess high levels of tolerance to all stress conditions evaluated under the study. These genotypes may be useful as donors for tolerance to drought stress at both seedling and vegetative stages in the breeding programs.

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