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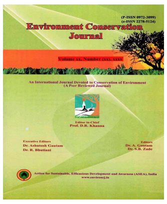

In-vitro selection of drought tolerant doubled haploid rice lines using polyethylene glycol (PEG)

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ABSTRACT

The present study was conducted to determine the response of 55 double haploid (DH) rice lines developed for drought tolerance from the cross Swarna × IR159B in polyethylene glycol (PEG) induced drought stress under *in-vitro* conditions (DH lines named as double haploid rice lines-DRL). Drought stress was created using PEG-6000 at different level of external water potential. Analyzed seedling traits of DRLs showed significant differences in response to different PEG concentrations. A decrement in plant growth at seedling stage with the increase in PEG concentration was observed as expected. Among 55DRLs, 14 DRLs were found to be drought tolerant sustaining the stress level till -7.5 bar as of the tolerant checks. Further, Drought linked SSRs were also evaluated in developed rice lines. Out of 8 SSRs, RM55 (R^2 value- 13.5%) and RM259 (R^2 value- 13.9%) found to be exhibiting significant association with the shoot/root ratio at - 7.5 bar stress level. Out of 14 DRLs, 9 DRLs were found to be showing drought tolerant in phenotypic and genotypic screening. Hence, PEG induced stress screening method used in this study will serve as the baseline for screening of rice lines for drought tolerance at very early stage without exploitation of much resource.

Introduction

Rice being a predominant food crop has devastating effect from drought stress limits the crop productivity by impeding plant growth and development, and thus reduces harvest size (Subba *et al.,* 2013). Approximately, 90% of the world's rice is produced and consumed in Asia. However, by 2025 production of 17 million hectares of traditionally grown irrigated rice will be affected by physical water scarcity and 22 million hectares will be hindered by "economic water scarcity" (Hibberd *et al.,* 2008; Prasad, 2011). Increase in water scarcity with the ongoing climatic changes will further worsen the scenario by posing a potential risk to productivity and food security in these rice growing areas (Li *et al.,* 2011). Studies of rice plants in response to identification of drought tolerance mechanism towards the development of promising drought tolerant lines under natural condition could be done by exploiting molecular, morphological, physiological and agronomic traits can make more crop per drop a reality (Degenkolbe *et al.,* 2009; Swamy and Kumar, 2013). Conventional breeding has led to the development of drought tolerant rice varieties still there is a cumbersome due to changing climatic regime accompanied with continuous nature of abiotic stresses. Thus, there is a need to fasten the breeding method. Double haploid (DH) technology *via in vitro* anther culture would accelerate the development of drought tolerant rice lines in short period of time. Looking to the success of this technology, since 2016, our lab is continuously working to develop DH lines in rice through *in vitro* anther culture for biotic and abiotic stresses

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which resulted into the release of variety CG Tejaswi Dhan high yielding and BLB resistance (Proceeding state seed sub-committee, Govt. of C.G. 2021). Many are in pipeline for high yielding, BLB, blast, drought tolerance, aroma (yet to be released). Field screening for drought tolerance is time consuming, labor intensive and requires suitable environmental conditions for the effective, repeatable phenotypic expression attributable to the genotype (Kacem *et al.,* 2017). It is therefore, necessary to use simple and effective early screening methods that relate to the field phenotypes. Selection of drought tolerant line *in vitro* is thought to be one of the ways to improve selection efficiency in addition to marker assisted selection.

Drought stress could be induced *in vitro* by using high molecular weight osmoticum *i.e*., PEG as it mimics in a way like soil drying resulting into selection of drought tolerant rice lines (Nepomuceno *et al.,* 1998; Widyastuti *et al.,* 2016).

To identify drought tolerant DH plants, molecular marker is also reliable tool which detect high degree of polymorphism in rice and hence are ideal for studies to identify tolerant genotype (Okoshi, 2004). In this study, attempt was made to identify the best promising drought tolerant line (DTL) derived from the cross Swarna and IR159B using varying level of external water potential given by PEG 6000 followed by genotyping of lines using random markers at seedling stage. The objective was to develop drought tolerant high yielding rice variety. Therefore, Swarna being a very popular yielder was selected and crossed with drought tolerant variety IR 159B. The above screening method together with the genotypic data can be employed to select superior drought tolerant lines varietal trials at very early stage, without exploitation of resources and yield loss due to stress.

Material and Methods

The experimental material consists of 55 DH rice lines developed through anther culture derived from the cross of Swarna × IR159B. Among 55 DH rice lines, 30 lines were mutagenized during its haploid callus stage using gamma irradiation (20 Grey) to increase variability for better selection. All the DH lines were named as DH rice lines no. 1-55 (DRL). Rice cultivars Dagaddeshi, RRF-127, IR-159B were used as tolerant check and Danteshwari, MTU1010, Swarna were taken as susceptible check. Mature rice seeds were harvested, manually dehusked using hand dehusker, and was treated with 0.2 % bavistin for two hours. Seeds were washed thrice with RO-system purified water followed by surface sterilization in 0.1% mercuric chloride for 8-10 minutes and again washed thrice with distilled water in laminar cabin. These seeds were placed in petri dish on to the moistened blotting paper for germination. After 3 days of germination, drought stress was induced *in vitro* using PEG (PEG6000) at 0.0 bar, -5.0, -7.5 and -10.0 bars of external water potential which was prepared by dissolving 196, 235 and 289 grams respectively in 1000 ml of distilled water (Hadas, 1976; Sabesan and Saravanan, 2016). After 10 days of PEG treatment, data were recorded at different level of external water potential on seedling traits such as root length, shoot length and root/shoot ratio. Shoot/root ratio (SL/RL) was calculated by measuring the ratio of shoot length over root length. The experiment was designed as factorial randomized design taking first factor as DTLs and second factor as different concentration of PEG treatments. Data analyzed with ANOVA using SPSS 16 software (SPSS *Inc*.). Further, least significant difference (LSD) among means was calculated at 0.05 level of significant.

For molecular characterization, genomic DNA was extracted from leaves of 10 days seedling from all DTLs and checks by modified CTAB method (Keb-Llanes *et al.,* 2002). The quality and quantity of DNA was checked in 0.8% agarose gel stained with ethidium bromide and UV spectroscopy using a Nano Drop Spectrophotometer. Eight SSR markers associated with drought tolerance QTLs were evaluated for screening of 55 DH lines derived from cross Swarna×IR159B (Table-1). Amplification reactions was carried out on thermal cycler (Applied Biosystems) by preparing 20µl final volume reaction containing 50 ng template DNA and EmeraldAmp GT PCR Master Mix (Takara Bio) (Table-1). This master mix includes an optimized buffer, PCR enzyme (2 U/µl), dNTP mixture (10 mM), gel loading dye (green), and a density reagent in a 2X premix format. The PCR condition were: denaturation at 94°C for 3 minutes,

Table 1: List of SSR markers associated with drought tolerance QTL

SN	Marker	Chr no.	Primers (5’ →3’)	Product size (bp)	Position (cM)
1	RM259	1	F: TGGAGTTTGAGAGGAGGG R: TGGAGTTTGAGAGGAGGG	162	54.2
2	RM472	1	F: CCATGGCCTGAGAGAGAGAG R: AGCTAAATGGCCATACGGTG	296	171.6
3	RM2634	2	F: GATTGAAAATTAGAGTTTGCAC R: TGCCGAGATTTAGTCAACTA	154	80.95
4	RM55	3	F: CCGTCGCCGTAGTAGAGAAG R: TCCCGGTTATTTTAAGGCG	226	168.2
5	RM451	4	F: GATCCCCTCCGTCAAACAC R: GATCCCCTCCGTCAAACAC	207	115.5
6	RM553	9	F: AACTCCACATGATTCCACCC R: GAGAAGGTGGTTGCAGAAGC	162	76.7
7	RM215	9	F: GAGAAGGTGGTTGCAGAAGC R: TGAGCACCTCCTTCTCTGTAG	148	99.4
8	RM271	10	F: TCAGATCTACAATTCCATCC R: TCGGTGAGACCTAGAGAGCC	101	59.4

35 cycles of denaturation for 45 sec at 94°C, annealing for 30 seconds at 50°C followed by 30 sec at 72°C and final elongation at 72°C for 10 minutes. The PCR products were detected using 1.5% agarose gel electrophoresis along with 100bp ladder and visualized by ethidium bromide staining under Gel documentation unit (Biorad). The genotypic data of DRL population were scored as “R” for parent 2 like bands *viz.*, IR-159B and “S” for parent 1 like band (Swarna). The scores were then used for analysis using SPSS 16.0 (*SPSS Inc.*) for associating markers (RM55, RM259) with the shoot/ root ratio of selected DRLs showing drought tolerance at 7.5 bar water potential treatment.

Results and Discussion

The phenotypic observations were recorded based on growth of root, shoot length in centimetres and root/shoot length ratio after 10days of PEG treatment by measuring it with scale in population along with parents and check (Fig-1). Data analyzed with ANOVA in factorial completely randomized design using SPSS 16.0 (*SPSS Inc.*) to test for significant difference among DRLs (G), PEG treatment (T) and their interactions (G×T). In the study, significant differences were observed for the seedling parameters (Table 2). Shoot length were found to be significant at 5% level of significance for DRLs, 1% level of significance for PEG treatments and for interaction between DRLs and PEG treatment. Similarly, for root length and shoot/root ration found to be significant at 1% level of significance for DRLs, PEG treatment and their interaction. Similarly, Akte *et al.*, 2016 reported significant difference for seedling parameters, different PEG treatment and interaction between them in rice. Khakwani *et al.*, 2011, Mansour and Elbagrmi, 2019 reported significant differences for wheat genotypes and PEG treatment.

Table 2: Analysis of variance for seedling trait of 55 DH rice lines during drought induced by polyethylene glycol (PEG)

Source	DF	Mean Square		
		Shoot length (cm)	Root length (cm)	Shoot/root ratio
DH rice line (G)	54	1.90^{*}	17.58^{**}	0.404^{**}
Treatment (T)	3	858.94^{**}	1580.039^{**}	21.89^{**}
Interaction (G × T)	162	1.38^{**}	4.52^{**}	0.16^{**}
Error	440	0.043	0.073	0.004

** **Significant at 1 % probability levels,** ***Significant at 5% level of significance**

Shoot response under different PEG concentration

The shoot bears most economic part of the crop and is an important parameter while selecting the superior genotypes against drought. Shoot length of 55 DRLs along with parents and check varieties were measured from the root base to the tip of the shoot after 10 days of PEG treatment at different concentration. In the study, it was observed that relative to the control, increasing PEG concentration steadily reduced shoot length (Table-

3). With the increase in moisture stress level using different concentration of PEG (-5 bar, -7.5 bar, -10 bar), mean shoot length found to be decreased by 68 % at -5 bar, 95 % at -7.5 bar and 99 % at -10 bar of external water potential with respect to control (0 bar) in DRL (Fig-2). Out of the 55 DRLs, 14 DRLs *i.e*., DRL-4 (0.53cm), DRL-6 (1.06cm), DRL-14 (0.66cm), DRL-18 (0.58cm), DRL-22 (1.03cm), DRL-23 (0.35cm), DRL-31 (0.61), DRL-36 (0.28cm), DRL-48 (1.06cm), DRL-49 (0.5cm), DRL-50 (1cm), DRL-51 (0.56cm), DRL-53 (0.26cm), DRL-55 (0.53cm) along with drought tolerant check (Dagaddeshi:1.2cm, RRF-127:1.3cm) and parent (IR159B:1.1cm) shown average shoot length at -7.5 bar external water potential. Also, DRL-10 (0.22cm) and DRL-42 (0.55cm) shown average shoot length at -10 bar external water potential. The lines showing shoot response together with drought tolerant check (Dagaddeshi and RRF-27) and parent (IR-158) at -7.5 bar and -10 bar moisture stress induced by PEG indicate drought tolerance. Shoot length of 24 DRLs along with drought susceptible check (Danteshwari, MTU1010) and parent (Swarna) showed response in -5 bar external water potential. Rest of the lines didn't produce any kind of shoot growth indicate drought susceptibility. Previous studies on PEG treatment in different crops reported that the increase in external water potential leads to the decrease in shoot length when compared with control (Jajarmi, 2009; Khakwani *et al.,* 2011; Govindaraj *et al.,* 2010; Sabesan and Saravanan, 2016, Mansour and Elbagrmi, 2019). Masour and Elbagrmi, 2019 reported slight increase in shoot length at level of -3 bar in 3 wheat cultivars and then reduced with the increase in PEG concentration. Similarly, Akte *et al*., 2016 reported decrement in shoot length from 15.76 cm in control to 15.76 cm in 4% PEG concentration in rice varieties. Decrement in shoot length with the increase in PEG concentration is due to decrease in turgor pressure resulting in reduced cell division and cell elongation (Lagerwerff *et al.,* 1961; Chandra, 2011; Nurhayati *et al*., 2017; Akte *et al.,* 2016; Sabesan and Saravanan, 2016). The water scarcity can in induced by PEG because it may cause effect on metabolic processes of plant *via* preventing nutrients transfer (Chandra, 2011; Govindaraj *et al.,* 2010). In contrast the normal shoot growth is also reported in the presence of high concentration of PEG (Purbajanti *et al.,* 2019; Hellal *et al.,* 2018). Similarly, in the present study 14 DRLs found to sustaining upto -7.5 bar PEG treatment. This may be due to increase in proline content resulting into drought tolerant genotype (Nurhayati *et al*., 2017; Kadir, 2007).

Root response under different PEG concentration

Roots are also an important seedling trait responsible for perceiving and transducing of water deficit signals to shoot which further triggers an array of physiological, morphological and molecular responses in the whole plant (Moumeni *et al.,* 2011). This combination of rapid sensing and signaling on both cellular and organ level enable the plant to tolerate water loss and thus survive in drought stress condition (Robbins and Dinneny, 2015). Root length of 55 DRLs along with parents and check varieties were measured from the root base to the tip of the root after 10 days of PEG treatment at different concentration. It was observed that the root length also declined with increased external water potential and consequently, all PEG treatments caused a decrease in root elongation in all DRL compared to their controls (Table-3). With the increase in external water potential using different concentration of PEG (-5 bar, -7.5 bar, -10 bar), mean root length found to be decreased by 21% at -5 bar, 59% at -7.5 bar and 96% at -10 bar external water potential with respect to control (0 bar) in DH rice lines (Fig-2). Among 55 DTL, 34 DTL showed root growth ranging from 1.05 cm (DTL-19) to 7.17cm (DTL-50) followed by 6.56cm (DTL-43) at -7.5 bars of PEG treatment. At -10 bar of PEG treatments, 17 DTL showed root growth ranging from 0.22cm (DTL-22) and 1.99cm (DTL-6). The lines showing root response together with drought tolerant check (Dagaddeshi and RRF-27) and parent (IR-158) at -7.5 bar and -10 bar of external water potential induced by PEG indicate drought tolerance. Similar to present study, several studies reported that the increased in PEG concentration leads to the root length deployment when compared with control (Jajarmi, 2009; Khakwani *et al.,* 2011; Govindaraj *et al.,* 2010; Sabesan and Saravanan, 2016; Akte *et*

Table 3: Effect of moisture stress on seedling traits of rice DH lines during drought induced by PEG

SN	**DH lines**	**Shoot length (cm)**				**Root length (cm)**				**Shoot/root ratio**			
		External water potential (Bars)				**External water potential (Bars)**				**External water potential (Bars)**			
		Control	**-5**	**-7.5**	**-10**	**Control**	**-5**	**-7.5**	**-10**	**Control**	**-5**	**-7.5**	**-10**
C1	DAGADDESHI	8.56	5.10	1.2	0.00	4.16	3.21	1.6	0.93	2.06	1.59	0.75	0.00
C2	RRF-127	7.11	4.20	1.3	0.00	6.37	6.01	1.67	1.12	1.12	0.70	0.78	0.00
C3	DANTESHWARI	7.06	3.20	0.00	0.00	4.23	3.00	0.00	0.00	1.67	1.07	0.00	0.00
C4	MTU1010	7.02	4.18	0.00	0.00	4.77	3.37	0.49	0.00	1.47	1.24	0.00	0.00
P1	SWARNA (SW)	5.32	3.60	0.00	0.00	5.30	2.61	0.56	0.00	1.00	1.38	0.00	0.00
P2	IR-159B (IR)	8.55	4.90	1.10	0.00	7.81	5.23	1.04	0.44	1.09	0.94	1.06	0.00
1	DRL-1	5.33	1.20	0.00	0.00	6.38	3.34	2.23	0.00	0.84	0.36	0.00	0.00
2	DRL-2	5.20	2.17	0.00	0.00	6.37	4.12	2.04	0.00	0.82	0.53	0.00	0.00
3	DRL-3	5.48	1.78	0.00	0.00	6.78	8.24	1.45	0.00	0.81	0.22	0.00	0.00
4	DRL-4	4.54	1.27	0.53	0.00	6.31	7.62	5.5	1.23	0.72	0.17	0.10	0.00
5	DRL-5	4.44	0.47	0.00	0.00	7.13	4.50	3.25	0.00	0.62	0.10	0.00	0.00
6	DRL-6	4.20	1.56	1.06	0.00	6.58	6.45	4.56	1.99	0.64	0.24	0.23	0.00
7	DRL-7	4.53	2.10	0.00	0.00	7.51	6.51	3.14	0.00	0.60	0.32	0.00	0.00
8	DRL-8	6.04	1.42	0.00	0.00	8.05	5.26	2.3	0.55	0.75	0.27	0.00	0.00
9	DRL-9	6.61	1.62	0.00	0.00	9.07	7.13	3.21	0.00	0.73	0.23	0.00	0.00
10	DRL-10	5.70	2.08	1.09	0.22	8.49	6.3	5.23	1.32	0.67	0.33	0.21	0.17
11	DRL-11	5.48	5.08	0.00	0.00	7.00	5.22	3.43	0.00	0.78	0.97	0.00	0.00
12	DRL-12	6.66	3.01	0.00	0.00	7.56	8.13	6.15	0.00	0.88	0.37	0.00	0.00
13	DRL-13	4.40	2.03	0.00	0.00	4.16	3.54	2.93	0.23	1.06	0.57	0.00	0.00
14	DRL-14	4.68	2.06	0.66	0.00	6.21	5.23	2.28	1.20	0.75	0.39	0.29	0.00
15	DRL-15	5.11	2.96	0.00	0.00	11.15	8.56	2.96	0.00	0.46	0.35	0.00	0.00
16	DRL-16	4.22	0.53	0.00	0.00	7.45	5.06	3.34	0.00	0.57	0.10	0.00	0.00
17	DRL-17	4.06	0.00	0.00	0.00	5.22	4.26	1.11	0.00	0.78	0.00	0.00	0.00
18	DRL-18	4.78	2.63	0.58	0.00	3.16	1.54	0.00	0.00	1.51	1.71	0.00	0.00
19	DRL-19	5.10	1.02	0.00	0.00	5.96	2.41	1.05	0.00	0.86	0.42	0.00	0.00
20	DRL-20	3.96	0.00	0.00	0.00	5.20	3.56	3.02	0.00	0.76	0.00	0.00	0.00
21	DRL-21	6.24	1.17	0.00	0.00	11.66	6.94	1.52	0.00	0.54	0.17	0.00	0.00
22	DRL-22	5.33	2.81	1.03	0.00	10.28	5.7	1.51	0.22	0.52	0.49	0.68	0.00
23	DRL-23	4.92	1.07	0.35	0.00	5.86	3.26	0.00	0.00	0.84	0.33	0.00	0.00
24	DRL-24	4.93	1.07	0.00	0.00	3.87	7.46	3.02	0.00	1.27	0.14	0.00	0.00
25	DRL-25	3.72	0.00	0.00	0.00	3.69	2.72	0.83	0.00	1.01	0.00	0.00	0.00
26	DRL-26	3.49	0.00	0.00	0.00	7.74	7.79	1.92	0.00	0.45	0.00	0.00	0.00
27	DRL-27	3.36	2.86	0.00	0.00	6.68	3.45	0.00	0.00	0.50	0.83	0.00	0.00

28	DRL-28	4.98	0.00	0.00	0.00	8.84	5.56	2.6	0.00	0.56	0.00	0.00	0.00
29	DRL-29	5.15	0.00	0.00	0.00	7.56	5.36	4.15	1.20	0.68	0.00	0.00	0.00
30	DRL-30	5.26	2.76	0.00	0.00	7.64	3.62	3.00	0.00	0.69	0.76	0.00	0.00
31	DRL-31	4.44	2.13	0.61	0.00	6.96	4.23	2.56	0.56	0.64	0.50	0.24	0.00
32	DRL-32	3.88	0.74	0.00	0.00	5.95	4.23	2.69	0.44	0.65	0.17	0.00	0.00
33	DRL-33	5.99	0.00	0.00	0.00	6.39	6.51	2.32	0.00	0.94	0.00	0.00	0.00
34	DRL-34	6.02	1.33	0.00	0.00	8.86	5.23	2.45	0.00	0.68	0.25	0.00	0.00
35	DRL-35	5.62	0.00	0.00	0.00	8.72	5.63	2.15	0.00	0.64	0.00	0.00	0.00
36	DRL-36	5.08	3.38	0.28	0.00	10.37	6.25	4.26	1.34	0.49	0.54	0.07	0.00
37	DRL-37	4.92	0.00	0.00	0.00	9.07	5.94	5.73	0.00	0.54	0.00	0.00	0.00
38	DRL-38	4.19	0.00	0.00	0.00	7.22	5.52	4.90	0.00	0.58	0.00	0.00	0.00
39	DRL-39	4.54	0.50	0.00	0.00	5.57	7.86	4.88	0.00	0.82	0.06	0.00	0.00
40	DRL-40	5.26	1.54	0.00	0.00	10.66	9.42	3.70	0.56	0.49	0.16	0.00	0.00
41	DRL-41	3.84	2.46	0.00	0.00	8.88	6.83	3.75	0.00	0.43	0.36	0.00	0.00
42	DRL-42	4.68	2.48	1.28	0.55	9.85	6.42	5.60	1.56	0.48	0.39	0.23	0.35
43	DRL-43	4.53	2.25	0.00	0.00	8.02	7.08	6.56	0.00	0.56	0.32	0.00	0.00
44	DRL-44	5.32	1.20	0.00	0.00	4.86	6.37	4.16	0.00	1.09	0.19	0.00	0.00
45	DRL-45	5.57	0.00	0.00	0.00	6.98	5.23	2.52	0.00	0.80	0.00	0.00	0.00
46	DRL-46	5.06	2.59	0.00	0.00	7.42	5.33	0.00	0.00	0.68	0.49	0.00	0.00
47	DRL-47	5.15	2.03	0.00	0.00	7.23	7.59	3.44	0.00	0.71	0.27	0.00	0.00
48	DRL-48	5.24	2.60	1.06	0.00	9.04	7.28	6.65	0.92	0.58	0.36	0.16	0.00
49	DRL-49	5.54	2.15	0.50	0.00	9.16	8.2	7.17	1.00	0.60	0.26	0.07	0.00
50	DRL-50	6.03	1.56	1.00	0.00	9.16	7.95	6.14	1.00	0.66	0.20	0.16	0.00
51	DRL-51	5.24	1.36	0.56	0.00	5.18	4.23	2.14	0.00	1.01	0.32	0.22	0.00
52	DRL-52	4.51	1.88	0.00	0.00	7.99	6.23	2.23	0.00	0.56	0.30	0.00	0.00
53	DRL-53	4.36	1.38	0.26	0.00	7.99	7.47	5.10	0.50	0.55	0.18	0.05	0.00
54	DRL-54	3.47	2.96	0.00	0.00	8.06	7.41	4.15	0.00	0.43	0.40	0.00	0.00
55	DRL-55	4.35	3.03	0.53	0.00	7.98	7.12	5.06	0.00	0.55	0.43	0.10	0.00
Treatment mean (55 lines)		4.92	1..57	0.21	0.01	7.40	5.83	3.24	0.29	0.71	0.30	0.05	0.01
		Mean Standard Error											
	Rice DH lines(G)	0.059				0.077				0.059			
	PEG treatment (T)	0.016				0.021				0.0004			
	Interaction (G × T)	0.119				0.155				0.036			
		LSD (P > 0.05)											
	Rice DH lines(G)	0.169				0.221				0.025			
	PEG treatment (T)	0.072				0.094				0.022			
	Interaction (G × T)	0.334				0.435				0.101			

al., 2016; Ghosh *et al.,* 2020). For instance, Akte *et al*., 2016 observed the decrement in root length of rice genotypes from 5.022 cm in control to 3.898 cm in 4% PEG concentration. Similarly, Wickramasinghe and Seran, 2019 reported the declining of root length in tomato seedling with the increasing PEG concentration.

Effect of water stress on shoot/root ratio

In addition to root length and shoot length, shoot to root ratio plays a major role in selecting drought tolerant lines. The interdependence of shoot and root is required for the optimal growth and development of the crop. The shoot relies on the root for water, nutrients and mechanical support while the roots depend on the shoot for organic nutrients (Hoad *et al.,* 2001). The shoot to root ratio reflects shoot and root growth patterns of crop under drought stress. A high shoot to ratio means high shoot growth and lower shoot-root ratio mean comparatively high root growth. In the study, DRLs under control (0 bar) has highest shoot/root ratio in DRL-19 (1.51) and lowest shoot/root ratio in DRL-54 (0.43) (Table-3). With the increase in external water potential using PEG treatment (-5 bar, -7.5 bar and -10 bar), we observed decrement in shoot/root ratio by 57 % at -5 bar, 93% at -7.5 bar and 98% at -10 bar of PEG concentration compared to control (Fig-2). Among 55 DRLs, 12 lines DRL-4 (0.10), DRL-6 (0.23), DRL-14 (0.29), DRL-22 (0.68), DRL-31 (0.24), DRL-36 (0.07), DRL-48 (0.16), DRL-49 (0.07), DRL-50 (0.16), DRL-51 (0.22), DRL-53 (0.05), DRL-55 (0.10) shown average shoot/root ratio at -7.5 bar of external water potential induced by PEG. DH lines DRL-10 (0.17) and DRL-42 (0.35) shown average shoot/root ratio at -10 bar of external water potential induced by PEG. The lines showing response were together with the drought tolerant check and parent indicates drought tolerance. Similarly, Thabet *et al*., 2018 reported decrement by increasing the PEG concentration from 1.01 cm in control to 0.77 cm for shoot to root ratio in barley genotypes. A decrease in shoot/root ratio under PEG-induced external water potential indicates that PEG induced osmotic stress positively influence drought growth compared to shoot growth.

Molecular analysis of DTLs using SSR marker

Plant responses to stress factors can be considered on a variety of levels of their organization, beginning with the molecular background (through cells and organs) and ending at the whole plant. Selection for drought tolerant lines based on phenotypic traits may be accelerated by using molecular markers associated with trait. The recent identification of major QTLs governing grain yield under drought has made possible the use of marker assisted selection (MAS) for improving drought tolerance in rice. In the present study, genotyping of 55 DTLs of Swarna × IR-159 B were performed using 8 SSR marker linked to drought tolerance (Table-1). Out of the 8 SSR markers used, two of them namely, RM55 and RM259 were found to exhibit polymorphism among parents of DTL. Here, Swarna like alleles were designated as “P1” and IR-159 B like alleles were designated as “P2” (Fig-3). The lower band (226 bp) was observed in IR159B (drought tolerant) and upper band (290bp) was observed in Swarna (drought susceptible) for RM55. Among DTLs, banding pattern showing 61.8% alleles (34 lines) were like Swarna and 38.2% allele (21 lines) were like IR159-B. Similarly, RM259 marker showing lower band (162 bp) observed in IR159B and upper band (210 bp) was observed in Swarna. Here, banding pattern

Table 4: Genotypic data of DH rice lines sustaining at -7.5 bar PEG treatment

SN	DH lines	Shoot/root ratio	Genotyping	
		PEG treatment (-7.5 bar)	RM55	RM259
P1	Swarna	0.00	P1	P1
P2	IR-159 B	1.06	P2	P2
1	DRL-4	0.10	2	2
2	DRL-6	0.23	2	1
3	DRL-10	0.17	2	2
4	DRL-14	0.29	2	2
5	DRL-22	0.68	1	1
6	DRL-31	0.24	2	2
7	DRL-36	0.07	2	2
8	DRL-42	0.23	1	1
9	DRL-48	0.16	2	2
10	DRL-49	0.07	2	2
11	DRL-50	0.16	2	2
12	DRL-51	0.22	2	1
13	DRL-53	0.05	1	1
14	DRL-55	0.10	1	1

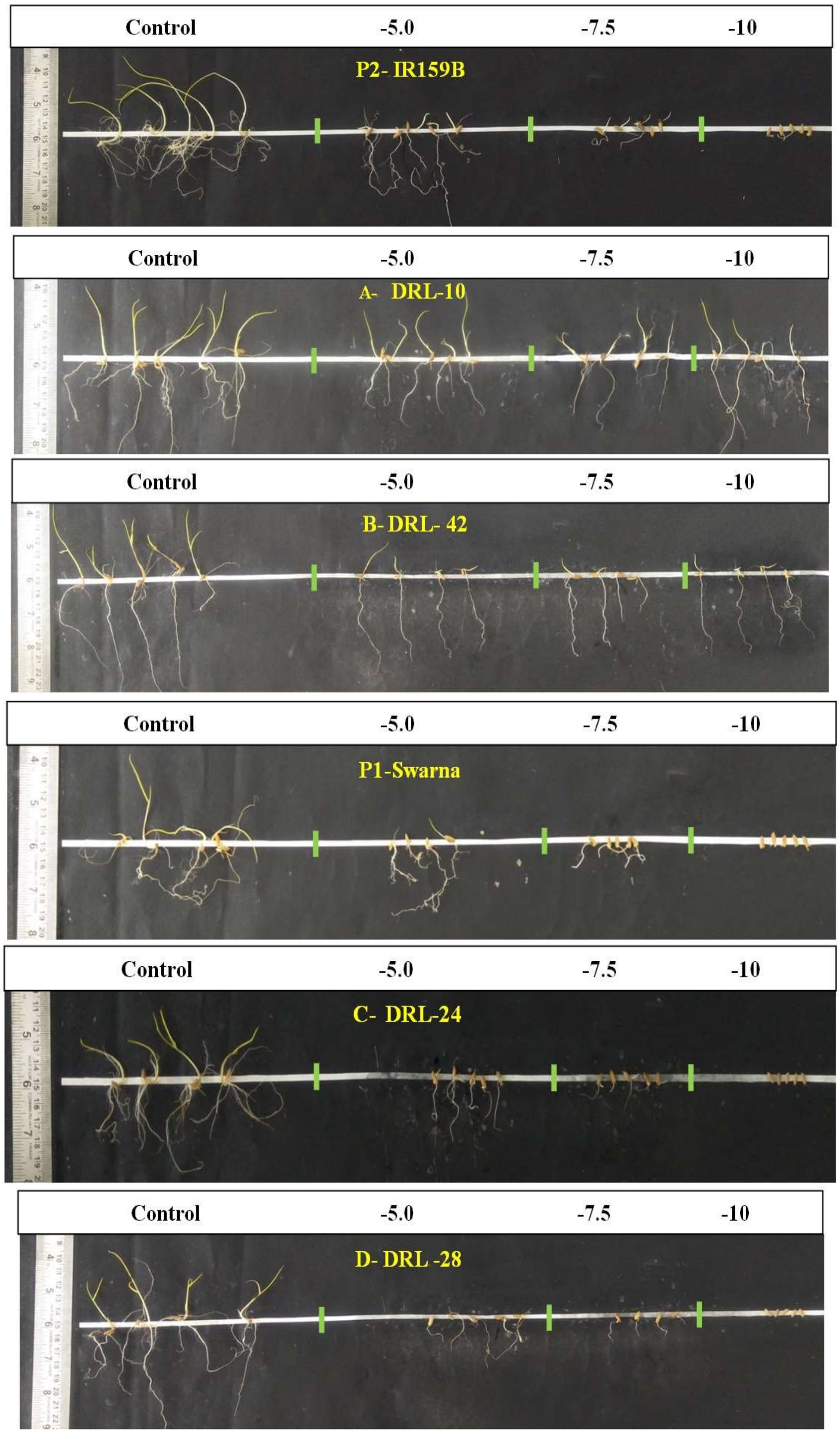

Figure 1: Response of seedling trait (Shoot and root length) for increased external water potential (0, -5.0, -7.5, -10 bar) using PEG.

Legends: P2- IR159B: Drought tolerant parent (showing shoot growth upto -7.5 bar and root growth upto -10 bar , A- DRL-10 indicating tolerance (showing shoot and root growth upto -10 bar), B- DRL-42 indicating tolerance (showing shoot and root growth upto -10 bar), P1- Swarna: Drought susceptible parent (showing shoot growth upto -5 bar and root growth upto -7.5 bar), C-DRL-24 indicating susceptibility (showing root growth upto -5 bar and root growth upto -7.5 bar), D-DRL-28 indicating susceptibility (showing shoot growth upto -5 bar and root growth upto 7.5 bar).

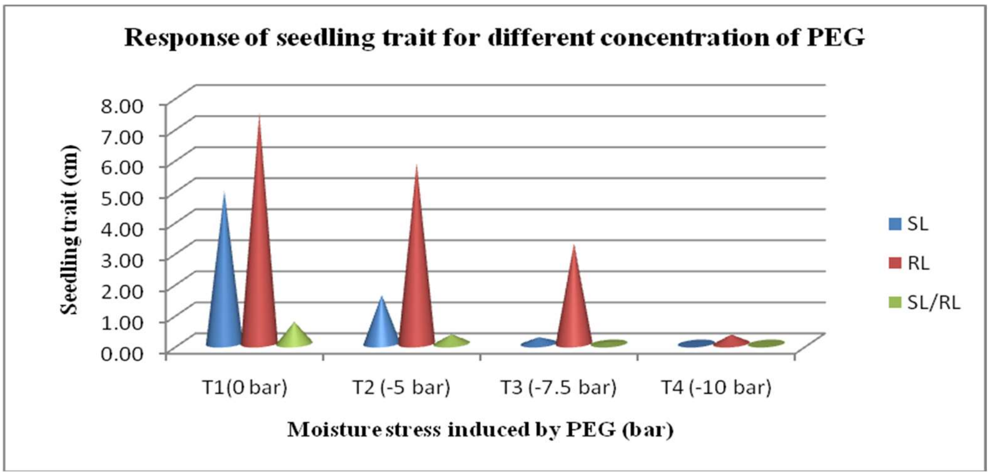

Figure 2: Effect of different levels of PEG induced water stress on 55 DH rice lines for seedling traits (SL-Shoot length, RL-Root length, SL/RL- Shoot-root ratio)

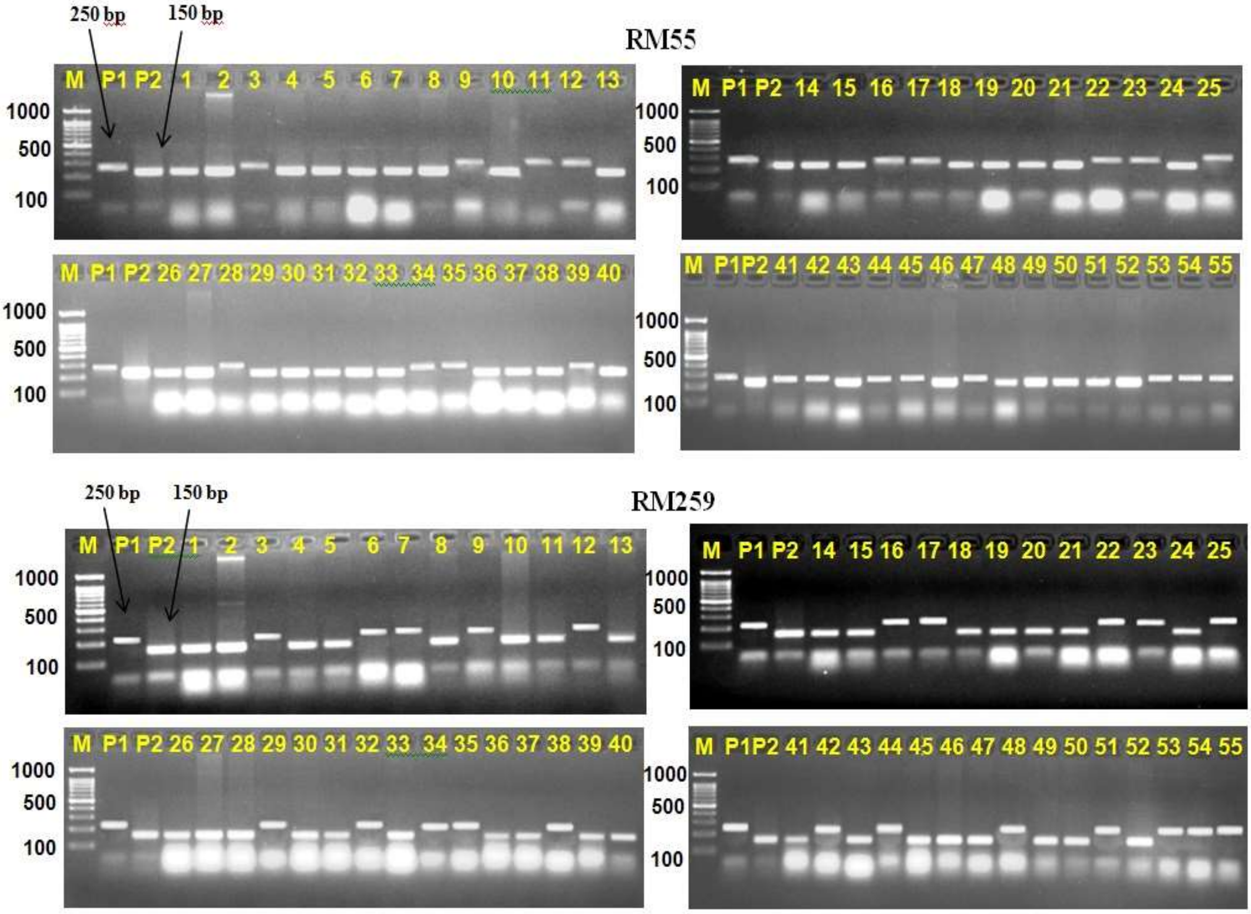

Figure 3: Molecular analysis of 55 DH rice lines derived from cross Swarna×IR159B

Legends: M: 100 bp ladder, P1: Swarna, P2: IR159B, 1-55: DH rice lines, SSR marker showing 290 bp (RM55) and 220bp (RM259) bands for Swarna like alleles indicating drought susceptible lines and 226bp (RM55) and 162bp (RM259) for IR159B like alleles indicating drought tolerant lines.

60% alleles (33 lines) were like Swarna and 40% alleles (22 lines) were like IR159B. Further, the scores generated in DRLs derived from cross Swarna and IR159B were used to find the association if any, with shoot/root ratio measured at -7.5 bar of external water potential induced using PEG. To study the association, 14 selected DRLs showing drought tolerance at -7.5 bar of external water potential were taken and analyzed using SPSS 16.0 software (SPSS *Inc.*) (Table-3). Phenotypic variance exhibited by RM55 is 13.5% (P value: 1.788E-10**, R^2: 0.135) and RM259 is 13.9% (P value: 1.267E-09**, R^2:0.138) for 7.5 bar stress level at 1% level of significance. Out of 14 selected DRLs, genotypic data of 9 DRLs (DRL-4, DRL-6, DRL-10, DRL-14, DRL-31, DRL-36, DRL-48, DRL-49, DRL-50) were found to be significant with the phenotypic data which could be further evaluated for drought tolerant trials in field. RM55 and RM259 marker was reported to be linked to drought tolerance in rice (Venuprasad *et al.,* 2009; Verma *et al.,* 2014, Sahoo *et al.,* 2019;). Wang *et al.* (2005) reported 16 candidate genes between markers RM212 and RM319 have potential role in drought tolerance and may be useful in marker assisted breeding for drought stress. Present investigation also shows the association of *in vitro* PEG screening with drought linked markers in DRLs developed for drought stress. It also indicates genetic stability in developed DRLs which could be utilized for new cultivar development in a short time span.

Conclusion

The study showed that application of PEG at different water potential has negative effect on *in vitro* rice growth due to negative osmotic pressure inside the cell. This inhibits plants to uptake available water to the seed and resulting in drought stress. Among 55 DRLs derived from cross Swarna and IR159B, 9 DRLs performed best by showing the significant seedling growth sustaining at - 7.5 bar external water potential. Drought linked markers *viz.*, RM55 and RM259 found to be reliable based on data generated. The results of the PEG analysis in the study demonstrate the use of PEG at different concentration of external water potential as an effective method for studying the effect of water stress on seed germination and seedling growth characteristics, and adjudged it as a simple cost-effective, time saving method for screening large sets of DH lines/rice lines within a very short period and precision. However, validation under real field conditions is needed to further authenticate these results.

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Conflict of interest

The authors declare that they have no conflict of interest.

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