Received: 13 Aug 2020 | Revised: 24 Sep 2020 | Accepted: 24 Sep 2020

Electronic Journal of Plant Breeding

Research Article

Evaluation of Backcross Inbred Lines (BILs) introgressed with drought tolerant QTLs using Polyethylene Glycol (PEG) induced water stress in rice

Himanshi Swain[1], M. Valarmathi[1], V. Balasubramani[1], N. Manikanda Boopathi[1]* and M. Raveendran[1]

[1]Dept. of Plant Biotechnology, Centre for Plant Molecular Biology and Bioinformatics, Tamil Nadu Agricultural University, Coimbatore 641003
***E-Mail**: nmboopathi@tnau.ac.in

Abstract
Drought is a never-ending climatic vagary that imposes worldwide high impact on crop yield reduction. Plants show a wide range of physiological responses including reduction of seed germination, leaf size and overall plant structure but with increased root proliferation. Incorporation of such traits for drought tolerance improvement in rice is found to be difficult and to evolve a new cultivar, it is essential to employ a rapid screening strategy for those traits. In this study, water stress was induced by using polyethylene glycol (PEG) and its impact on germination percentage and rate, root and shoot length, fresh and dry weight and water content among Backcross Inbred Lines (BILs; BC_2F_6) that were generated from backcrossing of a recurrent parent CBMAS1405 harbouring two drought-tolerant QTLs *qDTY1.1* and *qDTY2.1* with two different donor parents FR13A (*Sub1*) and 562-4 (*Pi9, Gm4, Xa21, xa13*). Four levels of osmotic stress (0 MPa, -0.5 MPa, -0.75 MPa, and -1 MPa) were generated using PEG-6000 and the seeds were grown. Data on the investigated traits indicated that water stress has a strong impact on the performance of the drought susceptible lines. However, the lines viz., 27-1-7-8-65-4-1 and 27-1-7-8-14-4-1, introgressed with target drought tolerance QTLs have shown better performance on par or higher than that of the drought-tolerant donor variety Apo (that harbours *qDTY1.1* and *qDTY2.1*). Thus, this study has helped to rapidly select the drought-tolerant breeding materials introgressed with drought-tolerant QTLs under simple laboratory conditions, and avoid extensive, costlier and laborious screening under field conditions. Though the results have to be confirmed with field studies, it may require lesser inputs and efforts, as the PEG screening strategy drastically reduced the sample size.

Key words
Drought stress, Rice, IWP, Apo, *qDTY1.1*, *qDTY2.1, PEG-6000*

INTRODUCTION

The crop yield loss by the end of 2030, will increase to around $7 billion with an annual global yield loss of 50 per cent due to major abiotic stress (Neog *et al.*, 2020, Verma and Deepti, 2016). Among the abiotic stresses, drought is one of the most persistent climatic vagaries affecting 40 per cent to 60 per cent of global agricultural land (Mollasadeghi *et al.*, 2011). In India, the world's second-largest populated country, 60-70% of the total population directly or indirectly depends on agriculture for their livelihood (Mekala and Viswanathan, 2017). Of the 160 million hectares of cultivated lands in India, 60 per cent of land is under the irrigated condition which includes larger areas of western and central India (90 per cent of South-Western monsoon) and southern and north-western India (50-75 per cent of their total annual precipitation) and the rest is under rainfed condition. Due to rapid climate change over the last few decades, India has experienced heavy and prolonged drought conditions in the year 1967, 1968, 1972, 1974, 1979, 1982, 1987, 2002, 2009 with a

https://doi.org/10.37992/2020.1103.145

production loss of 21.50 million tons (MT) in 2002 and 10.02 MT in 2009 (Ministry of Agriculture, 1988); (Mekala and Viswanathan, 2017, Swapna and Shylaraj, 2017). With rapid climate change pattern, increase in population, recurrent drought condition which cause 50% of rice yield, the identification of drought-resistant varieties are of high demand.

Drought stress creates a dramatic effect on the rice plant germination and growth. Though water stress leads to delay in the time of flowering, decreases the flowering rate, grain filling, and subsequently affect the gain production (Mahla *et al.*, 2017), the most vulnerable growth stage affected by water stress is the seed germination and development of primary seedling (Ahmad *et al.*, 2009). The quantity of leaves per plant, leaf size and photosynthesis rate decreases rapidly if the plant continues to witness drought stress at vegetative stage (Ahmad *et al.*, 2009, Zhang *et al.*, 2006). Therefore, it is essential to identify the early drought-resistant rice lines and use them in the further breeding procedure. To this end, various methods have been employed for artificial screening for selecting drought-resistant plants. Polyethylene Glycol (PEG), mannitol and sorbitol that are known to cause osmotic stress, have been frequently used for artificial water stress screening (Molnár *et al.*, 2004, Slama *et al.*, 2007). Among them, PEG with a molecular mass of 6000 and above have successfully been shown for inducing osmotic stress that mimics dry soil (Khakwani *et al.*, 2011). PEG is non-ionic, non-toxic, water-soluble polymers which form a hydrogen bond with water and decrease the water potential of the culture medium without causing any physiological damage to the seed (Lu and Neumann, 1998). Further, the larger molecular size of PEG prevents its absorption by the plant. Hence, PEG is considered as a better chemical in inducing artificial stress than any other chemicals (Tripathy Swapan, 2015). It has also been established that PEG significantly affects the photosynthetic rate of the plant as chlorophyll-a and chlorophyll-b content is highly affected (Jnandabhiram and Sailen Prasad, 2012).

Quantitative Trait Loci (QTL) mapping has shown its potential in identifying the genomics regions governing the drought tolerance and precisely interrogating them using marker assisted selection (Boopathi, 2020). Several numbers of QTLs that were known to control drought tolerance such as *qDTY12.1*(Bernier *et al.*, 2007), *qDTY2.1* and *qDTY3.1* (Venuprasad *et al.,* 2009), *qDTY1.1* (Vikram *et al.*, 2011) have been identified in rice. At this laboratory, the rice improved line, CBMAS1405 containing drought QTLs *qDTY1.1* and *qDTY2.1* was developed and it has been further improved to tolerate multiple stresses by interrogating QTLs that were tolerant to submergence, bacterial leaf blight, blast and gall midge through marker assisted backcross breeding (Valarmathi, 2019). The present study was conducted to evaluate those Backcross Inbred Lines (BILs) that were introgressed with drought-resistant QTLs *qDTY1.1* and *qDTY2.1* under PEG induced water stress.

MATERIALS AND METHODS

This study was conducted as factorial experiment using completely randomized design with three replications. The first factorial includes rice accessions (viz., recurrent parent (CBMAS14065), donor parents Apo and Improved White Ponni (IWP) and ten Backcross Inbred Lines (27-1-7-8-22-4-1, 27-1-7-8-42-1-1, 27-1-7-8-65-4-1, 27-1-7-8-121-2-1, 27-1-7-13-40-1-1, 27-1-7-8-14-4-1, 27-1-2-2-97-1-1, 27-1-2-2-1-3-1, 27-1-2-2-5-4-1, 27-1-2-2-19-2-1 which have shown to possess drought-resistant genes i.e., *qDTY1.1* and *qDTY2.1*). The second factor includes four levels of drought stress (that was induced by four different concentrations of PEG-6000 *viz*., 0 MPa (0 g in 100 ml), -0.5 MPa (19.6 g in 100 ml), -0.75 MPa (23.5 g in 100 ml) and -1 MPa (28.9 g in 100 ml). CBMAS14065 is a hybrid derivative of Improved White Ponni (IWP) and Apo and it contains two major drought-resistant QTLs *viz*. *qDTY1.1* and *qDTY2.1*(Muthu *et al*., 2020).

To examine the impact of drought stress on germination, ten seeds from each of the investigated rice accessions were transferred to labelled petri plates and 8 ml of PEG-6000 solutions of different concentrations were added. Seed germination was observed on 2nd, 4th and 6th day after sowing and germination percentage was calculated (Vikas *et al*., 2009). The seeds that have produced > 2 mm of root length were counted as germinated seeds (Goswami and Baruah, 1994, Hadas, 1976). On the 10th day, the germinated seedlings were collected from each replication and the root and shoot lengths were recorded. The fresh weight of the seedling was measured on 20th day and dry weight was measured after oven drying at 70°C for 48 hours (Hellal *et al*., 2018).

Germination Percentage and Germination Rate

Germination percentage was calculated to estimate the viability of seeds.

$$\text{Germination Percentage} = \frac{\text{Total number of germinated seeds}}{\text{total number of seeds used}} \text{ X } 100$$

Germination rate (provides a measure of the time course of seed germination) was calculated by using the formula suggested by Association of Official Seed Analysis (Aosa, 1983).

$$\text{Germination Rate} = \frac{\text{No of germinated seed}}{\text{day of first count}} + \frac{\text{No of germinated seed}}{\text{day of second count}} + \cdots + \frac{\text{No of germinated seed}}{\text{day of final count}}$$

Water content

The water content (WC) was calculated using the formula provided by Tounekti as (Tounekti *et al*., 2011) :

$$\mathrm{WC} = \frac{FW - DW}{\mathrm{DW}}$$

FW= Fresh weight; DW= Dry Weight
All the collected data from the investigated traits were subjected to simple statistical analysis using Microsoft Excel 2010.

RESULTS AND DISCUSSION

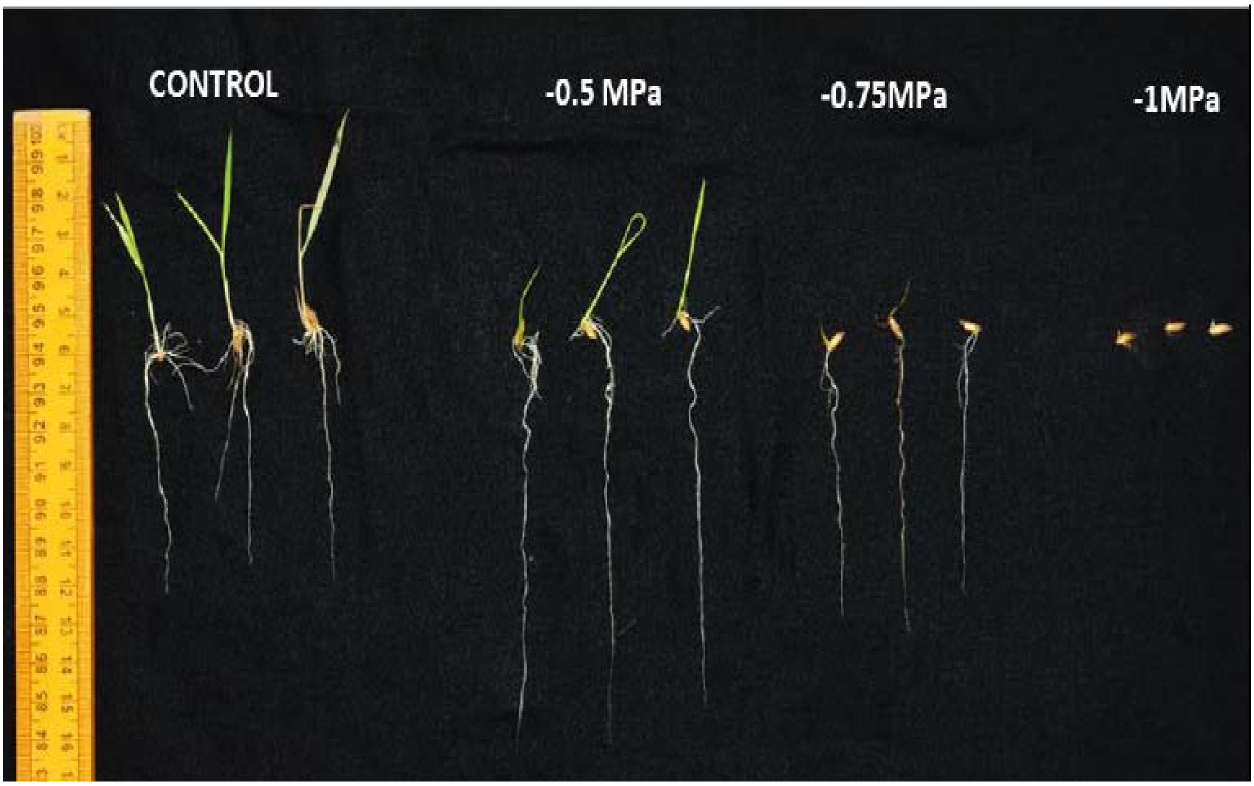
Evaluating the rice accession for better drought tolerance under field condition was found to be cumbersome as controlled and uniform drought stress cannot be implemented in the field conditions. However, by using *in vitro*-drought screening methods (such as use of known concentration of PEG), uniform conditions in terms of water stress can be achieved. Thus, the drought tolerant lines can easily be identified by examining the better germinated lines (Richards, 1978).

Germination Percentage and Germination Rate:Germination is one of the important parameters used to determine the resistance of rice genotype to water stress at the initial growth stage. Seed germination has a negative correlation with PEG concentration. With an increase in PEG concentration, the osmotic stress or water potential increase which adversely affect the seed germination (Islam *et al.*, 2018). The number of germinated seeds was observed on 2nd day, 4th day and 6th day and the germination percentage was calculated along with the germination rate. Table 1 illustrates the effect of PEG concentration on germination percentage. As expected, it was observed that the germination percentage was the highest at control condition (0 MPa) and lowest at -1 MPa. Germination Rate also decreases with an increase in PEG concentration. Lowest Germination Rate was recorded at -1 MPa which is represented in **Table 1**.

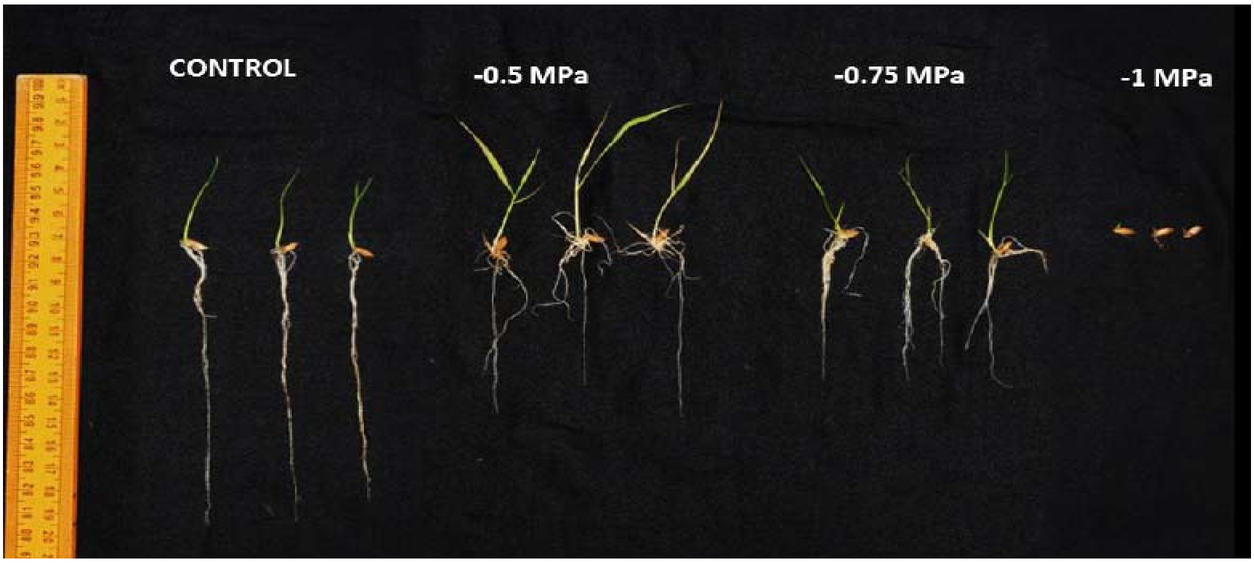
In this experiment, the donor parent Apo is used as a reference drought tolerant line. Apo had highest germination percentage of 97%, 90% and 70% at -0.5 MPa, -0.75 MPa and -1 MPa, respectively followed by the elite parent CBMAS14065 with a germination percentage of 90%, 90% and 67% at -0.5 MPa, -0.75 MPa and -1 MPa. The drought susceptible line, IWP showed incredibly low germination percentage of 80% (-0.5 MPa), 60% (-0.75 MPa) and 13% (-1 MPa) compared to that of Apo and CBMAS14065. Among the ten, five BILs which showed germination percentage close to that of Apo are 27-1-7-8-14-4-1, 27-1-7-8-65-4-1, 27-1-7-8-22-4-1, 27-1-2-2-1-3-1, and 27-1-7-8-42-1-1 (**Table 1**).

Root and shoot length decreased rapidly as PEG concentration increased (**Table 2**). The root length of different investigated rice accessions was significantly affected by an increase in drought stress. Maximum root and shoot length was observed in control condition but at high concentration (-1 MPa) the plumule failed to emerge from the seed and even the radicle size is less (< 0.05 cm) except for Apo (radicle length 0.1 cm) (**Fig. 1**).

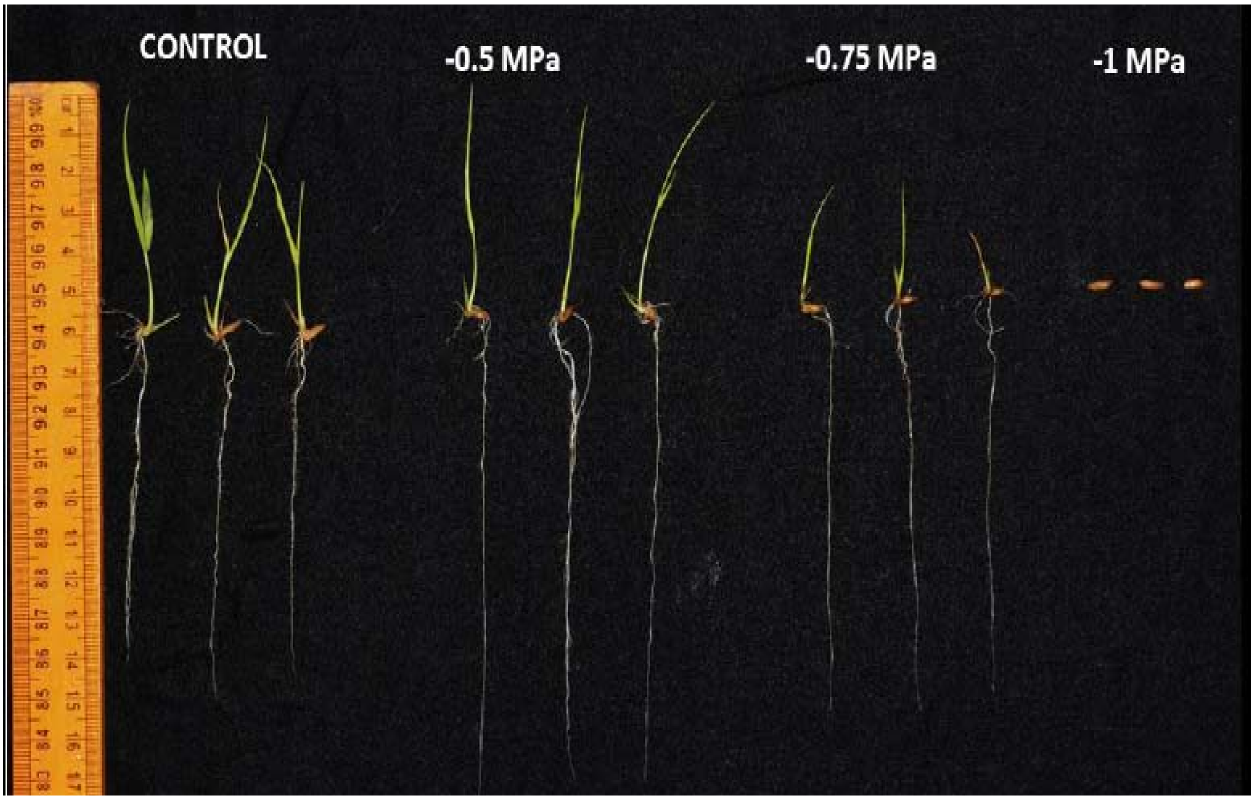
Improved White Ponni



CBMAS14065



Apo



27-1-7-8-14-4-1

27-1-7-8-65-4-1

Fig. 1. Performance of investigated parents and BILs under PEG-6000 simulated water stress

https://doi.org/10.37992/2020.1103.145

Table 1. Germination Percentage and Germination Rate of BILs at different concentration of PEG

	Germination percentage												Germination Rate			
	2nd Day				4th Day				6th Day							
	control	-0.5 MPa	-0.75 MPa	-1 MPa	control	-0.5 MPa	-0.75 MPa	-1 MPa	control	-0.5 MPa	-0.75 MPa	-1 MPa	control	-0.5 MPa	-0.75 MPa	-1 MPa
IWP	93.33±0.3	36.67±0.9	0±0	0±0	93.33±0.3	80±0.29	53.33±0.3	10±0.6	96.7±0.33	80±0.29	60.0±0.58	13.3±0.33	8.61	5.42	2.33	0.47
CBMAS14065	73.33±0.3	3.33±0.3	3.33±0.3	0±0	96.67±0.3	86.67±0.3	83.33±0.9	43.33±2.2	96.7±0.33	90.0±0	90.0±0.58	66.7±1.45	7.69	3.83	3.75	2.19
APO	86.67±0.9	23.33±1.5	6.67±0.3	0±0	100±0	93.33±0.3	90±0.6	50±1.2	100.0±0	96.7±0.33	90.0±0.58	70.0±1.53	8.5	5.11	4.08	2.42
27-1-7-8-22-4-1	70±1	3.33±0.3	0±0	0±0	93.33±0.7	86.67±0.9	60±0.6	50±0.6	93.3±0.67	90.0±0.58	66.7±0.88	53.3±0.88	7.39	3.83	2.61	2.14
27-1-7-8-42-1-1	63.33±0.7	0±0	0±0	0±0	86.67±0.7	93.33±0.3	80±1	3.33±0.3	93.3±0.33	86.7±0.33	83.3±0.67	23.3±0.33	6.89	4	3.39	0.47
27-1-7-8-65-4-1	63.33±1.2	6.67±0.7	10±1	0±0	90±0.6	76.67±0.3	63.33±1.3	33.33±0.9	96.7±0.33	83.3±0.33	83.3±0.88	36.7±0.67	7.03	3.64	3.47	1.44
27-1-7-8-121-2-1	50±1	13.33±1.3	0±0	0±0	90±0.6	86.67±0.7	63.33±1.5	10±0.6	93.3±0.33	90.0±0.58	73.3±1.76	10.0±0.58	6.31	4.33	2.81	0.42
27-1-7-13-40-1-1	60±1.5	16.67±0.9	13.33±0.9	0±0	73.33±1.2	86.67±0.7	70±1	6.67±0.7	93.3±0.33	93.3±0.33	80.0±0.58	13.3±0.67	6.39	4.56	3.75	0.39
27-1-7-8-14-4-1	80±1.2	30±1.5	3.33±0.3	0±0	90±0.6	90±0.6	86.67±0.7	16.67±0.9	100.0±0	100.0±0	90.0±0.58	20.0±1	7.92	5.42	3.83	0.75
27-1-2-2-97-1-1	53.33±0.3	10±0.6	3.33±0.3	3.33±0.3	70±0	56.67±0.7	63.33±0.3	13.33±0.9	93.3±0.33	76.7±0.33	70.0±0.58	13.3±0.88	5.97	3.19	2.92	0.72
27-1-2-2-1-3-1	26.67±0.9	16.67±1.2	0±0	0±0	60±0.6	70±0.6	83.33±1.2	13.33±0.7	93.0±0.33	90.0±0	90.0±0.58	23.3±0.33	4.38	4.08	3.58	0.72
27-1-2-2-5-4-1	80±0	46.67±1.3	13.33±0.7	0±0	90±0.6	90±0.6	90±0.6	13.33±0.3	93.3±0.33	100.0±0	93.3±0.33	16.7±0.33	7.81	6.25	4.47	0.61
27-1-2-2-19-2-1	66.67±0.7	13.33±0.3	6.67±0.7	0±0	83.33±0.3	76.67±0.7	76.67±1.3	3.33±0.3	93.3±0.33	86.7±0.33	80.0±1	13.3±0.33	6.97	4.03	3.58	0.31

Apo showed the highest root length of 6.8 cm, 6.5 cm and 0.1 cm under -0.5 MPa, -0.75 MPa and -1 MPa, respectively and the recurrent parent showed root length of 3.5 cm (-0.5 MPa) and 1.9 cm (-0.75 MPa). The lowest root length was recorded for IWP under -0.5 MPa (3.5 cm) and -0.75 MPa (1.9 cm) (**Table 2**). Similarly in case of shoot length, Apo showed a shoot length of 4 cm (-0.5 MPa) and 4.9 cm (-0.75 MPa) while CBAS14065 showed shoot length of 3.9 cm (-0.5 MPa) and 3.89 cm (-0.75 MPa). Similarly, IWP showed the minimum shoot length of 3.30 cm and 3.10 cm at -0.5 MPa and -0.75 MPa water stress condition respectively. The BILs whose root and shoot lengths were close to that of the donor parent were 27-1-7-8-14-4-1, 27-1-7-8-65-4-1 and 27-1-7-8-22-4-1 (**Table 2**).

Fresh Weight, Dry Weight and Water Content :Drought stress, generally, reduces the water content of a plant which concurrently affects the fresh weight and dry weight. The fresh weight of the resistant cultivar, Apo decreased by 7% and 10% and for CBMAS14065, it decreased by 42% and 48% when exposed to -0.5 MPa and -0.75 MPa, respectively. But the drought susceptible variety IWP showed a maximum decrease in the fresh weight when exposed to -0.5 MPa (63%) and -0.75 MPa (73%) (**Table 3**).

Table 2. Root length (in cm), shoot length (in cm) and root to shoot ratio of BILs at different concentration of PEG

	Root length				Shoot Length				Root:Shoot ratio			
	control	**-0.5 MPa**	**-0.75 MPa**	**-1 MPa**	**control**	**-0.5 MPa**	**-0.75 MPa**	**-1 MPa**	**Control**	**-0.5 MPa**	**-0.75 MPa**	**-1 MPa**
IWP	6.9±0.03	3.5±0.33	1.9±0.26	0.0±0	4.42±0.67	3.30±0.75	3.10±0.15	0.00±0	1.560302	1.060606	0.612903	0
CBMAS14065	6.8±0.22	6.5±0.17	5.9±0.4	0.0±0	3.82±0.11	3.90±0.06	3.89±0.19	0.00±0	1.780105	1.666667	1.51671	0
APO	8.0±0.18	6.8±0.27	7.7±0.65	0.1±0.04	4.30±0.3	4.00±0.07	4.90±0.21	0.00±0	1.860465	1.7	1.575964	0
27-1-7-8-22-4-1	7.5±0.32	6.3±0.32	6.0±0.44	0.0±0	4.51±0.33	3.98±0.07	4.32±0.12	0.00±0	1.662562	1.582915	1.388889	0
27-1-7-8-42-1-1	5.7±1.17	6.3±0.38	4.9±0.15	0.0±0	3.49±0.94	3.90±0.08	3.92±0.08	0.00±0	1.643312	1.615385	1.25	0
27-1-7-8-65-4-1	8.8±0.2	5.8±0.24	4.6±0.09	0.0±0	4.99±0.53	3.99±0.06	3.61±0.26	0.00±0	1.76392	1.453634	1.27116	0
27-1-7-8-121-2-1	7.6±0.3	5.3±0.27	4.4±0.26	0.0±0	4.71±0.47	4.00±0.05	4.10±0.32	0.00±0	1.613208	1.325	1.073171	0
27-1-7-13-40-1-1	8.9±0.2	8.2±0.15	3.2±0.24	0.0±0	5.54±0.16	5.20±0.35	2.50±0.11	0.00±0	1.60521	1.576923	1.28	0
27-1-7-8-14-4-1	7.9±0.32	8.6±1.05	3.5±0.3	0.0±0	4.59±0.59	5.90±0.12	3.90±0.19	0.00±0	1.72155	1.451977	0.897436	0
27-1-2-2-97-1-1	6.8±0.59	5.9±0.65	3.3±0.35	0.0±0	5.01±0.52	3.90±0.16	2.94±0.2	0.00±0	1.363636	1.512821	1.108844	0
27-1-2-2-1-3-1	4.9±0.12	6.5±0.2	4.0±0.45	0.0±0	3.31±0.54	4.90±0.19	3.25±0.29	0.00±0	1.479866	1.326531	1.230769	0
27-1-2-2-5-4-1	5.9±0.55	5.5±0.27	3.1±0.15	0.0±0	3.93±0.18	4.20±0.07	2.78±0.17	0.00±0	1.5	1.309524	1.122302	0
27-1-2-2-19-2-1	8.1±0.30	7.7±1.19	6.5±0.15	0.0±0	5.39±0.37	5.20±0.35	5.50±0.26	0.00±0	1.502783	1.478632	1.181818	0

Table 3. Fresh weight (in gm), dry weight (in gm) and water content of BILs at different concentration of PEG

	fresh weight				dry weight				water content			
	control	**-0.5 MPa**	**-0.75 MPa**	**-1 MPa**	**control**	**-0.5 MPa**	**-0.75 MPa**	**-1 MPa**	**control**	**-0.5 MPa**	**-0.75 MPa**	**-1 MPa**
IWP	109.5±5.5	40.9±1.9	29.4±3.3	0.0±0	31.6±0.86	17.76±0.53	8.53±0.55	0.0±0	2.5	1.3	2.4	0
CBMAS14065	85.83±0.8	49.76±2.2	44.96±2.2	0.0±0	25.3±2.09	19.02±0.43	12.8±0.88	0.0±0	2.4	1.6	2.5	0
APO	106.12±4.1	98.27±1.8	95.92±1.4	0.0±0	35.33±3.48	27.16±2.61	15.77±3.03	0.0±0	2.0	2.6	5.1	0
27-1-7-8-22-4-1	80.9±2.9	68.8±3.7	53.9±1.6	0.0±0	24.31±2.84	18.32±2.21	12.32±1.48	0.0±0	2.3	2.8	3.4	0
27-1-7-8-42-1-1	82.24±0.7	54.58±0.5	43.8±1.4	0.0±0	28.89±2.05	20.33±2.48	14.53±5.40	0.0±0	1.8	1.7	2.0	0
27-1-7-8-65-4-1	87.64±1.8	51.54±0.6	46.23±1.4	0.0±0	25.52±2.28	19.63±1.70	15.43±4.85	0.0±0	2.4	1.6	2.0	0
27-1-7-8-121-2-1	93.46±1.9	42.09±0.6	39.34±1.7	0.0±0	24.59±1.36	16.48±1.04	11.43±1.61	0.0±0	2.8	1.6	2.4	0
27-1-7-13-40-1-1	89.52±0.5	67.33±1	46.74±1.8	0.0±0	27.56±4.08	18.51±2.78	12.5±3.62	0.0±0	2.2	2.6	2.7	0
27-1-7-8-14-4-1	92.61±1.3	47.4±2.7	41.88±0.3	0.0±0	31.22±4.77	21.24±2.18	16.8±0.90	0.0±0	2.0	1.2	1.5	0
27-1-2-2-97-1-1	97.28±2.1	87.98±1	69.18±0.5	0.0±0	28.52±3.85	21.83±5.72	12.18±2.30	0.0±0	2.4	3.0	4.7	0
27-1-2-2-1-3-1	85.06±0.4	54.99±0.8	43.53±2.7	0.0±0	32.07±2.19	22.02±3.02	14.31±0.98	0.0±0	1.7	1.5	2.0	0
27-1-2-2-5-4-1	89.17±2.1	59.57±1.9	44.17±0.8	0.0±0	31.87±1.00	23.5±1.21	15.21±3.99	0.0±0	1.8	1.5	1.9	0
27-1-2-2-19-2-1	90.67±2.2	58.56±0.4	42.02±0.8	0.0±0	22.76±4.50	15.68±2.95	10.66±1.53	0.0±0	3.0	2.7	2.9	0

A similar trend in dry weight of investigated seedlings has also been found. Apo showed a minimum decrease of dry weight under -0.5 MPa (23%) and -0.75 MPa (55%). In case of CBMAS14065 the decrease in dry weight was more than that of Apo but less than that of IWP when exposed to -0.5 MPa (21%) and -0.75 MPa (49%) water stress. IWP showed a maximum decrease of dry weight at -0.5 MPa (44%) and -0.75 MPa (73%). In the case of BILs, the decrease of dry weight ranged from 23% - 33% at -0.5 MPa and 40-57% at -0.75 MPa. Among the BILs, 27-1-7-8-65-4-1 and 27-1-7-8-22-4-1 showed a minimum decrease of both fresh weight and dry weight (**Table 3**).

From the result of the present experiment, it can be concluded that with an increase in water stress, the physiological parameters like germination percentage, germination rate, root and shoot length, fresh weight and dry weight were highly affected. However, the effect of stress on drought-resistant varieties containing the QTLs *qDTY1.1* and *qDTY2.1* was different from that of IWP where both the drought-resistant QTLs were absent. Among all the 10 different BILs used in this experiment, 27-1-7-8-65-4-1 and 27-1-7-8-14-4-1 have shown better germination percentage, germination rate, root length, shoot length, fresh weight and dry weight under PEG induced water stress which is similar to that of Apo. Thus these two lines can be further tested in water-limited environments to validate their utility in the breeding program.

REFERENCES

Ahmad, S., Ahmad, R., Ashraf, M. Y., Ashraf, M. and Waraich, E. A. 2009. Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pak. J. Bot.*, **41**: 647-654.

Aosa, I. 1983. Seed vigor testing handbook. *Association of Official Seed Analysts. Contribution*, **32**.

Bernier, J., Kumar, A., Ramaiah, V., Spaner, D. and Atlin, G. 2007. A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci.*, **47**: 507-516. [Cross Ref]

Boopathi, N. M. 2020. Marker-Assisted Selection (MAS). In *Genetic Mapping and Marker Assisted Selection*: Springer. [Cross Ref]

Goswami, R. and Baruah, K. 1994. Effect of water potential treatments on germination and seeding growth of some upland rice cultivars. *Indian J. Plant Physiol.*, **37**: 61-61.

Hadas, A. 1976. Water uptake and germination of leguniinous seeds under changing external water potential in osmotic solutions. *J. Exp. Bot.*, **27**: 480-489. [Cross Ref]

Hellal, F., El-Shabrawi, H., Abd El-Hady, M., Khatab, I., El-Sayed, S. and Abdelly, C. 2018. Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. *J. Genet. Eng. Biotechnol.*, **16**: 203-212. [Cross Ref]

Islam, M., Kayesh, E., Zaman, E., Urmi, T. and Haque, M. 2018. Evaluation of rice (*Oryza sativa* L.) genotypes for drought tolerance at germination and early seedling stage. *The Agriculturists*, **16**: 44-54. [Cross Ref]

Jnandabhiram, C. and Sailen Prasad, B. 2012. Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India II. Protein and proline status in seedlings under PEG induced water stress. *Am. J. Plant Sci.*,

Khakwani, A. A., Dennett, M. and Munir, M. 2011. Early growth response of six wheat varieties under artificial osmotic stress condition. *Pak. J. Agric. Sci*, **48**: 121-126.

Lu, Z. and Neumann, P. M. 1998. Water-stressed maize, barley and rice seedlings show species diversity in mechanisms of leaf growth inhibition. *J. Exp. Bot.*, **49**: 1945-1952. [Cross Ref]

Mahla, R., Madan, S., Kaur, V., Munjal, R., Behl, R. K. and Midathala, R. 2017. Activities of sucrose to starch metabolizing enzymes during grain filling in late sown wheat under water stress. *J. Appl. Nat. Sci.*, **9**: 338-343. [Cross Ref]

Mekala, M. S. and Viswanathan, P. 2017. A Survey: Smart agriculture IoT with cloud computing. In *2017 international conference on microelectronic devices, circuits and systems (ICMDCS)*: IEEE. [Cross Ref]

Mollasadeghi, V., Valizadeh, M., Shahryari, R. and Imani, A. A. 2011. Evaluation of end drought tolerance of 12 wheat genotypes by stress indices. *World Appl. Sci. J.*, **13**: 545-551.

Molnár, I., Gáspár, L., Sárvári, É., Dulai, S., Hoffmann, B., Molnár-Láng, M. and Galiba, G. 2004. Physiological and morphological responses to water stress in Aegilops biuncialis and Triticum aestivum genotypes with differing tolerance to drought. *Funct. Plant Biol.*, **31**: 1149-1159. [Cross Ref]

Muthu, V., Abbai, R., Nallathambi, J., Rahman, H., Ramasamy, S., Kambale, R., Thulasinathan, T., Ayyenar, B. and Muthurajan, R. 2020. Pyramiding QTLs controlling tolerance against drought, salinity, and submergence in rice through marker assisted breeding. *PloS one*, **15**: e0227421. [Cross Ref]

Neog, P., Sarma, P. K., Saikia, D., Borah, P., Hazarika, G. N., Sarma, M. K., Sarma, D., Chary, G. R. and Rao, C. S. 2020. Management of drought in sali rice under increasing rainfall variability in the North Bank Plains Zone of Assam, North East India. *Clim. Change*, **158**: 473-484. [Cross Ref]

Richards, R. 1978. Variation between and within species of rapeseed (Brassica campestris and B. napus) in response to drought stress III. Physiological and physicochemical characters. *Aust. J. Agric. Res.*, **29**: 491-501. [Cross Ref]

Slama, I., Ghnaya, T., Hessini, K., Messedi, D., Savouré, A. and Abdelly, C. 2007. Comparative study of the effects of mannitol and PEG osmotic stress on growth and solute accumulation in Sesuvium portulacastrum. *Environ. Exp. Bot.*, **61**: 10-17. [Cross Ref]

Swapna, S. and Shylaraj, K. S. 2017. Screening for osmotic stress responses in Rice varieties under drought condition. *Rice Sci.*, **24**: 253-263. [Cross Ref]

Tounekti, T., Hernández, I., Müller, M., Khemira, H. and Munné-Bosch, S. 2011. Kinetin applications alleviate salt stress and improve the antioxidant composition of leaf extracts in Salvia officinalis. *Plant Physiol. Biochem.*, **49**: 1165-1176. [Cross Ref]

Tripathy Swapan, K. 2015. In Vitro Screening of Callus Cultures and Regenerants for Drought Tolerance in Upland Rice. *Res. J. Biotechnol.*, **10**: 6.

Valarmathi, M. 2019. Developing climate resilient rice through marker assisted QTL pyramiding. Post Doctorate, Center of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University.

Venuprasad, R., Dalid, C., Del Valle, M., Zhao, D., Espiritu, M., Cruz, M. S., Amante, M., Kumar, A. and Atlin, G. 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theor. Appl. Genet.*, **120**: 177-190. [Cross Ref]

Verma, A. and Deepti, S. 2016. Abiotic stress and crop improvement: current scenario. *Adv Plants Agric Res*, **4**: 00149. [Cross Ref]

Vikas, V., Satheesh, V., Subramanian, M., Kalarani, M. and Singh, V. 2009. Evaluation of rice hybrids and their parents for drought tolerance. *Ind. J. Plant Physiol*, **14**: 156-161.

Vikram, P., Swamy, B. M., Dixit, S., Ahmed, H. U., Cruz, M. T. S., Singh, A. K. and Kumar, A. 2011. qDTY 1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genet.*, **12**: 89. [Cross Ref]

Zhang, J., Jia, W., Yang, J. and Ismail, A. M. 2006. Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Res.*, **97**: 111-119. [Cross Ref]