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Drought Stress Screening in Backcross Inbred Lines of Rice (*Oryza sativa* L.) at Germination and Seedling Stage

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Drought is a key stress factor that limits rice production globally. The present study was carried out to identify drought tolerant rice lines at the germination and seedling stages using polyethylene glycol (PEG) induced stress. Twenty promising high yielding BC_2F_4 lines, stacked with drought yield

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QTLs $qDTY_{1}3_{1}$ and $qDTY_{12\cdot1}$, derived from the cross between Manu Ratna X Improved White Ponni, were subjected to drought stress. Drought was imposed during germination using three levels (0, 10 and 15%) of polyethylene glycol (PEG 6000) concentrations. The experiment was conducted in factorial completely randomized block design with three replications and the observations on number of seeds germinated, root length, shoot length, germination index, germination stress tolerance index, fresh weight stress tolerance index, dry weight stress tolerance index and seedling vigour index were evaluated after stress treatment. A significant difference was noted among lines for characters under drought stress. Among the lines, MIB-29-2-3-1, MIB-29-8-5-35, MIB-43-5-3-66 and MIB-119-5-7-33 were selected which showed higher germination percentage, root length, shoot length, germination stress tolerance index and and seeding vigour index. The selected lines can be further evaluated in the field to develop drought tolerant rice varieties.

Keywords: Water stress; backcross inbred rice lines; seeding vigour index; germination index; drought resilience; rice breeding; seedling stage drought resistance.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of more than half of the world's population and is cultivated in all continents except Antarctica. To meet the growing population's demand, rice production must be increased from 55 Mt to 577 Mt by 2032 [1]. Changing climatic patterns are affecting rice productivity and threatening global food security. Among the different stresses, drought is the most critical limitation to rice production in rainfed systems, impacting 10 million hectares of upland rice and over 13 million hectares of rainfed lowland rice in Asia alone [2]. Climatic changes including erratic rainfall patterns and climatic anomalies are causing a drastic reduction in rice production in India, especially in Kerala [3]. Therefore, in the current context of population growth and climate change, it is essential to develop high-yielding, drought-tolerant rice varieties.

Polyethylene glycol (PEG) is a commonly used chemical compound known to lower the osmotic potential of a nutrient solution, thereby inducing water stress in a controlled manner [4]. PEG causes osmotic stress and changes the plant tissue water potential resulting in decreased plant growth and biomass accumulation [5]. Drought stress in plants can be quantitatively assessed by measuring various metabolites, enzymes and pigments and comparing these levels with the normal conditions. Drought stress responses in plants include physiological changes like decrease in turgor, increased tolerance to desiccation and increased water uptake; photosynthetic changes due to stomatal closure, slower photosynthetic rate, reduced CO_2 uptake and pigment degradation and metabolic changes including increased antioxidant activity, accumulation of osmotically active solutes, increase in phenolic metabolites, upregulation of leaf flavonoids, increase in reactive oxygen species and reactive carbonyl compounds [6]. Higher molecular weight variants of PEG are preferred for inducing drought stress as it effectively blocks the diffusion through cell walls. This can lead to water loss *via* cytorrhysis rather than plasmolysis, as it is unable to penetrate plant cells [7,8].

Hence, this study was conducted under different concentrations of PEG 6000 using 20 selected BC_2F_4 rice lines to identify the superior drought tolerant backcross inbred lines that can be used to develop drought tolerant rice varieties in the future breeding programmes.

2. MATERIALS AND METHODS

2.1 Plant Materials

BC_2F_4 seeds derived from the cross between Manu Ratna and Improved White Ponni stacked with $qDTY_{1\cdot1}$ and $qDTY_{12\cdot1}$ [9] were evaluated under three levels of PEG 6000 concentrations (0%, 10% and 15%). Selected BC_2F_4 seeds were obtained from 20 superior BC_2F_3 lines subjected to drought stress at reproductive stage in field conditions.

2.2 Experimental Design

The experiment was conducted at the Department of Genetics and Plant Breeding, College of Agriculture, Vellayani using factorial completely randomized design with two factors and three replications. The main factor (factor A) was 20 lines while the sub factor (factor B) was three (0, 10 and 15%) levels of drought stress. Two replicates of five seeds from each line were subjected to different levels of PEG.

2.3 Growth Conditions

The seeds were placed on germination paper in petri plates and were subjected to three different PEG concentrations. The required amount of PEG 6000 was mixed with distilled water to prepare 10% and 15% solutions, while distilled water was used for the control treatment.

2.4 Morphological Observations

Seed germination was monitored every 24 hours for 14 days, after which the germination percentage was calculated. The shoot length (SL) and root length (RL) were measured using a scale in centimeters whereas seedling fresh weight was measured in (g) using digital weighing balance on the 14^{th} day. Seedlings were then dried for 72 hours at 80ºC and weighed in digital weighing balance to measure seedling dry weight. The germination and seedling characteristics of each treatment were compared to the control to identify drought tolerant rice lines. The different stress tolerance indices were computed as follows.

2.5 Germination Index (GI)

The germination index (GI) was calculated using the following equation [10]:

$$GI = \Sigma \left[\frac{n}{d}\right]$$

where n is the number of germinating seeds and d is the respective days of germination.

2.6 Germination Stress Tolerance Index (GSTI)

Germination stress tolerance index (GSTI) was calculated in terms of percentage [11] as follows:

$$GSTI = (PI_S / PI_C) \times 100$$

where, PI_S and PI_C are promptness index under stress and control condition. The promptness index (PI) was estimated using the following formula [12].

$$PI = (nd1 \times 1.0) + (nd2 \times 0.75) + (nd3 \times 0.50) + (nd4 \times 0.25)$$

where, nd1, nd2, nd3 and nd4 are number of seeds germinated on the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} day respectively.

2.7 Fresh Weight Stress Tolerance Index (FSTI)

Fresh weight stress tolerance index (FSTI) was estimated as follows [11]:

$$FSTI = (FWS_s / FWC_c) \times 100$$

where, FWS_s and FWC_c are fresh weight (mg) of seedlings under stress and control conditions respectively.

2.8 Dry Weight Stress Tolerance Index (DSTI)

Dry weight stress tolerance index (DSTI) was estimated as follows [11]:

$$DSTI = (DWS_s / DWC_c) \times 100$$

where, DWS_s and DWC_c are dry weight (mg) of seedlings under stress and control conditions respectively.

2.9 Seedling Vigour Index (SVI)

According to Abdul-Baki Anderson [13] SVI was calculated using the formula below:

$$SVI = (SL + RL) \times \text{Germination } \%$$

2.10 Statistical Analysis

The GRAPES 1.0.0 software [14] was used to conduct variance analysis (ANOVA) and significant differences were detected using the least significant difference (LSD) test at P-value<0.05. Mean values and standard errors (SE) are presented.

3. RESULTS AND DISCUSSION

Analysis of variance (ANOVA) revealed significant variations among the lines, treatments and interactions among the back inbred rice lines for seed germination, shoot length, root length and various tolerance indices (Table 1).

3.1 Effect of PEG Induced Drought Stress on Seed Germination

The effect of PEG induced drought stress on seed germination of different lines is presented in

Fig. 1. The number of seeds germinated (NSG) was highest in control (4.98) while the lowest was recorded at 15% PEG (3.10). The highest NSG (5.00) was observed in the line MIB-43-5-3-66 under three levels of treatment. This was statistically on par with lines MIB-29-8-5-30 (4.83), MIB-43-5-8-25(4.83), MIB-113-3-7-7 (4.67), MIB-119-5-7-18 (4.67) and MIB-29-2-3-1 (4.67). This suggests that these lines can tolerate drought conditions. MIB-113-3-7-70 (2.83) showed the least value for NSG, which was on par with MIB-29-8-5-10 (3.00). Also, the lines, MIB-29-8-5-31, MIB-29-8-5-35, MIB-43-5-3-32 and MIB-119-5-7-33 showed good germination with a mean value of 5.00 under 15 % PEG concentration. This may be due to the high resistance to drought conditions. The study revealed that the decline in the number of seeds germinated under moisture stress conditions intensified as PEG concentrations increased. Similar observation was reported by Herawati et al. [15], Sagar et al. [16] and Hartyanto et al. [17] in rice under PEG treatment. The decrease in germination of seeds caused by PEG was due to a reduction in the water potential gradient between the seeds and their environment [18]. This could cause osmotic imbalance, membrane impairment, decreased respiration, disrupt enzyme and metabolic activities in seeds during germination under drought stress [19,20]. The imbibition of water for germination is also reduced under higher negative osmotic potential [21].

3.2 Effect of PEG Induced Drought Stress on Vegetative Growth Traits

3.2.1 Root length

All lines exhibited decreased root length with increased PEG concentrations. When compared with the control the reduction in root length was high at 10% (Table2/Fig. 3). MIB-29-8-5-35 had the longest root length (9.09 cm), which was on par with MIB-43-5-3-32 (8.71 cm), MIB-119-57-33 (8.56 cm), MIB-29-2-3-1 (8.45 cm), MIB-29-8-5-30 (7.88 cm) and MIB-84-3-3-4 (7.86 cm). This suggested that these lines exhibit higher tolerance to drought stress during the early seedling stage. MIB-29-2-3-1, MIB-29-8-5-35, MIB-84-3-3-4 and MIB-119-5-7-33 showed an increased root length even under 15% drought stress conditions. This indicated their potential for tolerance against drought stress. The earlier studies by Sagar et al. [16] and Anik et al. [22] reported reduction in root length in PEG-treated seeds compared to the control. Reduced root lengths might be due to decreased cell development and elongation rates which could reduce the nutrient uptake under water stress conditions leading to unbalanced and poor plant stand [23,24].

3.2.2 Shoot length

In the study, all the lines showed a significant reduction in shoot length under PEG treated conditions compared to the control (Table2/Figure4). At 10% PEG concentration, a drastic reduction in shoot length was observed in all lines. The longest shoot length was observed in the genotype MIB-29-8-5-35 (6.05 cm), which was statistically comparable to MIB-43-5-8-71 (5.46 cm), MIB-29-8-5-30 (5.35 cm) and MIB-84-3-3-4 (5.28 cm). This revealed the potential of these genotypes to grow well under stress conditions. The findings were in accordance with Fatimah et al. [25], Purbajanti et al. [26] and Sagar et al. [16]. MIB-29-8-5-35, MIB-84-3-3-4 and MIB-119-5-7-33 showed higher shoot length even under 15% PEG concentration. In all lines root length was greater than shoot length, indicating that plumule growth is more sensitive to water stress than radicle growth. Similar findings were reported by Sagar et al. [16]. The radicle being the first organ to emerge from the seed will be faster in growth than the plumule. Additionally, the radicle will be in direct contact with water, while the plumule will not have direct access to water sources due to its later emergence and its position on the seed [27].

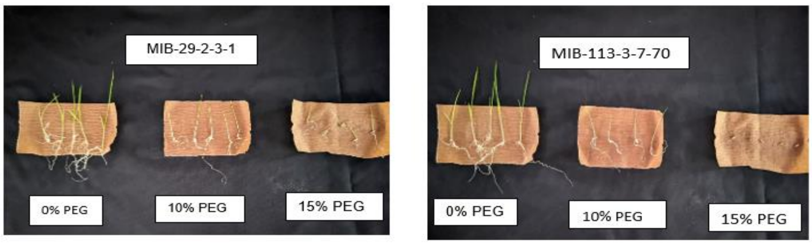

Fig. 1. Fourteen days old backcross inbred lines of rice under different concentrations of PEG

Table 1. ANOVA table for germination, vegetative growth and stress indices

S.O.V	NSG			RL			SL		
	Mean square	S.E(m)	C.D	Mean square	S.E(m)	C.D	Mean square	S.E(m)	C.D
Line	2.086	0.289	0.577	8.625	0.504	1.427	3.029	0.272	0.769
Treatment	37.708	0.112	0.224	511.331	0.195	0.553	590.68	0.105	0.298
Line X Treatment	1.787	0.5	1	11.493	0.874	2.471	4.394	0.471	1.332
Error	0.25			1.526			0.444		

S.O.V	GI			GSTI			FSTI		
	Mean square	S.E(m)	C.D	Mean square	S.E(m)	C.D	Mean square	S.E(m)	C.D
Line	0.264	0.069	0.196	655.114	4.771	13.496	398.805	1.974	5.584
Treatment	8.992	0.027	0.075	75552.924	1.848	5.227	40688.918	0.764	2.162
Line X Treatment	0.207	0.12	0.34	359.068	8.263	23.376	457.156	3.419	9.671
Error	0.029			136.563			23.375		

S.O.V	DSTI			SVI		
	Mean square	S.E(m)	C.D	Mean square	S.E(m)	C.D
Line	780.122	1.81	5.121	47502.798	35.58	100.65
Treatment	75908.494	0.701	1.983	5628832.15	13.78	38.982
Line X Treatment	621.369	3.136	8.87	79723.043	61.626	174.331
Error	19.665			7595.578		

Table 2. Mean performance of root length, shoot length and germination index of various lines under different PEG concentrations

Backcross Inbred rice lines	RL (cm)			SL (cm)			GI		
	PEG concentrations (%)								
	0	10	15	0	10	15	0	10	15
MIB-29-2-3-1	9.20	8.07	8.07	7.00	3.78	1.56	1.89	1.35	1.23
MIB-29-2-3-29	12.85	9.09	1.12	8.32	4.30	0.37	1.95	1.34	1.34
MIB-29-2-3-21	12.12	8.07	2.60	9.81	1.12	0.60	0.88	0.87	0.73
MIB-29-8-4-4	14.73	5.74	1.43	9.41	2.92	0.42	1.60	1.42	0.70
MIB-29-8-5-2	10.50	10.09	0.25	9.28	5.01	0.00	1.55	1.38	0.10
MIB-29-8-5-31	9.11	4.54	1.69	9.94	1.75	0.48	2.12	1.12	0.93
MIB-29-8-5-35	10.61	8.24	8.41	9.96	5.19	3.00	1.38	1.18	1.15
MIB-29-8-5-30	10.95	7.83	4.87	9.97	4.47	1.60	1.79	1.74	1.03
MIB-29-8-5-10	11.16	9.91	0.00	9.32	6.49	0.00	1.60	1.33	0.00
MIB-43-5-3-32	11.53	10.3	4.29	8.34	4.93	1.14	1.37	1.12	0.90
MIB-43-5-3-66	6.50	5.77	4.71	6.32	2.64	1.31	1.82	1.18	0.82
MIB-43-5-8-25	8.08	4.75	4.66	10.32	1.80	0.85	1.92	1.17	1.17
MIB-43-5-8-71	8.25	7.75	5.79	9.14	5.73	1.50	1.09	1.08	0.77
MIB-84-3-3-4	11.13	4.48	4.00	10.09	2.55	3.21	1.77	0.88	0.87
MIB-84-3-11-103	10.63	9.82	0.25	7.59	6.33	0.13	2.00	1.02	0.20
MIB-113-3-7-7	9.66	8.03	4.21	8.19	4.78	1.43	2.15	1.67	0.77
MIB-113-3-7-70	10.25	7.35	0.00	9.41	4.38	0.00	1.99	1.07	0.00
MIB-119-5-7-18	8.99	3.13	3.13	7.25	1.51	0.45	2.07	1.30	1.00
MIB-119-5-7-33	9.59	6.02	10.08	7.52	1.68	3.68	1.55	1.28	1.07
MIB-119-5-8-10	11.18	10.58	1.68	6.88	6.51	0.42	1.81	1.33	0.35
Mean	10.35	7.478	3.56	8.70	3.89	1.11	1.72	1.25	0.76

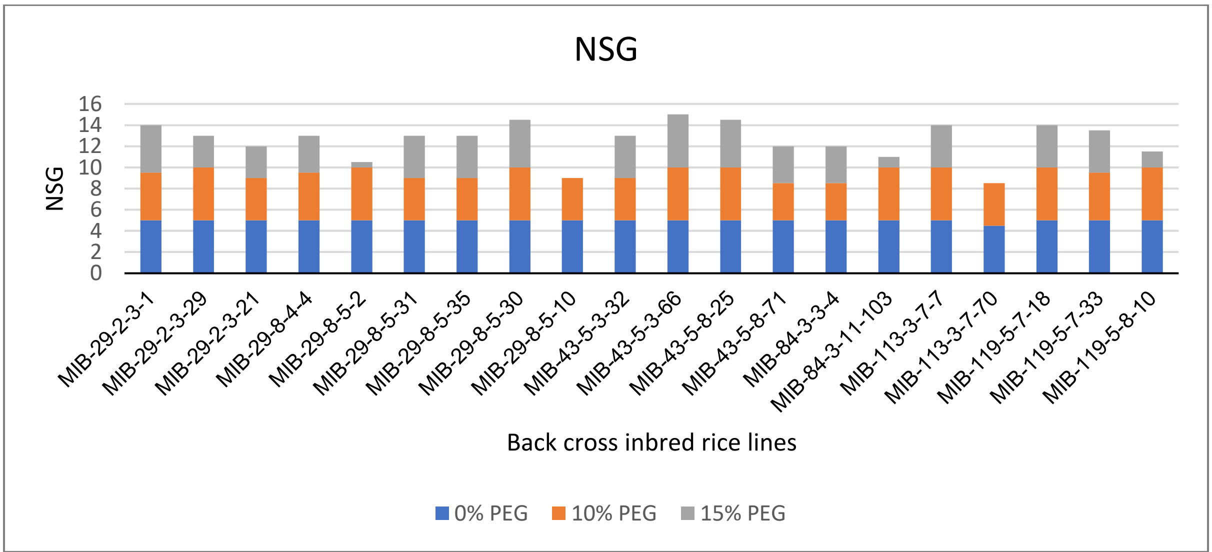

Fig. 2. Effect of PEG on Number of Seeds Germinated (NSG) in different backcross inbred rice lines

3.3 Stress Indices

3.3.1 Germination Index (GI)

A notable variation in GI was observed with respect to factor A and factor B as well as their interactions. The overall mean GI values ranged from 1.54 to 0.86 among the selected lines under PEG treatment. Drought stress caused a decline in GI and the highest GI was observed under control conditions while the lowest was under 15% PEG concentration. The highest germination index (GI) was observed in genotypes MIB-29-2-3-29 (1.54) followed by

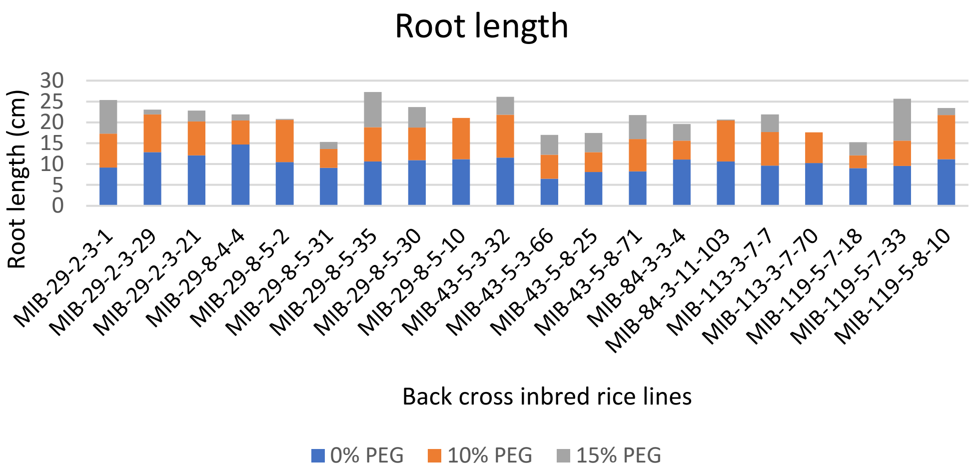

Fig. 3. Effect of PEG on root length in different backcross inbred rice lines

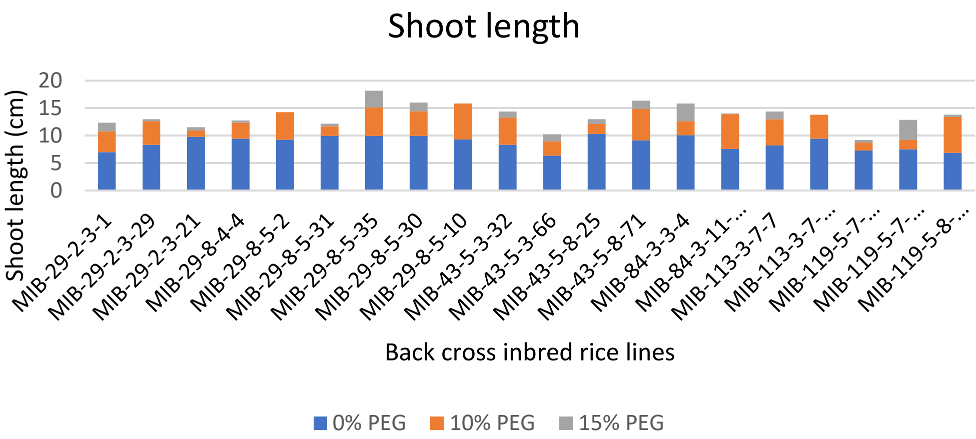

Fig. 4. Effect of PEG on shoot length in different backcross inbred rice lines

MIB-113-3-7-7 (1.53), MIB-29-8-5-30 (1.52), MIB-29-2-3-1 (1.49), MIB-43-5-8-25 (1.48), MIB-119-5-7-18 (1.46) and MIB-29-8-5-31 (1.39) indicating their potential tolerance to drought stress. MIB-29-2-3-1, MIB-29-2-3-29, MIB-29-8-5-35, MIB-43-5-8-25 and MIB-119-5-7-33 showed higher GI value even under 15% PEG concentration. The findings of Islam et al. [24] and Pope et al. [28] were in agreement with these results.

3.3.2 Germination Stress Tolerance Index (GSTI)

GSTI showed a decreasing trend with increasing PEG concentration in the study (Table 2) Among the lines tested, the highest GSTI values were recorded for MIB-29-8-5-35 (75.00) followed by MIB-29-2-3-1 (71.21) and MIB-119-5-7-33 (70.37). The lowest GSTI was observed in MIB-43-5-8-71 (37.50). The lines MIB-29-2-3-1, MIB-29-8-5-35, MIB-43-5-3-66 and MIB-119-5-7-33 showed a high GSTI even under 15% PEG concentrations. This indicated the ability of these lines to germinate under adverse drought conditions. The findings of Sagar et al. [16], Bukhari et al. [29] and Siddique et al. [30] also confirmed similar trends in germination stress tolerance and the relationship between PEG-induced stress and plant adaptability under drought conditions.

3.3.3 Fresh Weight Stress Tolerance Index (FSTI)

FSTI was the highest in MIB-29-8-5-30 (81.20) which was statistically comparable to MIB-43-5-8-25 (79.09). The least value for FSTI was found in in MIB-29-2-3-21 (55.50). The lines MIB-29-2-3-29, MIB-29-8-5-35, MIB-29-8-5-30, MIB-43-5-8-25 and MIB-119-5-7-33 showed higher FSTI value under 15% PEG concentrations. This showed the potential ability of the lines to perform well under drought conditions. Similar results were identified by Sagar et al. [16].

3.3.4 Dry Weight Stress Tolerance Index (DSTI)

DSTI is a parameter used to evaluate the ability of plants to maintain high biomass production under drought stress conditions. Higher the value of DSTI, higher will be the stress tolerance. There was significant variation in DSTI among various lines, PEG concentrations and their interactions. DSTI abruptly reduced under 15% PEG concentration. The highest DSTI was observed in MIB-84-3-3-4 (82.72). Meanwhile, the lines MIB-29-2-3-1, MIB-29-8-5-35, MIB-29-8-5-30, MIB-43-5-3-66 and MIB-43-5-8-71 maintained high DSTI value even under 15% PEG concentration. This shows the ability of these lines to perform well even under stressed conditions. In contrast, the lowest DSTI was recorded in MIB-29-2-3-21 (34.14) which was comparable with MIB-119-5-7-18 (35.13). The mean DSTI was found to be higher at a PEG concentration of 10% (53.75) than 15% PEG concentration (12.93). Similar results were given by Sagar *et al* [16]. Osmotic stress reduces water availability for plants, which leads to decreased cell division and elongation by lowering turgor pressure and inhibiting cell growth. Ultimately, this results in the reduction of both biomass and dry weight [31,32].

3.3.5 Seedling vigour index

SVI showed significant variation across the selected lines (Fig..5), PEG 6000 concentrations, and interactions (MIB-29-8-5-35 had the highest mean SVI (706.17), which was comparable to MIB-29-8-5-30 (654.36) and MIB-43-5-3-32 (613.24). The lines MIB-29-2-3-1, MIB-29-8-5-35, MIB-29-8-5-30, MIB-43-5-3-66, MIB-84-3-3-4 and MIB-119-5-7-33 indicated a higher value for SVI under higher stressed condition (15%). This showed their potential ability to tolerate the stress. In contrast, MIB-119-5-7-18 had the lowest SVI (334.23) which was on par with MIB-29-2-3-21 (408.53), MIB-29-8-5-31(428.04) and MIB-43-5-3-66 (431.14). The mean SVI was highest under control conditions (934.77) while 15% PEG concentration produced a lower mean SVI (188.88) than the 10% PEG concentration (491.89). Similar results were given by Sagar et al. [16], Violita Azhari [33] and Sathyabharathi et al. [34]. Early seedling vigour determines rapid and uniform seeding emergence, seedling growth and tolerance to adverse climatic factors [35].

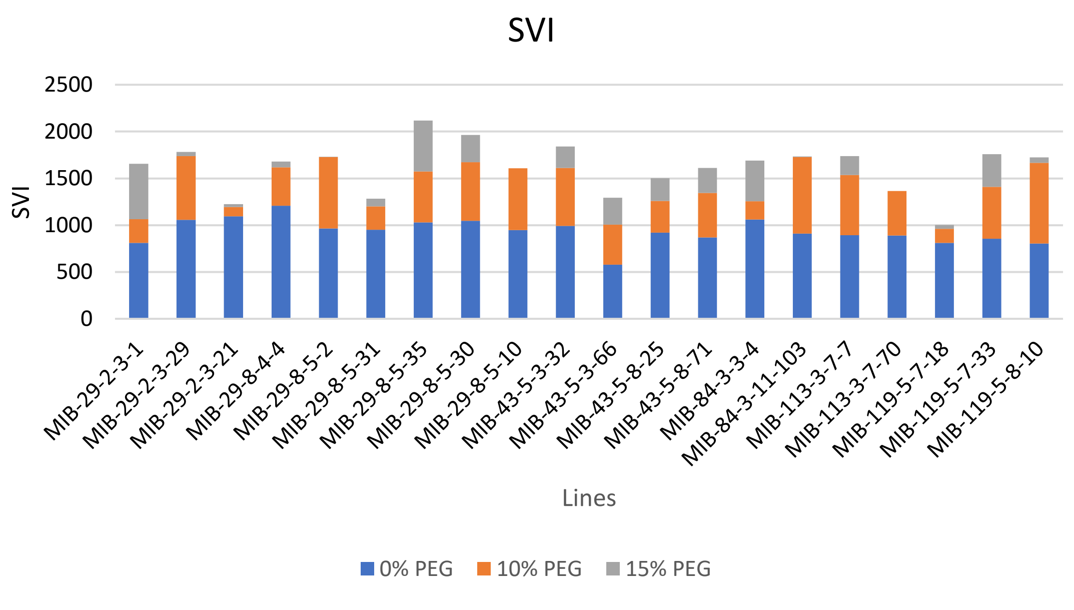

Fig. 5. Effect of PEG on seedling vigour index (SVI) in different backcross inbred rice lines

4. CONCLUSION

The backcross inbred rice lines exhibited significant variation in germination, early seedling growth and tolerance under different PEG induced drought stress conditions. The highest reduction in seed germination and growth traits was recorded under 15% PEG. The lines MIB-29-2-3-1, MIB-29-8-5-35, MIB-43-5-3-66 and MIB-119-5-7-33 showed better performance under 15% PEG concentration for the traits number of seeds germinated, root length, shoot length, germination index, germination stress tolerance index and seedling vigour index. These promising drought tolerant lines can be further advanced through pedigree method to develop high yielding drought resistant rice varieties.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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