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Original Article

Screening and selection of drought-tolerant rainfed lowland rice seedlings based on early seedling vigor under Polyethylene Glycol-Induced Stress

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ABSTRACT

A field-based drought tolerance screening is time-consuming and labor-intensive. Polyethylene Glycol (PEG) offers a simple laboratory-based method to simulate drought stress efficiently. This study was conducted to identify and select the five most drought-tolerant genotypes based on vigor-related traits under PEG-induced drought stress. These genotypes will be used in a subsequent study involving greenhouse and molecular screening for drought tolerance. Responses of 23 rainfed lowland rice (*Oryza sativa* L.) genotypes subjected to varying drought levels were investigated in two trials under laboratory conditions to evaluate against three drought levels (0MPa, -0.5MPa, -1MPa) at germination and early seedling growth stage of plant development. Germination percentage and rate, root length, seedling vigour index, root and shoot dry weight were gathered. Data from two trials were combined and analyzed statistically for all growth parameters obtained. Experimental units were arranged in factor factorial in RCBD with 3 replications. Results showed that all parameters gathered except for root-shoot ratio were significantly affected by the levels of PEG, genotypes and their interaction. The five drought-tolerant lines; AL-108, AL-87, AL-97, AL-55, AL-5 tolerated PEG-induced stressed at the highest drought level (-1MPa) and showed no significant difference with unstressed (0MPa) based on

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seedling vigour. AL52 and NSIC Rc82 were markedly affected at the highest PEG level (-1MPa) and hence considered as drought-sensitive.

Keywords: Polyethylene Glycol, Drought stress, Rainfed lowland rice varieties, Seedling Vigour

INTRODUCTION

Rice is one of the most significant crops in Asia and in the Philippines. The rainfed lowland rice system in Asia covers about 45M ha, which is almost 30% of the total rice area worldwide (Haefele and Bouman 2009, Hibberd et al 2008). Rainfed areas, where the main source of water depends on the occurrence of rainfall, are more prone to water scarcity. It is estimated that by 2025, 15M ha of traditionally irrigated land will suffer physical water scarcity and 22M ha will be under economic water scarcity (Prasad 2011).

Rice is exposed to many different environmental stresses, however drought is the most crucial during seed germination or the early vegetative stages. The impact of drought during these stages is very important as it will affect succeeding stages of crop growththat consequently results in a decrease in yield and production. Therefore, enhanced tolerance to drought stress has been an important goal in crop improvement programs.

The development of new rice genotypes tolerant to drought stress would increase yield and save water (Zhou et al 2006). Field screening for drought tolerance is resource-intensive and phenotypic expression of the genotypes is greatly affected by the environment. Unpredictable rainfall and changing weather conditions, particularly under rainfed scenarios, make field screening less efficient. Additionally, this requires a full-season of data, which is time-consuming and labor-intensive, hence the need for a simple and effective early screening method seems necessary (Kim et al 2001, Rai et al 2011). Laboratory or greenhouse screening offers a faster, more controlled approach, allowing the evaluation of large germplasm sets with minimal environmental influences.

The emergence of seeds under simulated drought stress conditions may also provide prospects in selecting and identifying seeds with less viability and vigour. PEG-based in vitro screening for drought tolerance has been proven to be a suitable method to effectively screen large sets of germplasm with good accuracy (Kulkarni and Deshpande 2007). Screening for drought tolerance in rice at germination stage using PEG-induced drought stress have been reported (Sebasan and Saravanan 2016, Akte et al 2016, Lum et al 2014, Gómez-Luciano et al 2012). PEG-based in vitro screening was effective in screening large sets of germplasm for drought tolerance with good accuracy (Kulkarni et al 2007). Gaurana-Nunez and Sta. Cruz (2022) demonstrated that drought-tolerant rice lines selected under PEG-induced drought stress consistently exhibited seven tightly-linked SSR markers associated with drought tolerance, highlighting the reliability of this selection method. A fast and simple screening method would be useful in selecting valuable genotypes with distinct growth characters that translate to drought tolerance and are suitable for breeding programs. Polyethylene glycol (PEG)-induced drought stress screening is potentially an effective method for identifying drought-tolerant in rice genotypes. PEG, with high molecular weight, mimics drought conditions by restricting water entry of water through the cell wall, making it valuable tool for regulating water potential in any germination test.

This study aims to identify and select the top five drought-tolerant genotypes based on vigor related traits. We hypothesized that tolerant genotypes under PEG-induced drought stress will also show drought tolerance in greenhouse conditions. The information generated will be used as input into the development of a rapid and suitable screening protocol for drought-tolerance.

MATERIALS AND METHODS

Experimental Treatments and Design

A laboratory experiment was conducted at Bay, Laguna during September – November, 2018. Twenty-three rice genotypes (Table 1) were used in this study. The 23 elite advanced lines (AL) were obtained from the University of the Philippines Los Baños (UPLB) research team, while two varieties PSB Rc14 (rainfed lowland cultivar, drought-tolerant check) and PSB Rc82 (irrigated lowland cultivar, drought-susceptible check) were sourced from the Philippine Rice Research Institute (PhilRice-UPLB).

Table 1. List of elite rice lines used in this study.

Entry	Rice Line (Index Number)	Cross Selection
1	AL-108	C 10461-10-3-3-3
2	AL-87	C 10205-B-9-3-2-2-1
3	AL-97	C 10202-B-1-2-2-2-1-1
4	AL-55	C 9643-B-5-2-1-1-1-1
5	AL-5	C 8852-B-18-1-1
6	AL-54	C93663-B14-1-1
7	AL-83	C102-B-14-3-3-3
8	AL-12	C 9301-B-12-2-2
9	AL-17	C 9301-B-2-2-1-2
10	AL-107	C 10461-10-3-3-1
11	AL-32	C9359-B-16-3-3-2
12	AL-57	C 9656-B-11-1-1-1-1
13	AL-88	C 10445-6-2-3-1
14	AL-9	C9301-B-11-1-2
15	AL-91	C 10451-1-1-3-2
16	AL-23	C9364-B-19-2
17	AL-27	C9354-B-3-2-1
18	AL-104	C 10460-4-1-1-2
19	AL-18	C 9301-B-8-2-2-1
20	AL-45	C9673-B-3-1-3
21	AL-56	C10026-B-1-1-2-2
22	AL-109	C 10461-13-1-1-3
23	AL-52	C9663-B-12-1-3-1
24	NSIC Rc14	Tolerant check
25	PSB Rc82	Susceptible check

Polyethylene glycol 6000 was used to impose different levels of water potential in evaluating drought tolerance at germination stage. A two-trial experiment was laid out in a factorial randomized complete design with subsampling in three replications. The first factor contained 23 rainfed rice elite lines along with susceptible check (PSB Rc82) and tolerant check (PSB Rc14) cultivar. The second factor included two levels of drought stress ie, -0.5MPa, and -1MPa of water potential which was imposed by dissolving 196 and 289g PEG respectively in 1L distilled water following the methods of Hadas (1976), Govindaraj et al (2010), and Sabesan and Saravanan (2016). A control (0MPa) was prepared using distilled water. Twenty-five seeds (25) of each rice genotype were transferred into sterilized germination dishes (9cm) in which filter papers were placed. Then 10mL of specified treatments were added to each dish. Seeds with root length of 2mm or more were considered as germinated. After 10 days, 10 randomly-germinated seeds were taken out of each germination plate. The stems and roots were separated to assess the morphological parameters. At this stage, germination percentage was calculated as the ratio of the number of seeds germinated to the total number of seeds used for germination, multiplied by 100. Germination rate was obtained as the ratio between the germinated seeds at 5th day and the number of seeds germinated on the 10th day multiplied by 100.

The shoot and root lengths of the seedlings were measured using a metric ruler (ie, length from shoot base to shoot tip or from root base to root tip). Root to shoot ratio was calculated as the ratio between the dry root and dry shoot weights. Vigour index, was estimated by multiplying the percentage of germination and the seedling length divided by 100.

Statistical Analysis

The ANOVA was used to detect the significant effects of the treatments. Treatment mean differences were analyzed using HSD at a 5% level of significance. All the analyses were done using SAS University Edition statistical software.

RESULTS AND DISCUSSION

Significant differences were observed under varying PEG concentrations (0MPa, -.5MPa and -1MPa) for all the characteristics under the study. Germination percentage and rate, root length, shoot length, leaf number, root number and root dry weight significantly differed by treatment, genotype, and their interaction except for shoot dry weight and root to shoot ratio. Shoot dry weight and root to shoot ratio significantly differed by treatment but not by genotypes and their interaction.

Effect of Drought on Germination Percentage and Rate

Drought is one of the important factors that affect the germination of seeds and the growth of seedlings. Low water potential in the soil can hamper water imbibition of the seed and may delay the seedling growth. It is known that water availability plays an important role in enzymatic reactions, solubilization and transport of metabolites, and also serves as a reagent in the hydrolytic breakdown of proteins, lipids and carbohydrates in the storage tissues of germinating seeds (Bewley and Black 1994).

Drought treatment, genotypes and their interaction significantly affected the percentage and rate of germination (Tables 2 and 3). Among the test genotypes, the highest germination percentages were obtained in: AL-108, AL-87, AL-97, AL-55 and

AL-5. These lines had significantly similar germination percentages with drought-tolerant check (PSB Rc14) with 79% germination percentage under -1MPa. From 0MPa to -1MPa check varieties PSB Rc14 (tolerant check) had 6% reduction in germination percentage while 24% reduction in NSIC RC 82 (susceptible check). Under -0.5MPa, no significant differences were observed among all genotypes except for AL-107 and AL-132. Although the germination percentage slightly decreased in most genotypes compared to the control, this suggests that PEG effectively induced stress in the germinating seeds.

Table 2. Means of germination percentage of different rainfed lowland rice genotypes under PEG-induced drought stress

Genotypes	Germination %			Percent Difference between 0MPa and -1MPa
	Water potential			
	0MPa	-0.5MPa	-1MPa	
AL-108	97abc	96ab	55abcd	14
AL-87	97ab	95ab	53abcde	15
AL-97	98a	97ab	63abc	11
AL-55	99a	97ab	68ab	9
AL-5	95abcd	97ab	63abc	10
AL-54	92abcd	91ab	47bcdef	16
AL-83	99a	91ab	49bcdef	17
AL-12	89bcd	89ab	40bcdefg	19
AL-17	91abcd	92ab	21fg	31
AL-107	95abcd	88b	27defg	28
AL-32	97ab	85b	27efg	28
AL-57	97ab	93ab	46bcdef	18
AL-88	99a	97ab	43bcdefg	20
AL-9	99a	97ab	29defg	27
AL-91	97abc	91ab	24fg	30
AL-23	97ab	90ab	43bcdefg	19
AL-27	96abc	93ab	40bcdefg	21
AL-104	88cd	88ab	23fg	30
AL-18	93abcd	97ab	44bcdefg	18
AL-45	96abcd	92ab	49bcdef	16
AL-56	97abc	98ab	37cdefg	22
AL-109	87d	89ab	47bcdef	15
AL-52	95abcd	91ab	17g	35
NSIC Rc14 (TC)	100a	98ab	79a	6
PSB Rc82 (SC)	99a	99a	35defg	24
Treatment Mean	99	93	43	
Genotypes (G)			780.22**	
PEG levels (T)			133363.24**	
G*T			432.27**	

Level of significance *$p<0.05$; **$p<0.01$ Legend: *TC*= tolerant check *SC*= susceptible check

Table 3. Means of germination rate of different rainfed lowland rice genotypes under PEG-induced drought stress

Genotypes	Germination rate			
	Water potential			Percent Difference between 0MPa and -1MPa
	0MPa	-0.5MPa	-1MPa	
AL-108	99	99	91a	2
AL-87	99	99	80b	5
AL-97	100	99	84b	4
AL-55	100	99	83b	5
AL-5	99	83	88b	3
AL-54	99	96	81b	5
AL-83	99	99	79b	6
AL-12	100	94	68b	9
AL-17	99	96	45b	19
AL-107	99	90	67b	10
AL-32	99	97	39c	22
AL-57	98	98	58b	13
AL-88	99	83	88b	3
AL-9	100	99	59b	13
AL-91	100	99	45c	19
AL-23	100	95	68b	9
AL-27	100	99	69b	9
AL-104	98	95	44c	19
AL-18	100	100	77b	6
AL-45	100	97	89a	3
AL-56	99	99	72b	8
AL-109	100	96	77b	7
AL-52	100	96	51b	16
NSIC Rc14 (TC)	99	100	83b	5
PSB Rc82 (SC)	99	97	40c	21
Treatment Mean	100	96	69	
Genotypes (G)			507.22**	
PEG levels (T)			45698.57**	
G*T			517.89**	

Level of significance *$p<0.05$; **$p<0.01$ Legend: *TC*= tolerant check *SC*= susceptible check

Germination percentage at -1MPa was below 50% except for the aforementioned five lines and tolerant check which is similar to the results obtained by Sabesan and Saravanan (2016) and Govindaraj et al (2010) in their evaluation of 40 indica varieties and 21 pearl millet, respectively, under different PEG levels. The reduction in the germination percentage under decreasing water potential is attributed to the low hydraulic conductivity of the environment due to PEG application wherein PEG acts as an osmoticum that lowers the osmotic and water potential of the environment for seed germination; as a result water imbibition is inhibited thus affecting seed germination (Govindaraj et al 2010). Furthermore, water stress limits the activity of amylase enzymes which play an important role during seed germination. Amylase acts by hydrolyzing the endosperm starch into metabolizable sugars, which provide the energy for the growth of roots and shoots (Nauriere et al 1992). The reduced activity of such enzymes due to water stress has negative effects on carbohydrate metabolism (Kaur et al 2000, Zeid and Shedeed 2006), thereby affecting seed germination.

Table 3. Means of germination rate of different rainfed lowland rice genotypes under PEG-induced drought stress

Genotypes	Germination rate			
	Water potential			Percent Difference between 0MPa and -1MPa
	0MPa	-0.5MPa	-1MPa	
AL-108	99	99	91a	2
AL-87	99	99	80b	5
AL-97	100	99	84b	4
AL-55	100	99	83b	5
AL-5	99	83	88b	3
AL-54	99	96	81b	5
AL-83	99	99	79b	6
AL-12	100	94	68b	9
AL-17	99	96	45b	19
AL-107	99	90	67b	10
AL-32	99	97	39c	22
AL-57	98	98	58b	13
AL-88	99	83	88b	3
AL-9	100	99	59b	13
AL-91	100	99	45c	19
AL-23	100	95	68b	9
AL-27	100	99	69b	9
AL-104	98	95	44c	19
AL-18	100	100	77b	6
AL-45	100	97	89a	3
AL-56	99	99	72b	8
AL-109	100	96	77b	7
AL-52	100	96	51b	16
NSIC Rc14 (TC)	99	100	83b	5
PSB Rc82 (SC)	99	97	40c	21
Treatment Mean	100	96	69	
Genotypes (G)			507.22**	
PEG levels (T)			45698.57**	
G*T			517.89**	

Level of significance *$p<0.05$; **$p<0.01$ Legend: *TC*= tolerant check *SC*= susceptible check

Germination rate, which indicates the speed of germination, was significantly influenced by the interaction effect of water potential levels and rice genotypes during germination. A higher rate of germination, even under drought conditions, is considered a desirable feature of rice genotypes. Water is the main factor stimulating seed germination. If water potential is lower than the desirable amount, water imbibition is hindered and germination will decline. Rate of germination

(Table 2) was reduced with increasing water deficit (100% in drought-susceptible control to 40% at -1MPa). Germination rate was severely affected at -1MPa and slightly decreased at -0.5MPa compared with the drought-tolerant control. Genotypes AL-108, AL-55, AL-97, AL-5 and NSIC Rc14 were able to maintain high germination rate even under -1MPa but the highest rate (91%) was obtained by AL-108. As water potential decreased germination percentage and germination rate were reduced in response to drought as observed in lentils (Kafi et al 2005), barley (Hellal et al 2018), wheat (Rana et al 2017) and rice (Akte et al 2016). In this study, PEG treatment had a negative effect on the seed germination of different rice genotypes. This could be due to hampered water and nutrient absorption, reduced solubility and oxygen diffusion due to decreasing water potential as claimed by Hellal et al (2018).

Effect of Drought on Root and Shoot Length

Root and shoot length measured at the end of the experiment in different rice genotypes were significantly different by varying drought levels, as well as the interaction between rice genotypes and drought treatment (Tables 4 and 5). The root and shoot are the two most important plant organs that are affected by water stress considering that a robust and longer root system is needed to reach for water deep down in the soil, while a vigorous shoot is needed for maintaining photosynthetic activity under stress conditions.

Table 4. Means of root length of different rainfed lowland rice genotypes under PEG-induced drought stress

Genotypes	Root length			
	Water potential			Percent Difference between 0MPa and -1MPa
	0MPa	-0.5MPa	-1MPa	
AL-108	6.33ab	5.44abc	2.50a	22
AL-87	6.60a	5.65ab	1.96ab	27
AL-97	6.56a	3.99bcdef	1.41ab	32
AL-55	5.55abcd	4.67abcdef	0.97ab	35
AL-5	4.32defg	3.18ef	1.70ab	22
AL-54	3.95g	3.75cdef	1.85ab	18
AL-83	5.95abc	5.24abcd	1.55ab	29
AL-12	5.36abcde	3.70def	0.97ab	35
AL-17	4.88cdefg	4.22abcdef	0.55b	40
AL-107	4.76cdefg	3.56def	0.76b	36
AL-32	4.69cdefg	4.53abcdef	0.72b	37
AL-57	4.99cdefg	4.14abcdef	1.05ab	33
AL-88	5.29abcdef	3.66def	0.82b	37
AL-9	4.27defg	4.88abcde	0.92ab	32
AL-91	5.09bcdefg	4.48abcdef	1.29ab	30
AL-23	4.85cdefg	3.30ef	0.92ab	34
AL-27	5.38abcde	5.62ab	1.72ab	26

Table 4. continued

Genotypes	Root length			
	Water potential			Percent Difference between 0MPa and -1MPa
	0MPa	-0.5MPa	-1MPa	
AL-104	5.48abcd	4.19abcdef	0.73b	38
AL-18	4.85cdefg	3.15f	1.15ab	31
AL-45	6.33ab	4.57abcdef	0.69b	40
AL-56	5.31abcde	3.64def	0.62b	40
AL-109	6.34ab	5.28abcd	1.19ab	34
AL-52	4.11efg	4.70abcdef	0.43b	40
NSIC Rc14 (TC)	3.99fg	3.60def	1.38ab	24
PSB Rc82 (SC)	6.31ab	5.84a	0.50b	43
Treatment Mean	5.26	4.36	1.13	
Genotypes (G)			5.77**	
PEG levels (T)			649.10**	
G*T			1.92**	

Level of significance *p<0.05; **p<0.01Legend: *TC*= tolerant check *SC*= susceptible check

Table 5. Means of shoot length of different rainfed lowland rice genotypes under PEG-induced drought stress

Genotypes	Shoot length			
	Water potential			Percent Difference between 0MPa and -1MPa
	0MPa	-0.5MPa	-1MPa	
AL-108	7.58bcdef	5.53a	0.17cd	48
AL-87	7.38cdef	4.79abc	0.43abcd	44
AL-97	7.00fgh	4.86abc	1.04a	37
AL-55	7.41cdef	5.01abc	0.95ab	39
AL-5	7.94abcd	5.38a	0.71abcd	42
AL-54	8.00abc	5.47a	0.36abcd	46
AL-83	7.54cdef	4.79abc	0.23cd	47
AL-12	7.15efgh	4.40abc	0.27bcd	46
AL-17	6.62gh	3.71bc	0.06d	49
AL-107	6.47h	3.60c	0.07cd	49
AL-32	7.06fgh	4.73abc	0.08cd	49
AL-57	7.36cdef	5.49a	0.12cd	48
AL-88	8.42a	5.65a	0.22cd	47
AL-9	6.54gh	4.86abc	0.22cd	47
AL-91	6.91fgh	4.62abc	0.14cd	48
AL-23	7.19efg	4.79abc	0.26cd	47
AL-27	7.93abcd	4.56abc	0.13cd	48
AL-104	7.24defg	3.78bc	0.12cd	48
AL-18	6.59gh	4.63abc	0.40abcd	44
AL-45	7.83abcde	5.54a	0.47abcd	44
AL-56	7.39cdef	4.75abc	0.39abcd	45

Table 5. continued

Genotypes	Shoot length			
	Water potential			Percent Difference between 0MPa and -1MPa
	0MPa	-0.5MPa	-1MPa	
AL-109	8.28ab	5.37a	0.76abc	42
AL-52	6.58gh	5.04ab	0.05d	49
NSIC Rc14 (TC)	7.64bcde	5.77a	0.36bcd	46
PSB Rc82 (SC)	7.61bcde	2.88d	0.02e	50
Treatment Mean	7.6	5.5	0.17	
Genotypes (G)			2.71**	
PEG levels (T)			1883.84**	
G*T			0.86**	

Level of significance *$p<0.05$; **$p<0.01$Legend: *TC*= tolerant check *SC*= susceptible check

Similarly, shoot length was severely affected by PEG-induced drought stress (Table 6). This conforms to the earlier findings of Lawlor (1970) wherein there was retardation of shoot and root growth in plants due to increasing moisture stress that was imposed due to the addition of high levels of PEG in the media. In this study, it was apparent that some genotypes such as AL-97 and AL-55 had the longest shoot length compared with the rest of the genotypes under -1MPa. Furthermore, shoot growth was more severely affected compared to roots in terms of length when grown under -1MPa. Reduction in shoot length is one of the well-known adaptive mechanism of plants to drought conditions. The decrease in shoot length aids in the reduction of transpiration and optimizes the distribution of assimilates for root growth (Chaves et al 2002).

Effect of Drought on Seedling Vigour Index

The Seedling *Vigor Index* (SVI) has been used as a drought index to evaluate the effect of drought tolerance on the seedling growth stage. Seedling vigor is the ability of a seed to emerge rapidly from soil or water, mainly measured by seed germination rate and early seedling growth (Huang et al 2004). It has several indicators root length, shoot length and dry weight during early seedling stage (Hellal et al 2018, Redoña and Mackill 1996), germination percentage and rate during seed germination stage (Wang et al 2010).

Rice genotypes, drought, and their interaction significantly affected the seedling vigour index of rice seedlings (Figure 1A and 1B). The seedling vigor index reduces as drought stress increases. Genotypes AL-5, AL-97 and AL-108 had the highest seedling vigour index and exceeded the performance of tolerant check NSIC Rc14 under the highest drought condition. The higher seedling vigor was due to the higher germination percentage, and root and shoot length of these genotypes. The test genotypes had varying reactions to the drought treatment (PEG) in the two trials. The susceptible check PSB Rc82 was confirmed to be susceptible at severe drought stress (-1MPa), while tolerant check (NSIC Rc14) sustained vigour but at a relatively lower level than the top three aforementioned genotypes.

Table 6. Means of root to shoot ratio of different rainfed lowland rice genotypes under PEG-induced drought stress

Genotypes	Root/Shoot Ratio			
	PEG levels			Percent Difference between 0MPa and -1MPa
	0MPa	-0.5MPa	-1MPa	
AL-108	0.83ab	1.30	3.44	31
AL-87	0.79abcd	1.02	3.14	27
AL-97	0.63efgh	1.16	1.96	22
AL-55	0.63efgh	0.98	1.87	25
AL-5	0.70abcdefgh	1.41	1.68	21
AL-54	0.57h	0.79	1.02	29
AL-83	0.79abcd	0.94	2.00	22
AL-12	0.78abcd	0.94	1.64	18
AL-17	0.76abcdefg	0.70	0.93	5
AL-107	0.67abcdefgh	0.69	1.89	24
AL-32	0.30i	0.94	0.75	22
AL-57	0.65efgh	0.92	1.81	22
AL-88	0.8ab	1.13	1.14	1
AL-9	0.86a	1.40	1.32	7
AL-91	0.81a	1.02	1.73	18
AL-23	0.72abcdefgh	0.98	1.76	21
AL-27	0.73abcdefgh	1.17	1.87	22
AL-104	0.60fgh	0.82	1.49	21
AL-18	0.53h	0.51	1.47	24
AL-45	0.69abcdefgh	0.38	1.71	21
AL-56	0.81a	1.04	1.21	10
AL-109	0.70abcde	1.04	1.44	17
AL-52	0.61efg	1.10	0.79	24
NSIC Rc14	0.80abc	1.13	1.56	16
PSB Rc82	0.71abcdefgh	1.12	1.59	19
Treatment Mean	0.83	1.30	3.44	
Genotypes (G)	3.92^{ns}			
PEG levels (T)	190.71**			
G*T	4.69^{ns}			

Level of significance *$p<0.05$; **$p<0.01$ Legend: *TC*= tolerant check *SC*= susceptible check

The increasing level of PEG resulted in the decreasing seedling vigour in all genotypes. The higher concentration of PEG (-1MPa) inhibits the survival and growth of rice seedlings by lowering the water potential, thereby reducing the availability of water supply during seed imbibition. Early researches revealed that PEG-induced osmotic stress causes hydrolysis of storage compounds that leads to the lowering of internal osmotic potentials of the seed (Hampson and Simpson 1990). The higher level of Polyethylene glycol (6000) hampers the nutrient transfer to the embryo due to limited water (Nurhayati et al 2017). Higher germination rate and good seedling height contributed to good vigour index. Hence, as the germination rate and seedling growth are negatively affected by increasing level of drought, seedling vigour inevitably reduced. Shoot length, root length, germination percentage and dry weight were reduced with increasing magnitude when seedlings were exposed to increasing drought level. Similar results were obtained by Hellal et al (2018) in barley and Faijunnahar (2017) in wheat. Regression analyses also showed a significant relationship $R^2=0.91$ (Figure 1A), with rate of change in seedling vigour index relative to PEG level. There is a negative relationship between PEG levels and seedling vigour, which clearly shows that as the stress level increases, the seedling vigour index decreases with increasing drought levels. Therefore, this study confirmed that the use of PEG could certainly work as a drought inducer in rice during the seedling stages by affecting important seedling morphological traits that are affected by a drought regime. Namuco et al (2009) demonstrated that plant vigour effectively increases drought tolerance in rice. The measure of plant vigour was also shown to be an efficient and cost-effective method in the early screening of wheat genotypes (Jenks et al 2007, Choi 2003). Overall, five rainfed lowland rice genotypes were identified that showed potential tolerance to drought stress, namely: AL-108, AL-87, AL-97, AL-55 and AL-5 while AL-52 and NSIC Rc82 were found to be susceptible to PEG-induced drought stress.

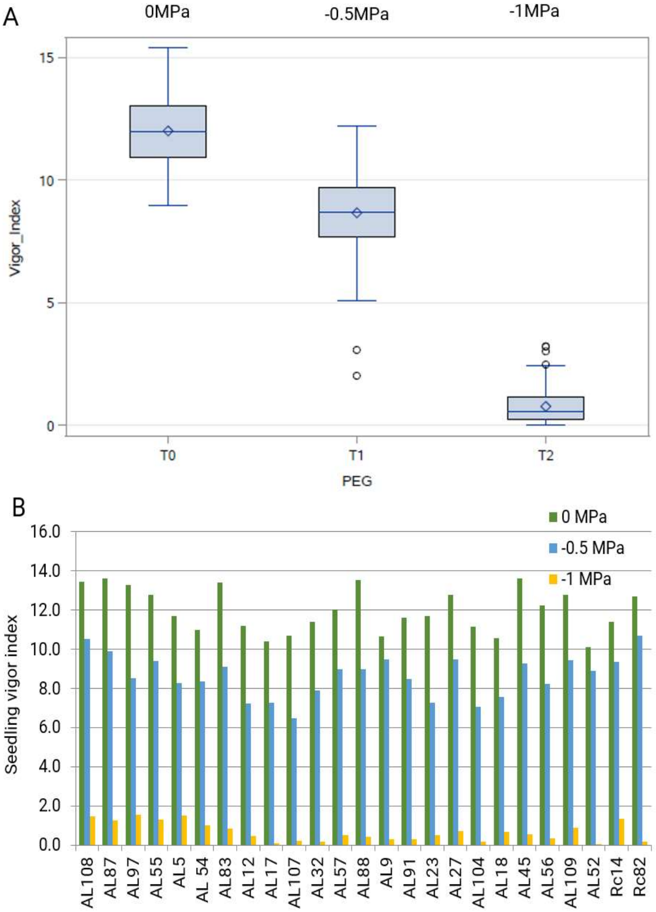

Figure 1. A) Relationship between vigor index with PEG levels B.) Effects on vigour index on different rainfed lowland rice genotypes under drought stress

Effect of Drought on Seedling Biomass

The root-shoot ratio was significantly affected by PEG levels but not by the genotypes and its interaction (Table 6).

Clearly, severe drought negatively affected the seedling dry biomass and this is more apparent in severe drought regimes. The reduction in plant dry weight is supported by reduced plant growth as a function of shorter roots and shoots under drought conditions. It has been established that components of plant growth ie, plant height, number of tillers and leaf area are affected, first by delaying germination and subsequently the reduced growth of shoots and overall reduction of dry matter (Shekari 2000).

The highest root-shoot ratio was observed higher in AL-108 (3.44) and lowest by AL-108 (0.75) under severe stress (-1MPa). This increase in root-shoot ratio under increasing PEG levels is attributed to the increase in root dry weight and decrease in shoot dry weight. It is a known mechanism of drought-tolerant plants to inhibit shoot length and allocate the reserve energy for root growth to allow more water uptake and reduce water loss in the leaves. Susceptible check (PSB Rc82) had a root-shoot ratio significantly similar to tolerance check NSIC Rc14, though it had a lower vigor index. Therefore, this genotype might be having tolerance to drought in terms of root/shoot ratio.

CONCLUSION

Varying drought levels imposed by using PEG reduced germination percentage, germination rate, root length, shoot length and seedling vigour index. These seedling parameters significantly reduced at -0.5MPa and -1MPa drought regimes. However, the root-shoot ratio increased with increasing drought stress. Five rice genotypes were found to have tolerance to drought stress namely: AL-108, AL-87, AL-97, AL-55 and AL-5 based on all parameters evaluated. Among the five tolerant genotypes AL-97 had relatively higher tolerance based on seedling vigour, however, considering root-shoot ratio, AL-108 was the most tolerant under -1MPa.

On the other hand, AL-52 and NSIC Rc 82 were susceptible to PEG-induced drought based on their seedling vigour. However, susceptible check PSB Rc82 might be showing a degree of tolerance based on root-shoot ratio. NSIC Rc14, known as a tolerant genotype, also had excellent growth and seedling vigour performance under different levels of PEG. Field evaluation is recommended to further validate tolerance observed under PEG-induced drought conditions.

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AUTHOR CONTRIBUTIONS

MGN was responsible for the study design and manuscript preparation.

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AVAILABILITY OF DATA AND MATERIALS

The data and materials generated or analyzed during this study are available upon request from the corresponding author.

ETHICAL CONSIDERATION

This study does not involve human participants, animals, or any other ethical concerns. Therefore, no ethical approval was required.

COMPETING INTEREST

The author declares no competing interests.

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