

## RESEARCH ARTICLE

# Assessment of germination performance and early seedling growth of Malaysian *indica* rice genotypes under drought conditions for strategic cropping during water scarcity

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## ABSTRACT

Drought is a major abiotic constraint on rice (*Oryza sativa* L.) production in Malaysia. Malaysian Agricultural Research and Development Institute (MARDI) has released more than 50 *indica* rice genotypes so far. However, little has been studied about their drought tolerance. The study aimed to evaluate the germination and early seedling growth performances of 15 rice genotypes under polyethylene glycol (PEG) 6000-induced drought stresses. Four osmotic potentials of PEG 6000 (0, -0.3, -0.6, -0.9 MPa) were used in the study denoted as control, low, moderate and severe stresses respectively. Data on seed germination percentage, germination index, germination energy, germination rate, seedling height, seedling vigor I and II, fresh and dry weight of seedlings were measured. Studied parameters varied greatly among genotypes with different osmotic stresses. The findings suggested most of the rice genotypes showed an apparent reduction in germination and growth traits, while MR211 showed less reduction under all of the stresses. The Multivariate clustering grouped genotypes into five different clusters, where cluster V (MR220, MR269, MR253, MR297, MR303 and MR284) incorporated six genotypes showed better germination and growth under different osmotic potentials, followed by cluster IV (MR211 and MR307). Cluster II incorporated one genotype MARDI WARNA98 which had minimal germination and growth attributes. Principal component analysis (PCA) was performed to identify the maximum contributing variables for diversity, revealed maximum variation by first two components (81.8% and 7.7%) respectively. Therefore, PEG 6000 can be used as an efficient tool for discrimination and identification of drought tolerance in rice.

**Key words:** Drought, early seedling growth, germination, *indica* rice, *Oryza sativa*.

## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most significant staple crops worldwide for food security (Asibi et al., 2019). Almost 90% of the global rice is cultivated and consumed in Asia, while its production requires a semi-aquatic environment with high water requirements (Kumar et al., 2021). Its cultivation relies heavily on rainy subsistence farming, which is becoming progressively more vulnerable to droughts (Almazroui et al., 2020). Drought is a multilateral stress affecting the metabolism of plants at different stages, and it has been considered as a vital issue for plant breeders and agronomists (Wang et al., 2019). Water stress severely affects growth, development and yield in rice (Pamuta et al., 2022). Therefore, it is extremely important to maintain high productivity and grain quality of high-yielding rice genotypes under water stress.

Malaysia is one of the most vulnerable countries in the Asia-Pacific region to disasters such as droughts, landslides, floods, and climate change. Food security is now seriously threatened by drought stress in the developing world including Malaysia (Fen et al., 2015). The Malaysian government has

set a goal of 100% self-sufficiency level (SSL) in rice production line in order to meet demand, while currently achieves a considerably lower level of 75% SSL (Rahim et al., 2017). According to a previous study by Herman et al. (2015), one of the main causes of declining rice production in Malaysia is El Niño phenomenon, which disrupted agricultural activity in granary areas and caused 20% decrease in overall production. Moreover, the country experienced prolonged dry seasons and severe droughts due to an increase in global temperature as a result of climate change and severe depletion of watersheds (Payus et al., 2020). Therefore, a fundamental method to choose the tolerant genotypes to ensure enhanced production involves screening a wide group of readily accessible genotypes under drought conditions.

Seed germination and seedling growth properties are immensely vital factors for yield determination (Rauf et al., 2007). It has been reported by Swain et al. (2014) that seed germination and early seedling growth are certainly the most critical stages, strongly impaired by water stress. Therefore, selection of drought resistant genotypes in an earlier growing season seems to be most vital and efficient (Xie et al., 2013). Polyethylene glycol (PEG) 6000 is a non-ionic water polymer, unexpected to penetrate into plant tissue rapidly thus is widely used to induce water stress (Bagher et al., 2012), it has been used in the current study. Previous studies by Tang et al. (2019) suggested that in vitro screening with PEG is one of the trustworthy routes to screen drought-tolerant genotypes. More significantly, the approaches of using PEG 6000 to screen the drought tolerant rice genotypes are affordable and efficient (Shereen et al., 2019).

Rice genotypes with increased drought tolerance are required to reduce yield losses and boost overall production. Hence, the current research has been done to assess germination and early seedling growth responses of 15 Malaysian *indica* rice genotypes at different drought conditions to screen their drought tolerance, so that the tolerant genotypes can be suggested to plant during water scarcity.

## MATERIALS AND METHODS

### Plant material

A laboratory experiment was conducted at the Department of Biology, Universiti Putra Malaysia. Seeds of 15 rice (*Oryza sativa* L.) genotypes supplied by Malaysian Agricultural Research and Development Institute (MARDI), were used for this study: MARDI WANGI88 (accession number-MRGB13020), MARDI WARNA98 (MRGB13141), MR157 (MRGB08636), MR167 (MRGB08646), MR185 (MRGB08455), MR211 (MRGB11629), MR219 (MRGB11633), MR220 (MRGB11634), MR253 (MRGB12095), MR263 (MRGB12133), MR269 (MRGB12120), MR284 (MRGB12140), MR297 (MRGB13019), MR303 (MRGB13001) and MR307 (MRGB13005).

### Viable seed separation and seed sterilization

Flotation method was used for the separation of viable seeds prior to sterilization. Seeds of all genotypes were surface sterilized for 30 s through immersion in 70% ethanol. Afterward, the seeds were rinsed once with distilled water removing the ethanol. Then the seeds were stirred with 40% sodium hypochlorite solution for 20 min. Finally sterile distilled water was used to rinse the sterilized seeds four to five times (Abiri et al., 2016).

### Germination under different drought conditions

Germination of 15 rice genotypes was induced with four different water potential of polyethylene glycol (PEG 6000) (0, -0.3, -0.6 and -0.9 MPa) denoted as control, low, moderate and severe, respectively. Deionized water (0 MPa) was used as control. PEG 6000 was dissolved in deionized water according to the formula of Michel and Kaufmann (1973):

$$\Psi_s = - (1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T$$

where C is the concentration of PEG 6000 in g kg<sup>-1</sup> H<sub>2</sub>O and T is the temperature (25 °C).

Germination assays were performed by evenly distributing 10 seeds in glass jar for each of three replicates lined with Whatman filter paper (90 mm diameter). Sterile deionized water (8 mL) for control and desired amounts of osmotic solutions were placed in each jar to mimic drought stress. Seeds were germinated at 25 ± 2 °C, relative humidity of 50%-70% and 12:12 h photoperiod in growth room (Khan et al., 2019) for 14 d. Seeds were checked regularly for germination and were considered germinated if the emerged radicles measured

approximately 2 mm (Chunthaburee et al., 2014). The following germination attributes and seedling growth parameters were measured after 14 d growth:

Germination percentage (GP, %) = (Seeds normally germinated/Total seeds sown) × 100 (Abdul-Baki and Anderson, 1970)

Germination index (GI) =  $\sum Gt/Dt$ , where Gt is number of seeds germinated on a specific day, Dt is the corresponding number of days from the start of the experiment (Tang et al., 2019)

Germination energy (GE, %) = (Seeds germinated within 3 d/Total seeds sown) × 100 (Tang et al., 2019)

Germination rate (GR, %) = (Seeds germinated at 4 d/Total seeds sown) × 100 (Tang et al., 2019)

Seedling vigor index (SVI) = Seedling height (SH) × Germination percentage (GP) (Abdul-Baki and Anderson, 1970)

Seedling vigor index II (SVII) = Germination percentage (GP) × Dry weight (DW) (Pant and Bose, 2016)

An electronic scale was used to measure fresh seedling weight (FW) while the dry seedling weight (DW) was weighed after drying at 80 °C in an oven until constant weight is obtained (Yan, 2015). Seedling height (SH) was measured with a plastic ruler (Abdul-Baki and Anderson, 1970).

### Experimental design and statistical analyses

The experiment was designed in completely randomized design (CRD) with three replicates for each genotype per treatment. After collecting data, they were tested for normality, which indicated that data were normally distributed. ANOVA was done with a DSAASTAT version (1.101) (Onofri and Pannacci, 2014). Duncan's multiple range test (DMRT) ( $p \leq 0.05$ ) was used to compare the means where ANOVA indicated significant difference. Pearson's correlation was studied to find out the inter-correlations among the traits using SPSS window version 26 (IBM Corp., Armonk, New York, USA). Minitab version 19.0 (Minitab Ltd., Coventry, UK) was used for cluster analysis and principal component analysis (PCA) to find the similarity among genotypes and to display the ratio of total variance explained by various components and variables respectively.

## RESULTS

### Germination performances under different drought levels

The detailed comparative analysis of germination performances of 15 rice genotypes was performed in different PEG 6000 concentrations (Table 1). The study found that as the level of drought increased, germination performances (GP, GI, GE and GR) decreased significantly; however, the response of the genotypes was different at different drought levels. All the genotypes presented germination percentage (GP) higher than 70% under low drought except MARDI WARNA98. Similarly, under moderate and severe drought all genotypes had GP higher than 50% and 40%, respectively, except MARDI WARNA98. Maximum GP was observed in MR211 in all of the stresses. Germination index (GI) was maximal in MR211 in all of the drought levels, while MARDI WARNA98 was minimal. Most of the genotypes scored germination energy (GE) higher than 50 under low drought stress representing higher speed of germination except MARDI WANGI88, MARDI WARNA98 and MR220. Under moderate drought, 14 genotypes resulted in GE higher than 30 except MARDI WARNA98, representing lower speed compared to low drought. In addition, under severe drought stress, GE value was minimal for all genotypes among all the drought levels. Similarly, germination rate (GR) value was also observed to decrease as the drought level increases in all genotypes.

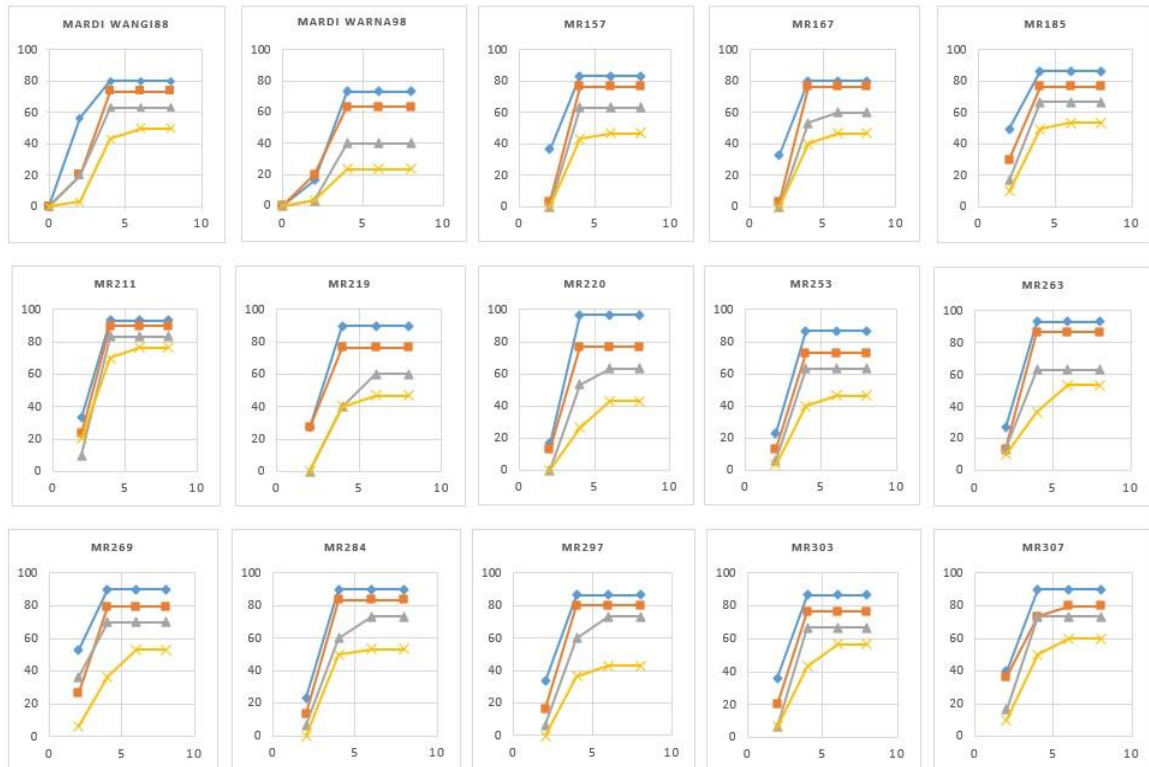
### Germination time course

The cumulative germination percentage decreases with reduced water potential irrespective of the genotype investigated. The finding suggested that, the genotype MARDI WARNA98 had minimal GP in all of the stress levels. Germination of rice was recorded to be delayed and inhibited increasingly at reduced water potential. The minimal effect was observed in MR211. The cumulative germination time course at PEG 6000 induced water potentials for each rice genotype studied has been presented in Figure 1.

**Table 1.** Germination performances of 15 rice genotypes under different drought conditions. Means  $\pm$  SE in the same column with the same letter do not differ significantly according to DMRT ( $P \leq 0.05$ ). SE: Standard error of the mean; GP: germination percentage; GI: germination index; GE: germination energy; GR: germination rate.

Genotypes	Treatments	GP	GI	GE	GR
MARDI WANGI88	Control	80.0 $\pm$ 5.77b-f	3.6 $\pm$ 0.22a-e	76.7 $\pm$ 3.33a-e	80.00 $\pm$ 5.77a-f
	Low	73.3 $\pm$ 3.33d-h	2.6 $\pm$ 0.17g-m	46.7 $\pm$ 3.33h-n	73.33 $\pm$ 3.33b-g
	Moderate	63.3 $\pm$ 3.33g-k	2.2 $\pm$ 0.19k-s	36.7 $\pm$ 3.33k-p	63.33 $\pm$ 3.33e-k
MARDI WARNA98	Severe	50.0 $\pm$ 0.00k-n	1.5 $\pm$ 0.11s-w	30.0 $\pm$ 5.77m-p	46.67 $\pm$ 3.33i-m
	Control	73.3 $\pm$ 3.33d-h	2.5 $\pm$ 0.21h-n	46.7 $\pm$ 6.67h-n	73.33 $\pm$ 3.33b-g
	Low	63.3 $\pm$ 3.33g-k	2.3 $\pm$ 0.10j-q	46.7 $\pm$ 6.67h-n	63.33 $\pm$ 3.33e-l
MR157	Moderate	40.0 $\pm$ 5.77n	1.2 $\pm$ 0.19vwx	20.0 $\pm$ 0.00p	40.00 $\pm$ 5.77mn
	Severe	23.3 $\pm$ 3.33o	0.8 $\pm$ 0.20x	16.7 $\pm$ 8.82p	23.33 $\pm$ 3.33n
	Control	83.3 $\pm$ 3.33a-e	3.4 $\pm$ 0.25a-f	80.0 $\pm$ 0.00a-d	83.33 $\pm$ 3.33a-e
MR167	Low	76.7 $\pm$ 6.67c-g	2.5 $\pm$ 0.19h-o	60.0 $\pm$ 5.77d-j	76.67 $\pm$ 6.67a-f
	Moderate	63.3 $\pm$ 3.33g-k	1.9 $\pm$ 0.05m-v	40.0 $\pm$ 5.77j-p	63.33 $\pm$ 3.33e-k
	Severe	46.7 $\pm$ 3.33lmn	1.3 $\pm$ 0.12u-x	20.0 $\pm$ 5.77p	43.33 $\pm$ 3.33k-n
MR185	Control	80.0 $\pm$ 5.77b-f	3.1 $\pm$ 0.29a-i	70.0 $\pm$ 10.00a-g	80.00 $\pm$ 5.77a-f
	Low	76.7 $\pm$ 6.67c-g	2.4 $\pm$ 0.38i-p	53.3 $\pm$ 8.82f-l	76.67 $\pm$ 6.67a-f
	Moderate	60.0 $\pm$ 5.77h-l	1.8 $\pm$ 0.13n-w	36.7 $\pm$ 3.33k-p	53.33 $\pm$ 3.33g-m
MR211	Severe	46.7 $\pm$ 3.33lmn	1.3 $\pm$ 0.10u-x	23.3 $\pm$ 3.33op	40.00 $\pm$ 5.77mn
	Control	86.7 $\pm$ 3.33a-d	3.7 $\pm$ 0.23ab	83.3 $\pm$ 6.67abc	86.67 $\pm$ 3.33a-d
	Low	76.7 $\pm$ 3.33c-g	3.0 $\pm$ 0.10b-j	70.0 $\pm$ 5.77a-g	76.67 $\pm$ 3.33a-f
MR219	Moderate	66.7 $\pm$ 6.67f-j	2.4 $\pm$ 0.29i-o	60.0 $\pm$ 5.77d-j	66.67 $\pm$ 6.67d-i
	Severe	53.3 $\pm$ 3.33j-n	1.7 $\pm$ 0.17p-w	26.7 $\pm$ 3.33nop	50.00 $\pm$ 5.77h-m
	Control	93.3 $\pm$ 3.33ab	3.6 $\pm$ 0.35abc	90.0 $\pm$ 5.77a	93.33 $\pm$ 3.33ab
MR220	Low	90.0 $\pm$ 0.00abc	3.3 $\pm$ 0.22a-g	73.3 $\pm$ 3.33a-f	90.00 $\pm$ 0.00abc
	Moderate	83.3 $\pm$ 3.33a-e	2.8 $\pm$ 0.21f-l	60.0 $\pm$ 5.77d-j	83.33 $\pm$ 3.33a-e
	Severe	76.7 $\pm$ 6.67c-g	2.6 $\pm$ 0.18g-m	43.3 $\pm$ 3.33i-o	70.00 $\pm$ 11.55c-h
MR253	Control	90.0 $\pm$ 0.00abc	3.4 $\pm$ 0.07a-f	83.3 $\pm$ 3.33abc	90.00 $\pm$ 0.00abc
	Low	76.7 $\pm$ 3.33c-g	2.9 $\pm$ 0.20e-l	60.0 $\pm$ 5.77d-j	76.67 $\pm$ 3.33a-f
	Moderate	60.0 $\pm$ 5.77h-l	1.6 $\pm$ 0.26r-w	20.0 $\pm$ 11.55p	40.00 $\pm$ 15.28mn
MR263	Severe	46.7 $\pm$ 3.33lmn	1.3 $\pm$ 0.05u-x	16.7 $\pm$ 8.82p	40.00 $\pm$ 0.00mn
	Control	96.7 $\pm$ 3.33a	3.3 $\pm$ 0.13a-g	66.7 $\pm$ 3.33b-h	96.67 $\pm$ 3.33a
	Low	76.7 $\pm$ 3.33c-g	2.5 $\pm$ 0.11g-m	46.7 $\pm$ 3.33h-n	76.67 $\pm$ 3.33a-f
MR269	Moderate	63.3 $\pm$ 3.33g-k	1.8 $\pm$ 0.19n-w	30.0 $\pm$ 10.00m-p	53.33 $\pm$ 12.02g-m
	Severe	43.3 $\pm$ 3.33mn	1.2 $\pm$ 0.05wx	20.0 $\pm$ 5.77p	26.67 $\pm$ 3.33n
	Control	86.7 $\pm$ 6.67a-d	3.2 $\pm$ 0.37a-h	76.7 $\pm$ 8.82a-e	86.67 $\pm$ 6.67a-d
MR284	Low	73.3 $\pm$ 3.33d-h	2.5 $\pm$ 0.29g-m	56.7 $\pm$ 8.82e-k	73.33 $\pm$ 3.33b-g
	Moderate	63.3 $\pm$ 3.33g-k	2.0 $\pm$ 0.13m-u	36.7 $\pm$ 6.67k-p	63.33 $\pm$ 3.33e-k
	Severe	50.0 $\pm$ 5.77k-n	1.5 $\pm$ 0.24t-x	23.3 $\pm$ 6.67op	43.33 $\pm$ 12.02k-n
MR297	Control	93.3 $\pm$ 3.33ab	3.4 $\pm$ 0.12a-f	80.0 $\pm$ 5.77a-d	93.33 $\pm$ 3.33ab
	Low	86.7 $\pm$ 3.33a-d	2.9 $\pm$ 0.24d-l	60.0 $\pm$ 5.77d-j	86.67 $\pm$ 3.33a-d
	Moderate	63.3 $\pm$ 3.33g-k	2.2 $\pm$ 0.19l-t	46.7 $\pm$ 3.33h-n	63.33 $\pm$ 3.33e-k
MR303	Severe	53.3 $\pm$ 3.33j-n	1.7 $\pm$ 0.26o-w	33.3 $\pm$ 8.82l-p	46.67 $\pm$ 8.82i-m
	Control	90.0 $\pm$ 5.77abc	3.8 $\pm$ 0.22a	83.3 $\pm$ 3.33abc	90.00 $\pm$ 5.77abc
	Low	80.0 $\pm$ 5.77b-f	2.9 $\pm$ 0.26c-k	60.0 $\pm$ 10.00d-j	80.00 $\pm$ 5.77a-f
MR307	Moderate	70.0 $\pm$ 5.77e-i	2.8 $\pm$ 0.22f-l	53.3 $\pm$ 3.33f-l	70.00 $\pm$ 5.77c-h
	Severe	53.3 $\pm$ 3.33j-n	1.6 $\pm$ 0.24q-w	26.7 $\pm$ 3.33nop	43.33 $\pm$ 8.82k-n
	Control	90.0 $\pm$ 5.77abc	3.4 $\pm$ 0.26a-f	86.7 $\pm$ 3.33ab	90.00 $\pm$ 5.77abc
MR307	Low	83.3 $\pm$ 3.33a-e	2.9 $\pm$ 0.19e-l	66.7 $\pm$ 3.33b-h	83.33 $\pm$ 3.33a-e
	Moderate	70.0 $\pm$ 5.77e-i	2.3 $\pm$ 0.27j-r	53.3 $\pm$ 6.67f-l	66.67 $\pm$ 6.67d-i
	Severe	53.3 $\pm$ 6.67j-n	1.6 $\pm$ 0.16r-w	33.3 $\pm$ 3.33l-p	43.33 $\pm$ 3.33k-n
MR307	Control	86.7 $\pm$ 6.67a-d	3.3 $\pm$ 0.13a-g	76.7 $\pm$ 3.33a-e	86.67 $\pm$ 6.67a-d
	Low	80.0 $\pm$ 5.77b-f	2.9 $\pm$ 0.32e-l	70.0 $\pm$ 10.00a-g	80.00 $\pm$ 5.77a-f
	Moderate	73.3 $\pm$ 3.33d-h	2.2 $\pm$ 0.34k-s	43.3 $\pm$ 41.53i-o	60.00 $\pm$ 10.00f-m
MR307	Severe	43.3 $\pm$ 3.33mn	1.3 $\pm$ 0.10u-x	20.0 $\pm$ 5.77p	40.00 $\pm$ 5.77mn
	Control	86.7 $\pm$ 3.33a-d	3.5 $\pm$ 0.15a-f	83.3 $\pm$ 3.33abc	86.67 $\pm$ 3.33a-d
	Low	76.7 $\pm$ 3.33c-g	2.9 $\pm$ 0.24e-l	73.3 $\pm$ 3.33a-f	76.67 $\pm$ 3.33a-f
MR307	Moderate	66.7 $\pm$ 3.33f-j	2.2 $\pm$ 0.03l-t	50.0 $\pm$ 0.00g-m	66.67 $\pm$ 3.33d-j
	Severe	56.7 $\pm$ 3.33i-m	1.7 $\pm$ 0.28q-w	30.0 $\pm$ 10.00m-p	43.33 $\pm$ 8.82k-n
	Control	90.0 $\pm$ 5.77abc	3.6 $\pm$ 0.29a-d	83.3 $\pm$ 6.67abc	90.00 $\pm$ 5.77abc
MR307	Low	80.0 $\pm$ 5.77a-d	3.2 $\pm$ 0.12a-h	63.3 $\pm$ 3.33c-i	76.67 $\pm$ 3.33a-f
	Moderate	73.3 $\pm$ 6.67d-h	2.6 $\pm$ 0.32g-m	56.7 $\pm$ 8.82e-k	73.33 $\pm$ 6.67b-g
	Severe	60.0 $\pm$ 5.77h-l	2.0 $\pm$ 0.13m-u	36.7 $\pm$ 3.33k-p	53.33 $\pm$ 8.82g-m





**Figure 1.** Germination time course of 15 Malaysian *indica* rice genotypes for four different drought stresses. Time-course changes in seed germination exposed to increasing doses PEG6000 induced drought levels has been plotted between germination percentage (x-axis) and days of germination (y-axis). Blue, orange, grey and yellow color represents Control (0 MPa); Low (-0.3 MPa); Moderate (-0.6 MPa); and Severe (-0.9 MPa) conditions respectively.

### Seedling growth response under drought

To further analyze the effect of different drought stresses on these genotypes, seedling growth traits were recorded (Table 2). The highest seedling height (SH) was observed in MR303, while MARDI WARNA98 showed minimum SH in low drought. In moderate drought, MR303 and MR284 revealed maximum SH. MR211 resulted in maximum SH in severe drought, whereas MARDI WARNA98 represented minimum SH in all of the drought conditions. Furthermore, the maximum fresh weight (FW) was observed in MR211, while minimum FW was recorded in MR219 under low, moderate and severe droughts. The dry weight (DW) was maximal in the genotypes MR307, MR297 and MR211, while it was minimal in the genotypes MARDI WARNA98, MR157 and MR220 under low, moderate and severe stresses respectively. Further, to assess the effect of drought on seedling growth performance (seedling size, health and growth rate), seedling vigor I and II (SVI, SVII) were recorded in Table 2. The SVI was observed to be maximal in MR211 and minimal in MARDI WARNA98 in all stress levels. The genotype MR211 revealed maximum seedling vigor II (SVII) in all of the three drought levels. However, SVII was observed minimal in MARDI WARNA98.

### Relationship of germination parameters and seedling growth traits under drought stress

Table 3 presents the correlations among all the studied traits. The results revealed that germination percentage (GP) presented significant and positive relationship with other germination parameters and growth attributes. The highest significant correlation was observed between GP and GR. All other parameters were positively correlated to each other.

**Table 2.** Seedling growth performance under different drought levels. Means  $\pm$  SE in the same column with the same letter do not differ significantly according to DMRT ( $p \leq 0.05$ ). SE: Standard error of the mean; SH: seedling height; FW: fresh weight; DW: dry weight; SVI: seedling vigor I; SVII: seedling vigor II.

Genotypes	Treatments	SH cm	FW mg	DW mg	SVI	SVII
MARDI WANGI88	Control	8.3 $\pm$ 0.21g-q	58.8 $\pm$ 0.60d-g	31.5 $\pm$ 0.29d-g	666.07 $\pm$ 60.43h-m	2519.33 $\pm$ 158.83d-i
	Low	6.9 $\pm$ 0.68n-v	52.9 $\pm$ 1.06i-p	27.0 $\pm$ 0.43k-o	501.27 $\pm$ 28.92l-s	1975.00 $\pm$ 62.13j-p
	Moderate	5.8 $\pm$ 0.32s-w	48.3 $\pm$ 0.33o-u	24.7 $\pm$ 0.89o-t	366.87 $\pm$ 40.02o-v	1558.67 $\pm$ 30.03o-u
	Severe	5.3 $\pm$ 0.76t-w	42.7 $\pm$ 0.33w-zA	22.4 $\pm$ 0.81r-w	266.50 $\pm$ 38.19u-x	1121.67 $\pm$ 40.45u-y
MARDI WARNA98	Control	6.7 $\pm$ 0.67o-v	55.2 $\pm$ 0.40g-k	26.4 $\pm$ 0.99l-q	491.17 $\pm$ 52.50m-t	1930.00 $\pm$ 34.08k-q
	Low	6.4 $\pm$ 1.02p-w	50.4 $\pm$ 0.32l-s	24.1 $\pm$ 1.48o-u	409.47 $\pm$ 84.56o-u	1535.60 $\pm$ 178.01o-u
	Moderate	4.4 $\pm$ 0.61wxy	42.7 $\pm$ 0.33 w-zA	21.5 $\pm$ 0.97uvw	176.30 $\pm$ 37.44vwx	856.60 $\pm$ 116.28yz
	Severe	3.3 $\pm$ 0.69xy	40.7 $\pm$ 0.37yzA	20.4 $\pm$ 0.88w	76.70 $\pm$ 17.51x	480.53 $\pm$ 89.95z
MR157	Control	9.6 $\pm$ 0.63b-l	58.6 $\pm$ 1.15d-h	28.4 $\pm$ 0.32h-m	807.20 $\pm$ 84.55c-j	2366.00 $\pm$ 122.02e-k
	Low	9.3 $\pm$ 0.86c-m	53.9 $\pm$ 0.64h-n	24.1 $\pm$ 0.94o-u	704.27 $\pm$ 58.22g-m	1842.67 $\pm$ 125.22m-s
	Moderate	9.1 $\pm$ 0.40d-m	48.1 $\pm$ 0.33p-u	20.9 $\pm$ 0.14vw	575.83 $\pm$ 19.45k-o	1321.80 $\pm$ 74.44t-y
	Severe	7.4 $\pm$ 0.77m-t	42.2 $\pm$ 1.16x-zA	20.5 $\pm$ 0.75w	340.57 $\pm$ 9.44q-v	960.93 $\pm$ 93.63w-z
MR167	Control	9.6 $\pm$ 0.54b-l	56.4 $\pm$ 0.85g-i	26.7 $\pm$ 0.64k-p	765.60 $\pm$ 45.12e-k	2139.33 $\pm$ 163.26g-n
	Low	9.1 $\pm$ 0.83d-m	53.1 $\pm$ 2.10i-o	24.4 $\pm$ 0.81o-u	704.10 $\pm$ 112.25g-m	1865.67 $\pm$ 122.52l-s
	Moderate	8.7 $\pm$ 1.15f-o	47.6 $\pm$ 1.17q-v	23.5 $\pm$ 0.87q-w	522.17 $\pm$ 98.97l-r	1416.67 $\pm$ 171.50s-w
	Severe	6.8 $\pm$ 0.62o-v	43.2 $\pm$ 0.46v-z	21.0 $\pm$ 0.58vw	322.13 $\pm$ 54.30r-v	980.00 $\pm$ 75.72wxy
MR185	Control	11.6 $\pm$ 0.42ab	61.2 $\pm$ 1.41def	32.4 $\pm$ 0.47b-f	1001.47 $\pm$ 10.02abc	2814.00 $\pm$ 147.09cde
	Low	9.0 $\pm$ 0.28d-n	57.6 $\pm$ 1.22f-i	29.5 $\pm$ 0.78f-k	691.07 $\pm$ 37.73h-m	2262.33 $\pm$ 142.19g-m
	Moderate	8.2 $\pm$ 1.16h-q	49.4 $\pm$ 0.80n-t	25.7 $\pm$ 0.79l-q	565.20 $\pm$ 139.83k-p	1703.47 $\pm$ 121.26n-t
	Severe	6.0 $\pm$ 0.69r-w	47.2 $\pm$ 1.17r-w	22.1 $\pm$ 0.26s-w	316.10 $\pm$ 32.81r-w	1179.77 $\pm$ 64.21u-y
MR211	Control	11.3 $\pm$ 0.44abc	67.8 $\pm$ 1.28a	38.2 $\pm$ 0.42a	1059.53 $\pm$ 78.51a	3563.33 $\pm$ 101.71a
	Low	10.2 $\pm$ 0.29a-i	59.4 $\pm$ 0.87d-g	30.9 $\pm$ 0.07e-j	915.30 $\pm$ 26.12a-g	2784.00 $\pm$ 6.00c-f
	Moderate	9.1 $\pm$ 1.00d-m	52.5 $\pm$ 0.63j-q	27.1 $\pm$ 0.64k-o	760.60 $\pm$ 101.20e-k	2255.33 $\pm$ 101.04g-m
	Severe	8.7 $\pm$ 0.56e-o	47.8 $\pm$ 1.74q-v	26.0 $\pm$ 0.99l-q	670.43 $\pm$ 80.94h-m	1988.07 $\pm$ 143.81j-o
MR219	Control	11.0 $\pm$ 0.46a-d	58.9 $\pm$ 1.19d-g	33.3 $\pm$ 0.34b-e	993.90 $\pm$ 41.36abc	2999.70 $\pm$ 30.15bc
	Low	10.0 $\pm$ 0.19a-k	48.4 $\pm$ 0.78o-u	31.0 $\pm$ 1.59e-i	763.60 $\pm$ 23.52e-k	2371.60 $\pm$ 118.49e-k
	Moderate	6.8 $\pm$ 0.53o-v	41.7 $\pm$ 0.92yzA	25.5 $\pm$ 1.80m-w	402.27 $\pm$ 20.99o-u	1514.67 $\pm$ 103.55p-v
	Severe	4.9 $\pm$ 0.56vwx	38.0 $\pm$ 1.89A	22.2 $\pm$ 1.64s-w	232.33 $\pm$ 39.26u-x	1046.00 $\pm$ 144.34v-y
MR220	Control	11.0 $\pm$ 0.04a-d	62.7 $\pm$ 1.49cde	35.1 $\pm$ 0.58b	1060.90 $\pm$ 34.22a	3393.33 $\pm$ 168.26ab
	Low	10.1 $\pm$ 0.06a-j	54.9 $\pm$ 1.12g-m	31.3 $\pm$ 0.94d-h	770.73 $\pm$ 36.28d-k	2407.00 $\pm$ 164.32e-j
	Moderate	8.4 $\pm$ 0.30g-p	46.8 $\pm$ 0.62r-x	28.6 $\pm$ 0.32g-l	529.07 $\pm$ 14.87l-r	1814.33 $\pm$ 105.76m-s
	Severe	2.5 $\pm$ 0.57y	39.6 $\pm$ 1.42zA	20.4 $\pm$ 0.72w	106.20 $\pm$ 18.33wx	880.13 $\pm$ 35.24xyz
MR253	Control	11.9 $\pm$ 0.56a	66.0 $\pm$ 0.99abc	38.2 $\pm$ 0.62a	1036.13 $\pm$ 96.03ab	3305.33 $\pm$ 197.45ab
	Low	10.5 $\pm$ 0.74a-g	54.9 $\pm$ 2.05g-m	31.8 $\pm$ 0.39c-f	771.20 $\pm$ 87.25d-k	2330.33 $\pm$ 117.80f-l
	Moderate	9.1 $\pm$ 0.64d-m	50.1 $\pm$ 2.50m-s	28.1 $\pm$ 0.58i-n	570.13 $\pm$ 17.94k-p	1778.00 $\pm$ 103.94n-t
	Severe	7.9 $\pm$ 1.29j-r	44.1 $\pm$ 0.24u-z	24.7 $\pm$ 0.35o-t	401.53 $\pm$ 85.16o-u	1238.33 $\pm$ 56.11u-y
MR263	Control	11.2 $\pm$ 0.11a-d	59.1 $\pm$ 0.24d-g	38.2 $\pm$ 0.62a	1045.20 $\pm$ 26.42a	2766.67 $\pm$ 84.52c-f
	Low	10.3 $\pm$ 0.29a-h	54.5 $\pm$ 0.74g-m	31.8 $\pm$ 0.39c-f	891.27 $\pm$ 18.54a-h	2280.67 $\pm$ 89.09g-m
	Moderate	8.1 $\pm$ 1.26i-r	50.4 $\pm$ 0.54l-s	28.1 $\pm$ 0.58i-n	511.43 $\pm$ 81.34l-r	1498.67 $\pm$ 64.67q-v
	Severe	6.6 $\pm$ 0.26o-v	45.5 $\pm$ 1.30s-y	24.7 $\pm$ 0.35o-t	351.33 $\pm$ 7.54p-v	1168.33 $\pm$ 15.90u-y
MR269	Control	10.3 $\pm$ 1.07a-h	61.4 $\pm$ 1.47def	31.5 $\pm$ 1.29d-g	936.87 $\pm$ 147.87a-f	2820.67 $\pm$ 120.17cde
	Low	9.1 $\pm$ 0.22d-m	55.1 $\pm$ 0.60g-l	28.6 $\pm$ 0.33g-l	724.60 $\pm$ 42.30f-l	2286.77 $\pm$ 143.66g-m
	Moderate	7.9 $\pm$ 0.43j-r	48.7 $\pm$ 1.26o-u	26.8 $\pm$ 2.29k-o	551.03 $\pm$ 31.97k-q	1902.93 $\pm$ 299.60k-r
	Severe	5.3 $\pm$ 0.43t-w	44.7 $\pm$ 1.38t-y	25.3 $\pm$ 0.90n-r	283.93 $\pm$ 39.44s-x	1343.33 $\pm$ 57.83t-x
MR284	Control	11.1 $\pm$ 0.72a-d	66.5 $\pm$ 1.30abc	38.5 $\pm$ 0.90a	1009.97 $\pm$ 126.09abc	3473.00 $\pm$ 265.77a
	Low	10.5 $\pm$ 0.89a-g	55.6 $\pm$ 1.37g-k	31.1 $\pm$ 0.98e-h	878.00 $\pm$ 109.44a-i	2598.00 $\pm$ 187.16c-g
	Moderate	9.9 $\pm$ 0.45a-k	49.4 $\pm$ 2.56n-t	26.2 $\pm$ 1.26l-q	689.17 $\pm$ 46.62h-m	1837.20 $\pm$ 198.58m-s
	Severe	5.2 $\pm$ 0.42u-x	42.8 $\pm$ 1.59w-zA	22.1 $\pm$ 0.47s-w	272.33 $\pm$ 34.86t-x	1174.27 $\pm$ 127.59u-y
MR297	Control	11.1 $\pm$ 0.42a-d	63.2 $\pm$ 2.93bcd	34.1 $\pm$ 1.18bcd	967.33 $\pm$ 94.62a-e	2968.67 $\pm$ 324.67bcd
	Low	10.7 $\pm$ 0.39a-f	56.9 $\pm$ 2.57f-j	31.9 $\pm$ 0.44c-f	862.77 $\pm$ 91.85a-j	2549.53 $\pm$ 164.14c-h
	Moderate	7.8 $\pm$ 0.69k-s	50.9 $\pm$ 0.49k-r	28.7 $\pm$ 0.89g-l	578.77 $\pm$ 78.62k-o	2099.00 $\pm$ 35.51h-n
	Severe	6.2 $\pm$ 0.27q-w	45.5 $\pm$ 0.58s-y	25.1 $\pm$ 0.49n-s	269.43 $\pm$ 29.41t-x	1087.60 $\pm$ 83.49u-y
MR303	Control	11.4 $\pm$ 0.70abc	67.2 $\pm$ 0.90ab	38.4 $\pm$ 0.31a	988.17 $\pm$ 93.12a-d	3326.00 $\pm$ 103.13ab
	Low	10.9 $\pm$ 0.80a-e	58.1 $\pm$ 0.51e-h	30.7 $\pm$ 0.88e-j	840.37 $\pm$ 93.61a-j	2355.33 $\pm$ 115.70e-k
	Moderate	9.9 $\pm$ 0.35a-k	52.3 $\pm$ 1.75j-q	26.1 $\pm$ 0.94l-q	658.70 $\pm$ 31.39i-m	1733.17 $\pm$ 70.79n-t
	Severe	7.5 $\pm$ 0.39l-s	47.6 $\pm$ 0.95q-v	23.3 $\pm$ 1.21q-w	425.87 $\pm$ 43.44n-u	1329.50 $\pm$ 141.56t-x
MR307	Control	11.4 $\pm$ 0.30abc	68.2 $\pm$ 1.64a	40.4 $\pm$ 1.17a	1023.27 $\pm$ 40.97abc	3620.00 $\pm$ 134.54a
	Low	10.3 $\pm$ 0.26a-h	57.1 $\pm$ 0.67f-j	34.6 $\pm$ 0.87bc	819.20 $\pm$ 42.49b-j	2774.00 $\pm$ 243.14c-f
	Moderate	8.7 $\pm$ 0.47e-o	49.7 $\pm$ 0.78n-s	28.0 $\pm$ 1.03j-n	643.07 $\pm$ 79.89j-n	2064.00 $\pm$ 253.28i-n
	Severe	7.2 $\pm$ 0.34m-u	44.6 $\pm$ 1.69t-y	24.5 $\pm$ 1.06o-u	430.33 $\pm$ 53.06n-u	1457.17 $\pm$ 81.25r-v

**Table 3.** Pearson correlation coefficients among the germination and morphological parameters from 15 rice genotypes exposed to three different drought levels. \*\*Correlation is significant at the 0.01 level (2-tailed), \*Correlation is significant at the 0.05 level (2-tailed). GP: Germination percentage; GI: germination index; GR: germination rate; GE: germination energy; SH: seedling height (cm); FW: fresh weight (mg); DW: dry weight (mg); SVI: seedling vigor I; SVII: seedling vigor II.

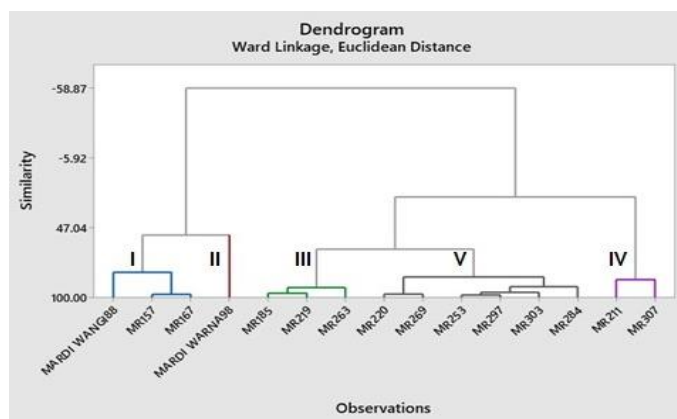
Traits	GP	GI	GR	GE	SH	FW	DW	SVI	SVII
GP	1								
GI	0.904**	1							
GR	0.964**	0.939**	1						
GE	0.873**	0.930**	0.921**	1					
SH	0.775**	0.620*	0.734**	0.753**	1				
FW	0.748**	0.765**	0.801**	0.847**	0.755**	1			
DW	0.659**	0.607*	0.570*	0.572*	0.614*	0.690**	1		
SVI	0.921**	0.768**	0.871**	0.839**	0.948**	0.801**	0.702**	1	
SVII	0.895**	0.806**	0.823**	0.774**	0.744**	0.782**	0.922**	0.880**	1

### Cluster analysis and principal component analysis (PCA) of studied traits

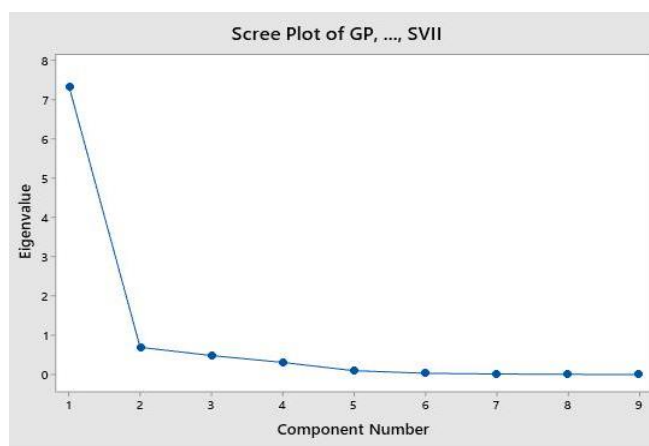
The cluster analysis based on Euclidian distance (Ward Method) groups similar data into same clusters thereby enabling to determine the relationship between genotypes (Belay et al., 2021). The cluster analysis classified the genotypes into five clusters (Figure 2). Cluster I was composed of MARDI WANGI88, MR157 and MR167, and among them MR157 and MR167 are closely related to each other. MARDI WARNA98 forms one cluster (cluster II), it has poor germination and seedling growth traits. Similarly, MR185, MR219 and MR263 are closely related in terms of the studied parameters and belong to cluster III. MR 211 was the genotype with best performance in terms of almost all of the parameters grouped in cluster IV with MR307. Cluster V grouped MR220, MR269, MR253, MR297, MR303 and MR284, among them, two couples of genotypes were most similar to each other: MR220 and MR269, MR253 and MR297.

Principal component analysis (PCA) enables to aggregate a linear combination of multiple indicators into fewer comprehensive indices (Wang et al., 2021). Thus, the purpose of PCA is to obtain a small number of linear combinations of all variables that represent the bulk of data variability. The nine principal components along with Eigen value has been presented in Figure 3, which makes clearly apparent that two principal components (PCs) showed most of the variability.

The first principal component (PC1) with an Eigen value of 7.363 accounted for 81.8% variability, whereas second principal component (PC2) with an Eigen value of 0.693 accounted for 7.7% variation of studied parameters (Table 4). From the biplot, PC1 was positively influenced by all measured traits, whereas PC2 negatively contributed to all germination and positively contributed to all growth traits (Figure 4). Many researchers have used biplot analysis to compare different genotypes and plant species for different criteria (Singh et al., 2016). According to the biplot, dry weight (DW) and seedling vigor II (SVII) were the major contributors to variability, while the rest of the parameters seemed to have a minimal contribution to variability. In the PC1 group, GP (0.352) recorded the highest variability. By contrast, DW (0.284) had lowest variability. Maximum variability in the PC2 group recorded in DW (0.708) whereas GP (-0.152), GI (-0.331), GR (-0.337) and GE (-0.333) contributed negatively. The PC1 group showed the maximum variability (81.8%) for drought tolerance contributing traits than the other groups (Table 4). Two variables named DW and SVII collectively strongly contributed to both PCs.



**Figure 2.** Cluster analysis revealed as dendrogram for 15 genotypes showing similarity among them.

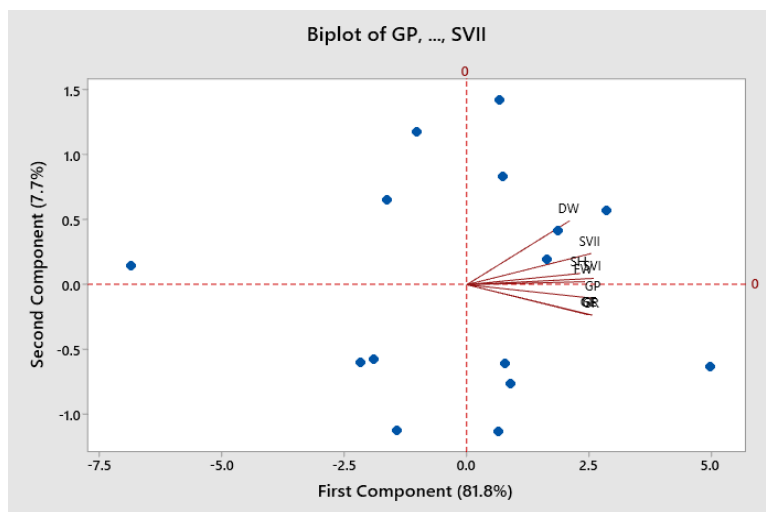


**Figure 3.** Scree plot of PCA between eigenvalue and component number.

**Table 4.** Eigenvalues, variability and factor loadings of PC1 and PC2 generated by PCA executed on all of the nine parameters of rice genotypes in four different drought levels. PC: Principal component; GP: germination percentage; GI: germination index; GR: germination rate; GE: germination energy; SH: seedling height (cm); FW: fresh weight (mg); DW: dry weight (mg); SVI: seedling vigor I; SVII: seedling vigor II.

Variable	PC1	PC2
GP	0.352	-0.152
GI	0.334	-0.331
GR	0.347	-0.337
GE	0.342	-0.333
SH	0.314	0.125
FW	0.325	0.033
DW	0.284	0.708
SVI	0.351	0.070
SVII	0.345	0.346
Eigenvalue	7.363	0.693
Proportion	0.818	0.077
Cumulative	0.818	0.895





**Figure 4.** Biplot of the first two principal components of PCA executed on seed germination and growth attributes of 15 rice genotypes. GP: Germination percentage; GI: germination index; GR: germination rate; GE: germination energy; SH: seedling height; FW: fresh weight; DW: dry weight; SVI: seedling vigor I; SVII: seedling vigor II.

## DISCUSSION

The results from the current study showed that the seed germination performances were poor with high osmotic stress compared to the control and low stress conditions. The germination percentage (GP), germination energy (GE), germination rate (GR), and germination index (GI) were found to reduce as PEG concentrations increased (Table 1). Reportedly, the reduction in germination with increased PEG level was associated with high seed nutrient imbalance, toxic ions, and reduced soluble osmotic potential (Yang et al., 2018). The current study suggested maximum GP in the control treatment thereby representing germination was going well because of water content. The study found that different genotypes responded differently to four osmotic stresses as hypothesized. The highest reduction in seed germination and seedling growth traits was recorded under -0.9 MPa osmotic potential, contrary lowest reduction was observed under -0.3 MPa. As the intensity of the stress increases, the ability of plants to absorb and use water is reduced, thus their tolerance mechanisms becomes failure to support proper growth (Islam et al., 2018). Seedling fresh and dry weight, seedling length are some of critical parameters severely affected under abiotic stress. Hence, investigation of these parameters are mostly important in studying early growth of plants under stress (Gola, 2018). In a study by Zheng et al. (2016), drought stress markedly altered the early seedling growth in rice, resulting in significant reduction in shoot and root length, fresh and dry weights. In the current study of investigation, all the mentioned traits reduced with increased PEG induced drought level (Table 2). The decline in seed germination and seedling growth under water stress is a common response supported by many workers (Kosar et al., 2018). Water deficiency under high osmotic stresses initially lowers water absorption capability of seed, activity of hydrolytic enzymes, interrupts nutrient supply to embryo which all collectively leads to reduced germination, cell division and elongation (Fahad et al., 2017).

Previous studies by Iqbal et al. (2018) suggested that the cluster analysis grouped rice genotypes into different clusters, thereby ensuring the presence of high to moderate diversity among the genotypes. The present study suggested five clusters, where cluster IV incorporated two genotypes (MR211 and MR307), which had the highest value for almost all germination and growth parameters (Figure 2). Six genotypes (MR220, MR269, MR253, MR297, MR303 and MR284) were placed together in cluster V. The genotypes of cluster IV and V presented better tolerance in the current study. The PCA is a powerful statistical procedure to reduce the dimensions of the variables and to divulge constructive evidence-driven feedback from a highly correlated dataset (Bahrami et al., 2014). The PCA simplifies the complex data by transforming the number of correlated variables into a smaller number of variables called principal components. A scree plot was shown denoting the variance explained by nine components, while the maximum variation was clearly apparent in first two components

(Figure 3 and Table 4). The cumulative variance observed by these two principal components (PCs) was 89.5%. Hence, selection of genotypes from these two PCs will be useful (Ahmadzadeh et al., 2011). In the present study, PCA clarified that two representative parameters, seedling vigor II and dry weight of seedlings (SVII and DW) from both of the PCs, were sufficient to capture most of the variation in the data (Figure 4). Thus these two traits could be used to screen the rice genotypes for drought tolerance (Bhattarai and Subudhi, 2019).

## CONCLUSIONS

As a significant food crop, rice has begun to place more emphasis on drought resilience; however, its selection under field circumstances is difficult due to the low heritability and lengthy process. In vitro screening for drought tolerance could be an alternative, where the genotypes with better performance under different drought levels could be suggested in field conditions. Findings from the current study suggest that different rice genotypes react differently to drought stresses, which may indicate that certain rice genotypes have better tolerance. The results demonstrate that polyethylene glycol (PEG) 6000 affects rice seed germination and early seedling growth, however, the effect depends on osmotic potentiality of PEG 6000. Highest concentration of PEG 6000 reduced germination and other growth attributes greatly compared to lower concentration. The study discriminated eight genotypes as drought tolerant, among them MR211 and MR307 were most tolerant, whereas among four susceptible genotypes MARDI WARNA98 was most drought sensitive. Three genotypes (MR185, MR219 and MR263) were moderately tolerant. Therefore, the study enabled to assess the tolerance of studied rice genotypes under different drought conditions and categorize them as their tolerance level.

### Author contribution

Conceptualization: F.Z.E., R.N. Methodology: F.Z.E., R.N. Software: F.Z.E. Validation: R.N. Formal analysis: F.Z.E. Investigation: R.N. Resources: R.N. Data curation: F.Z.E., R.N. Writing-original draft: F.Z.E. Writing-review & editing: R.N. Visualization: F.Z.E. Supervision: R.N. Project administration: R.N. Funding acquisition: R.N. All co-authors reviewed the final version and approved the manuscript before submission.

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