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Research Article

Assessment Of Drought Tolerance in Different Rice Genotypes During Germination and Early Growth Stages by Using Polyethylene Glycol-6000

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Abstract

Rice is a crucial staple food for over half of the global population, particularly in Southeast Asia. However, rice production is increasingly affected by biotic and abiotic stresses, including drought, exacerbated by global climate change. This study explored the effects of negative osmotic pressure induced by polyethylene glycol (PEG-6000) on rice seed germination and growth traits. The experiment was conducted at Gokuleshwor Agriculture and Animal Science College Laboratory, Baitadi, Nepal, using four rice varieties: Black rice, Subha mansuli, Hardinath-4, and Rato basmati. Four osmotic potentials (0, -0.2, -0.4, -0.6 MPa) were applied under controlled conditions. Data on seed germination, root length, shoot length, root-to-shoot ratio, and vigor index were analyzed using two-way ANOVA and LSD tests. Results showed significant differences among rice genotypes and PEG treatments. Rato basmati exhibited the highest germination percentage (78.27%), root length (4.44 cm), shoot length (4.44 cm), and vigor index (718.78), even at -0.6 MPa osmotic potential, indicating superior drought tolerance. Increasing PEG-induced osmotic stress reduced germination and seedling growth across all genotypes. However, Rato basmati's better performance under -0.6 MPa suggests it as a drought-tolerant genotype, making it a potential candidate for future drought-tolerance breeding programs in rice.

Keywords: Rice genotypes, Drought tolerance, Polyethylene glycol (PEG), Germination, early growth stage.

Introduction

The most prevalent of the two rice species produced as cereal is *Oryza sativa*, followed by *O. glaberrima*, sometimes known as African rice. It was domesticated in the Yangtze River region of China between 13,500 and 8,200 years ago (Nornile, 1997; Vaughan *et al.*, 2008; Zhang *et al.*, 2012). More than half of the world's population relies on it as a staple meal, particularly in Southeast Asia; also, the most significant crop in Nepal (Sharif *et al.*, 2014). It is cultivated in 1.49 million hectares and has an average productivity of 3.76 mt ha-1 in 2019 (MoALD, 2019). It provides roughly 20% to the Agricultural Gross Domestic Product (AGDP) and 7% of the GDP (MoALD, 2020; CBS, 2018). It accounts for 50-80% of daily caloric intake (Fukagawa and Ziska 2019). Consequently, over half of the world's rice crop is impacted by drought stress (Mostajeran and Rahimi-Eichi, 2009). According to Tripathi *et al.*

(2018), if the existing rice production area remains the same, the yield of rice must increase from 3.0 mt ha^{-1} to 6.0-7.0 mt ha^{-1} by 2035. The situation to meet the ever-increasing requirements of humans for food, shelter, and nourishment is becoming more dire every day due to global climate change, population growth, and drought stress (HongBo *et al*., 2005). Water stress at critical growth stages, especially at the reproductive stage, exhibits serious harmful effects on rice, making it a crop that is vulnerable to drought (Khodarahmpour, 2011). Abiotic stress, particularly drought, is thought to be responsible for 70% of yield losses in rainfed highland ecosystems, where water stress is one of the main obstacles to rice production and yield stability (Singh Rajput *et al*., 2022). One of the most crucial phases of plant development is germination. Plant productivity and yield are also reliant on the germination stage of growth. Lack of moisture in the arid regions and the establishment of seedlings that quickly process information and function well are the two factors limiting seedling growth (Zimudzi *et al*., 2012). These days, the severity of the crisis has been increased because of global climate change (Pan *et al*., 2003). The impact of dry circumstances on plant growth and grain output is contingent upon the intensity of the drought and the stage of plant development during the drought. One of the stages of growth that is susceptible to water shortage is seedling emergence. The yield and maturity period are largely dependent on the pace and degree of the plant (Rauf *et al*., 2007).

The PEG (Polyethylene glycol) is used in plant biology research to induce osmotic stress in plants. By adding PEG, it can mimic drought conditions, which allows to study the plants' response to water stress and the developing of strategies to improve drought tolerance in plant crops. Drought stress during the early stages of growth can be replicated using polyethylene glycol (PEG). Because PEG has a high molecular weight and is osmotic, it has been widely employed in numerous studies as an artificial stress inducer (Violita and Azhari, 2021). By lowering water potential, the PEG inhibits seed germination and growth; this effect is more noticeable on shoots than on primary roots (Khajeh-Hosseini *et al*., 2003). PEG is commonly employed to modulate water potential in germination studies (Wang *et al*., 2018) due to its high molecular weight, inability to penetrate through cell walls, and ability to generate consistent water stress without inflicting direct physiological harm (Violita and Azhari, 2021). According to Bashir and Khanzada (1997), genotypes with larger seed weights often had greater germination percentages. The development of drought-tolerant crop varieties is the best alternative for crop production, yield improvement, and yield stability under situations of low soil moisture. The most appealing method for quickly developing new varieties would be a physiological approach, but breeding for particular, sub-optimal settings requires a deeper comprehension of the process that determines yield (Siddique *et al.*, 2000). A well-known and proven method for assessing genotypes in laboratory settings is the use of polyethylene glycol (PEG), an inert, non-ionic polymer, to simulate water stress conditions (Shereen *et al.*, 2019).

The goal of this study is to investigate how varying amounts of PEG in rice varieties affect germination, root length, shoot length, root: shoot ratio, and vigor index. This is because germination and early plant establishment are crucial for rice production. As well as to find out better performing genotypes with higher negative osmotic potential of PEG-6000. The results will help to select the most tolerant genotypes for breeding purposes to develop drought-tolerant genotypes of rice in the future.

Material And Methods

Experimental Details

The study was conducted at Gokuleshwor Agriculture and Animal Science College Laboratory, Baitadi, Nepal. Four different rice varieties (Table 1) were included in the study. Four different osmotic potentials (negative) of PEG-6000, i.e.,0, -0.2, -0.4, and -0.6 MPa were included in the study (Table 2).

Table 1: The code name, and name of different rice genotypes present in the study

Genotype code	Genotype name
G1	Black rice
G2	Subha mansuli
G3	Hardinath -4
G4	Rato basmati

Table 2: The code name, and name of different treatments of PEG-6000 present in the study

PEG Treatments code	PEG treatment name
O_1	Control
O_2	-0.2 MPa
O_3	-0.4 MPa
O_4	-0.6 MPa

Seed Germination Experiment

Three replications of 25 sterilized (with 5% sodium hypochlorite) seeds were germinated on the Whatman No.1 filter paper in Petri dishes. The 10 ml treatment solution and distilled water were poured on the filter paper and afterward, the solution or distilled water was given according to needs. The Petri dishes were sealed with parafilm to prevent evaporation. Seed are incubated at room temperature for 10 days by maintaining a 12-hour dark period and seed germination was computed at 24-hour intervals by considering seed germinated to 1-2 mm.

Statistical Analysis

The collected data were entered in MS Excel and further analysis was done using R software. Two-way analysis of variance (ANOVA) was then used to infer the significance of the data. The least significant difference test at 5% probability was used to compare the means where ANOVA indicated significant differences.

Results

The different genotypes of rice impact on their GP, RL, SL, R/S, and VI under different osmotic potentials (Table 3) in Petri dish conditions. Rato basmati has the highest root length (4.44 cm), shoot length (4.44 cm), vigor index (718.78), and second-highest germination percentage (78.27%) followed by Subha mansuli (78.51%); however, lowest germination percentage (40.36%), shoot length (1.58 cm), and vigor index (204.32) were found in Black rice with higher root shoot ratio (1.87) (Table 3).

The Table 4 represents the impact of different osmotic potentials of PEG-6000 on GP, RL, SL, R/S, and VI of different rice genotypes (Table 4). The highest germination percentage (84.94%), root length (5.24 cm), shoot length (4.74 cm), root shoot ratio (1.35), and vigor index (868.39) were found in control treatment; while the lowest germination percentage (42.56%), root length (0.95 cm), shoot length (2.37 cm), root shoot ratio (1.02), and vigor index (187.63) were found in -0.6 MPa osmotic potential (Table 4). The examined characters revealed considerable differences between the genotypes, and it was concluded that rising PEG concentrations caused a significant decrease in the germination percentage, root length, shoot length, root: shoot, and vigor index.

Table 3: The impact of different genotypes of rice on their seed germination and growth traits grown under different osmotic potentials of PEG-6000

Genotypes	**GP %**	**RL (cm)**	**SL (cm)**	**R:S**	**VI**
Subha mansuli (G2)	78.51^{a}	2.27^{b}	3.24^{b}	0.68^{b}	458.97^{b}
Rato basmati(G4)	78.27^{a}	4.44^{a}	4.44^{a}	0.97^{b}	718.78^{a}
Hardinath-4 (G3)	69.27^{b}	3.91^{a}	4.12^{ab}	1.13^{b}	594.34^{a}
Black rice (G1)	40.36^{c}	2.74^{b}	1.58^{c}	1.87^{a}	204.32^{c}
LSD	7.23	0.85	0.72	0.53	194.92
CV (%)	13.05	30.74	3.35	1.16	23.72
Mean	66.59	3.34	25.83	55.26	494.11

Note: GP= Germination percentage, RL= Root length, SL=Shoot length, R:S = Root Shoot ratio, VI= Vigor index, LSD= Least Significance difference (5%), CV= Coefficient of Variation.

Table 4: The impact of different osmotic potentials on seed germination and growth traits of different rice genotypes

Osmotic potential	**GP %**	**RL (cm)**	**SL (cm)**	**R:S**	**VI**
Control (O_1)	84.94^{a}	5.24^{a}	4.74^{a}	1.35^{a}	868.39^{a}
-0.2 MPa (O_2)	75.95^{b}	3.23^{b}	3.75^{b}	1.28^{a}	532.98^{b}
-0.4 MPa (O_3)	62.92^{c}	3.11^{b}	2.81^{bc}	1.23^{ab}	387.43^{c}
-0.6 MPa (O_4)	42.56^{d}	0.95^{c}	2.37^{c}	1.02^{b}	187.63^{d}

Note: GP= Germination percentage, RL= Root length, SL=Shoot length, R:S = Root Shoot ratio, VI= Vigor index

Table 5: The impact of rice genotypes by different osmotic potentials interaction on seed germination and growth traits of rice genotypes included in the study.

Interaction	GP	RL	SL	R:S	VI
G_1*O_1	56.67^{bcdef}	4.72^{ab}	2.57^{cdef}	1.92^{a}	423.57^{defg}
G_1*O_2	49.52^{defg}	2.95^{bcde}	1.65^{def}	2.05^{a}	239.83^{efgh}
G_1*O_3	31.43^{fg}	2.23^{bcde}	1.19^{ef}	1.89^{a}	110.53^{gh}
G_1*O_4	23.81^{g}	1.07^{e}	0.91^{f}	1.62^{a}	43.34^{h}
G_2*O_1	100.00^{a}	3.61^{abcde}	4.35^{abc}	0.84^{a}	796.67^{bc}
G_2*O_2	82.86^{ab}	2.72^{bcde}	3.87^{bcd}	0.71^{a}	546.73^{cde}
G_2*O_3	82.62^{ab}	1.34^{de}	2.53^{cdef}	0.5^{a}	317.10^{defgh}
G_2*O_4	48.57^{efg}	1.4^{cde}	2.23^{cdef}	0.66^{a}	175.49^{fgh}
G_3*O_1	91.67^{a}	6.07^{a}	6.63^{a}	0.92^{a}	1159.07^{a}
G_3*O_2	76.90^{abc}	2.87^{bcde}	4.07^{abcd}	0.70^{a}	525.78^{cdef}
G_3*O_3	62.62^{bcde}	4.51^{abc}	3.14^{bcdef}	2.05^{a}	474.18^{cdef}
G_3*O_4	45.71^{efg}	2.21^{bcde}	2.65^{cdef}	0.85^{a}	218.34^{efgh}
G_4*O_1	91.43^{a}	6.55^{a}	5.41^{ab}	1.21^{a}	1094.25^{ab}
G_4*O_2	94.52^{a}	4.39^{abcd}	4.31^{abc}	1.01^{a}	819.59^{abc}
G_4*O_3	75.00^{abcd}	4.37^{abcd}	4.37^{abc}	1.01^{a}	647.92^{cd}
G_4*O_4	52.14^{cdef}	2.46^{bcde}	3.69^{bcde}	0.64^{a}	313.37^{defgh}

Note: GP= Germination percentage, RL= Root length, SL=Shoot length, R:S = Root Shoot ratio, VI= Vigor index

The genotype 'Subha mansuli' with control treatment recorded the highest values for seed germination percentage (100%) while the lowest was on genotype 'Black rice' with -0.6 MPa osmotic potential (23.81%) (Table 5). Higher shoot length (6.63 cm), and vigor index (1159.07) were on genotype 'Hardinath-4' under control treatment; however, lower values for these traits were recorded for the genotype 'Black rice' with-0.6 MPa osmotic potential. Regarding the root length, maximum (6.55 cm) was observed for the genotype 'Rato basmati' under control treatment while a minimum root length (1.07 cm) was noted for the genotype 'Black rice' with -0.6 MPa osmotic potential. A high proportion of root shoot ratio (2.05) was noted on the genotype 'Black rice' with -0.2 MPa osmotic potential and low proportion (0.5) was found for the genotype 'Subha mansuli' with -0.4 MPa osmotic potential. Hardinath-4 with the interaction of control treatment has shown significant results on germination percentage (91.67%), root length (6.07 cm), shoot length (6.63 cm), and vigor index (1159.07) however with high negative osmotic potential i.e -0.6 Mpa showed poor results on germination and seedling traits. The germination and seedling traits were decreasing with increasing PEG concentrations. However, the Rato Basmati genotype exhibited the least reduction in the germination percentage, root length, shoot length, and vigor index with the increasing order of negative osmotic potentials of PEG-6000 than other genotypes, suggesting a superior drought tolerance genotype of rice.

Discussion

For agricultural plants, seed germination is a crucial stage in the growth from seeds to seedlings. In both favorable and unfavorable situations, increased seed germination promotes plant survival and increases yields (Gašparovič et al. 2021). When the seeds are metabolically active, the germination process takes place, and water is a crucial component in promoting that activity; the seeds' metabolism becomes disturbed or even ceases if there is not enough water (Taiz & Zeiger, 2010). Several essential enzymes and nourishment that has been saved for the developing embryo influence the process of seed germination; these enzymes' functions are disturbed by the rising negative osmotic potential, which causes seeds to lose their ability to (Billah et al. 2021). The other main cause of the decline in seed germination is the lower water imbibition and unfulfilled moisture requirements of the seeds (Mahpara *et al.*, 2022). In our study, the maximum germination percentage was found in Subha mansuli, and control treatment representing as PEG-6000 induced the decrease in the germination of seedlings in rice. Similar result was also observed in (Violita and Azhari, 2021) study where seven days after treatment, more than 90% of the rice seeds germinated (0% PEG). Osmotic agents like PEG are crucial for controlling hormones, mineral elements, protein metabolism, and signal transduction effects (Sagar et al.

2020a). Slowing down the moisture rate of seeds is the main function of PEG (Basu et al. 2010).

In our research findings, root length decreased as PEG concentration rose and water stress had a major impact on root length for several rice varieties (Table 4). The findings of this study regarding root length are consistent with those of (Viera *et al*., 2022; Pope *et al*., 2024) reported that water stress causes a decrease in both root and shoot length. Subha mansuli and Rato basmati showed comparatively longer roots than any other genotype of rice when tested against water stress at varying PEG concentrations. This suggests that all of these cultivars fared better than average when subjected to PEG-6000 concentrations. Water stress can hinder the development of radicles by reducing the water potential gradient between the seed and the environment, resulting in shorter seedling heights (Murillo-Amador *et al*., 2002; Sokoto and Muhammad, 2014). Water stress can severely affect root cell growth, hindering nutrient uptake and photosynthesis. Photosynthesis is essential for biomass buildup and root elongation. Water stress reduces plants' ability to absorb and use water, making drought tolerance mechanisms ineffective for normal development (Islam *et al*., 2018; Magar *et al*., 2019; Sagar *et al*., 2020b).

Our study results decrease in the shoot length of rice varieties with higher negative osmotic potential. This suggested that a decrease in shoot length with PEG concentration induces the stress to induce the seedling growth of rice. In the case of root shoot ratio, it was not highly significant, but a slight decrement in the ratio was found (Table 4) with an increment in negative osmotic potential of PEG-6000 concentrations. A tolerance metric for assessing the impact of water stress on seedling growth is the seedling vigor index (Kouighat *et al*., 2021). Under water stress conditions, the results showed significant heterogeneity in the rice types' seedling vigor index. This is in line with (Zahedifar and Zohrabi, 2016), who found that the seed vigor index is most affected by water stress. A higher seed vigor index was obtained in Rato basmati and control treatment. An essential indicator of seed quality, the seedling vigor index assesses the likelihood of uniform and quick plant emergence. Some researchers have proposed that plants with a greater vigor index may enhance agricultural yield and growth and that the early vigor of seedlings with strong development might be employed as a favorable attribute of interest for the selection of tolerant types (Yousefi *et al*., 2020).

The examined genotypes' germination rates and seedling parameters were generally decreased by G × PEG-induced drought stress interactions. Except for root shoot ratio, other traits were found to be maximum values for control treatment with varied genotypes interaction. Such as Subha mansuli with control treatment noted the highest germination percentage (100%); while shoot length and vigor index were responded to higher values in the interaction between Hardinath-4 with control treatment (Table 5). This implies that distinct seedling qualities are susceptible to the interplay between the control treatment and various rice genotypes.

Conclusion

From our study, we can conclude that the 'Rato basmati' variety of rice has shown the best performance in terms of germination and early growth stage. However, the increase in the negative osmotic potential of PEG-6000 has resulted significant decrease in the germination percentage and seedling traits of rice. The highest reduction in seed germination and growth traits was observed in the -0.6 MPa osmotic potential of PEG-6000. 'Rato basmati' variety of rice with the interaction of -0.6 Mpa osmotic potential has shown better results for rice traits which implies better drought tolerance than other genotypes included in the study. Therefore, 'Rato basmati' variety of rice can be used for further breeding programs to improve the drought tolerance of rice crop.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

Authors' Contribution

AK. Limbu designed the research plan; Ak. Limbu, A. Dhami, J. Rawal, P. RC, A. Mandal and R. Awasthi performed the experimental works and collected the required data. AK. Limbu and J. Rawal analyzed the data; AK. Limbu, A. Dhami, J. Rawal and J. Adhikari prepared the manuscript. AK. Limbu and J. Adhikari finalized the manuscript. The final form of the manuscript was approved by all authors.

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