

## Research Article

# In vitro screening of rice landraces for increased drought tolerance at early growth stages using Polyethylene glycol (PEG 6000)

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## ABSTRACT

Drought stress at germination stage can negatively impact the emergence and uniformity of seedlings leading to poor seedling establishment. Screening for drought tolerance at germination stage helps to identify potential rice germplasm tolerant to water scarcity during early growth. An experiment was conducted at the laboratory of Department of Agronomy, Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Nepal from 18<sup>th</sup> to 27<sup>th</sup> July 2023 in order to assess the seed germination of 20 rice landraces under drought stress condition. The experiment was carried out in a two factorial Completely Randomized Design (CRD) with three replications. The genotypes were evaluated against three levels of drought stress simulated at three concentrations of Polyethylene glycol (PEG 6000) @ 0% (Control), 10% and 20%. 20% PEG condition significantly ( $p \leq 0.05$ ) recorded the lowest values for Germination Percentage (82%), Vigor Index (443.65), Coefficient of Velocity of Germination (9.91), Germination Index (160.78), Root Length (3.63 cm), Shoot Length (1.67 cm) and the highest values for Mean Germination Time (3.22 days) and Root-Shoot ratio (2.67 cm). Conversely, Control exhibited the fastest Mean Germination Time (1.75 days) and highest Germination Index (219.7). Highest Vigor Index (1329.35) and Shoot Length (7.83 cm) along with lowest Root-Shoot ratio (0.80 cm) was noted in 10% PEG. The Control and 10% PEG were statistically at par for parameters Germination Percentage and Root Length. Genotype Manamure significantly exhibited the highest Germination Index (224.33) and the fastest Mean Germination Time (1.67 days) whereas genotype Rato Anadi Lamcho showed an inferior performance with the lowest Vigor Index (786.37), Germination Index (146.78) and the slowest Mean Germination Time (3 days). Genotype Pafele was observed with maximum values of Germination Percentage (94.67%), Vigor Index (726.8), Germination Index (198.33), Shoot Length (2.713) and fastest Mean Germination Time (2.38 days) under 20% PEG condition. Using PEG 6000 to assess early growth traits is a cost-effective approach in achieving rapid screening for tolerant rice germplasm.

**Keywords:** Climate change, Drought stress, Early growth stage, Osmotic Potential, PEG

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## INTRODUCTION

Rice (*Oryza sativa* L.) is an anomaly among the domesticated cereals as it is a tropical C3 grass that evolved in a semi-aquatic, anaerobic (flooded), low-radiation habitat. It requires larger amount of water throughout its life cycle as compared to other crops. Rice is highly sensitive to a variety of abiotic stresses, including drought (Lafitte *et al.*, 2004). In the wake

of the rapid increase in population and changing climate, drought stress has become more prominent. Approximately, 23 million ha of rain-fed rice are affected by drought globally (Serraj *et al.*, 2011). Drought affects rice at morphological, biochemical and molecular levels and thereby affects its yield (Pandey & Shukla, 2015). Effect of drought on rice physiology depends on the magnitude and the timing of the stress, i.e. stage of plant development when stress occurs (Blum, 2005). Drought stress at germination stage can negatively impact emergence and uniformity of seedling causing poor seedling establishment and ultimately reducing the overall effectiveness of crop management practices that leads to yield losses.

Rain-fed rice accounts for 44% of the total rice area in Nepal. As water for agriculture is becoming increasingly scarce, there is a risk in fulfilling the rising demand of rice and ensuring food security of the country. It is now critical to develop new rice varieties that are resilient to stress and high-yielding in rain-fed environments. Traditional cultivars have a richness of genetic variety for responding to water deficits since they have endured drought events for generations. The traditional varieties or landraces have a high capacity to tolerate biotic and abiotic stresses, with high yield stability and an intermediate yield level under a low input agricultural system (Manohara *et al.*, 2019). However, Nepal has not made use of the great genetic variety of its rice germplasm in rice breeding. It is necessary to characterize and assess such variety of regional landraces that can be used in breeding projects.

There is a high environmental influence on the phenotypic expression of genotypes and a lot of resources (land, labor, power) is required in drought tolerance screening under field condition, which is not always practical or efficient, hence there is a need for a simple and effective early screening method (Kim *et al.*, 2001). The *in vitro* screening method proves to be an ideal method in order to screen a large set of germplasm accurately and with less effort. Seed germination under simulated drought conditions can reveal seed weaknesses and predict relative differences among seed lots in field emergence. Using Polyethylene glycol, a non-penetrating inert osmotic agent of high molecular weight that can reduce the water potential of a media without being absorbed or being phytotoxic (Lawlor, 1970) is one of the reliable approach to study resistance during the germinal phase for drought tolerance screening. PEG mimics the osmotic stress condition by creating a higher negative osmotic potential that lowers the rate of water imbibition in seed. The objective of this research was to assess drought tolerance in rice landraces using *in vitro* germination assays and identify rice landraces that exhibit enhanced drought tolerance.

## **MATERIALS AND METHODS**

### **Laboratory condition**

The experiment was conducted at the laboratory of Department of Agronomy and Plant Breeding, Lamjung Campus, Lamjung, Nepal from 18<sup>th</sup> to 27<sup>th</sup> July 2023. The research site is located at an altitude of 857 masl and 28.13°N latitude; 84.42°E longitude in the mid-hills region of the western Nepal which has a humid tropical climate. According to the report of nearest meteorological station, the average temperature is 22.5°C with maximum of 28.64°C and minimum of 16.39°C.

### **Genetic materials**

A total of 20 different genotypes of rice landraces of unknown drought tolerance were used for the experiment. The seeds were collected from Purkot Community Seed Bank, Tanahun, and Ghanpokhara Community Seed Bank, Lamjung.

The twenty genotypes of rice landraces used in the experiment are listed in Table 1.

**Table 1: Rice genotypes used in the experiment**

Symbol	Name of Genotype (in Nepali)	Symbol	Name of Genotype (in Nepali)
1	Aapjhutte	11	Kalo patle
2	Kaathe	12	Rato Anadi Dalle
3	Lekali Basmati	13	Parmali
4	Maanamure	14	Darmali
5	Gaure	15	Juhari
6	Kartike	16	Kalo Jhinuwa
7	Rato Anadi Lamcho	17	Pafele
8	Dalle Masino	18	Chiniya
9	Piyale	19	Pahelo Mansaar
10	Jhumka	20	Dalle

### Experimental design and procedures

Seeds of each genotype were soaked in respective solutions for 12 hours. The seeds were then washed thoroughly and shade dried for the next 24 hours. Three replicates of 25 sterilized seeds were germinated on a layer of Whatman™ No.42 (diameter 90mm) filter paper in Petri dishes (150 × 15 mm). 5 ml of treatment solution or distilled water was poured on the filter paper and afterwards the solution or distilled water was given according to the needs. The Petri dishes were sealed with lids to prevent evaporation. Seeds were incubated at 25 ± 2°C and 12 hours light dark period for 10 days. The experiment was laid out in a Completely Randomized Design (CRD) with three replications and three different moisture levels as shown in Table 2.

**Table 2: Moisture levels maintained in seed germination experiment**

Moisture level	Moisture condition
M1	Control
M2	10% PEG
M3	20% PEG

### Preparation of desired concentration of treatment

During screening, water deficit stress was artificially induced by desired strengths of Polyethylene Glycol 6000 (PEG 6000). Three different osmotic potentials, i.e., 0, -0.6MPa and -1.2MPa was included in the study by using PEG-6000 (Kaufmann & Eckard, 1971). Distilled water was used as a control (0 MPa) and was also used for preparation of the desired concentration of treatment solution. Osmotic potential of -0.6 MPa was created by using 10% PEG, i.e. adding PEG-6000 @10g per 100 ml distilled water and repeating the process until 100g per 1000 ml solution was prepared. Similarly, osmotic potential of -1.2 MPa was created by using 20% PEG, i.e. adding 200g per 1000 ml solution.

### Data collection

#### Germination Percentage (G %)

Germination was recorded every 24 hours for 10 days and was considered complete once the radicle protruded about 2 mm in length.

$$G\% = \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds tested}} \times 100 \dots\dots\dots(1)$$

**Mean Germination Time (MGT)**

MGT is a quantitative indicator of the average length of time required for maximum germination of a seed. The lower the MGT, the faster a population of seeds has germinated.

$$MGT = \frac{\sum n \times d}{N} \dots\dots\dots(2)$$

Where, *n* = number of seeds germinated on each day,  
*d* = number of days from the beginning of the test, and  
*N* = total number of seeds germinated at the termination of the experiment

**Coefficient of Velocity of Germination (CVG)**

CVG gives an indication of the rapidity of germination.

$$CVG = \frac{N + N_2 + \dots + N_i}{100} \times (N_1T_1 + N_2T_2 + \dots + N_iT_i) \dots\dots\dots(3)$$

Where, *N* is the number of seeds germinated every day and *T* is the number of days from seeding corresponding to *N*.

**Germination Index (GI)**

The germination or emergence index (GI or EI), is a measure for percentage and rate of germination.

$$GI = (10 \times N_{10}) + (9 \times N_9) + (8 \times N_8) + \dots\dots\dots + (1 \times N_1) \dots\dots\dots(4)$$

Where, *N*<sub>10</sub>, *N*<sub>9</sub>, *N*<sub>8</sub>.....*N*<sub>1</sub> = Number of germinated seeds in 10, 9, 8.....1 days respectively.

**Root Length (RL) and Shoot Length (SL)**

Ten normal seedlings were taken by random per each replicate on the final day of the experiment, i.e. the 10<sup>th</sup> day. The root and shoot length of the straightened seedlings were measured precisely using a ruler and length was taken in cm scale.

**Root- Shoot Ratio (R: S)**

Root to shoot ratio was calculated as;

$$R:S = \frac{\text{Root length}}{\text{Shoot length}} \dots\dots\dots(5)$$

**Statistical Analysis**

All the recorded data were tested for normality using Shapiro-Wilk test, which indicated that data were normally distributed. Two-way analysis of variance (ANOVA) was conducted using R 4.3.0 programming to infer the statistical significance of the data. Least Significant Difference (LSD) test at 5% probability (*p*<0.05) was used to test the significant difference among treatments followed by a post-hoc test of Duncan’s Multiple Range Test (DMRT) at (*p*<0.05) for mean comparison. Basic statistics and data visualization were performed on Microsoft Excel program.

**RESULTS AND DISCUSSION****Effect of water stress on germination percentage**

The germination percentage reduced from 95.27% at 10% PEG to 82% at 20% PEG. Genotypes Dalle Masino and Chiniya showed the highest germination percentage of 97.33% while the lowest percentage of 66.22% was shown by Lekali Basmati (Table 3).

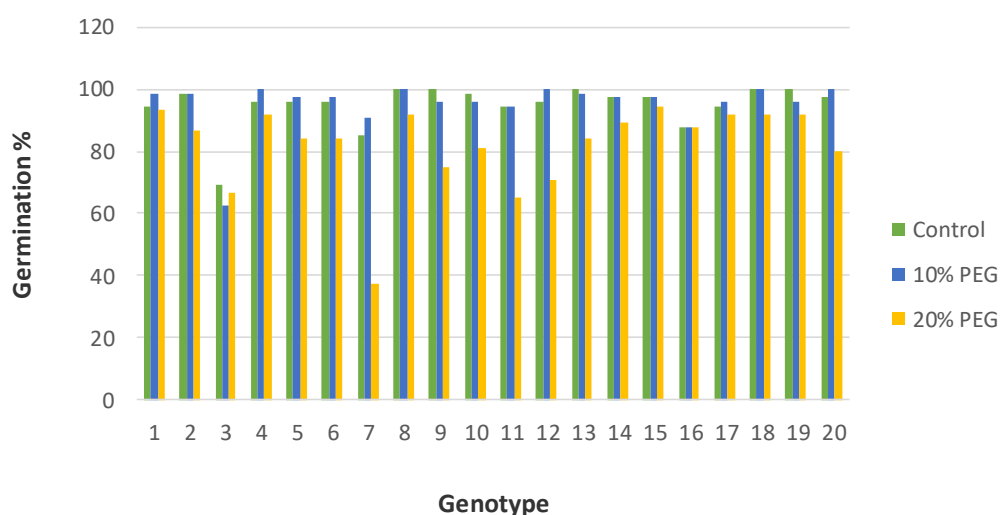
**Table 3: Evaluation of germination parameters of 20 rice genotypes under 3 osmotic stress conditions induced by different concentrations of PEG (6000)**

Treatment	GP	VI	MGT	CVG	GI	RL	SL	R:S
<b>1. Genotype</b>								
Aapjhutte	95.55 <sup>abc</sup>	1006.77 <sup>cde</sup>	1.77 <sup>l</sup>	9.98 <sup>ghi</sup>	220.78 <sup>ab</sup>	5.02 <sup>ef</sup>	5.43 <sup>cdef</sup>	1.18 <sup>gh</sup>
Kaathe	94.67 <sup>abc</sup>	1191.80 <sup>b</sup>	2.06 <sup>ij</sup>	11.29 <sup>defg</sup>	212.22 <sup>bcde</sup>	5.90 <sup>c</sup>	6.37 <sup>a</sup>	1.48 <sup>def</sup>
Lekali Basmati	66.22 <sup>i</sup>	835.16 <sup>hi</sup>	2.46 <sup>def</sup>	6.74 <sup>j</sup>	141.44 <sup>k</sup>	7.94 <sup>b</sup>	4.74 <sup>hij</sup>	3.40 <sup>a</sup>
Maanamure	96.00 <sup>ab</sup>	1029.02 <sup>cd</sup>	1.67 <sup>l</sup>	9.44 <sup>hi</sup>	224.33 <sup>a</sup>	4.77 <sup>fg</sup>	5.84 <sup>bcd</sup>	1.01 <sup>gh</sup>
Gaure	92.44 <sup>bcd</sup>	1013.98 <sup>cde</sup>	2.85 <sup>ab</sup>	14.95 <sup>a</sup>	189.11 <sup>ij</sup>	4.77 <sup>fg</sup>	6.00 <sup>ab</sup>	0.95 <sup>gh</sup>
Kartike	92.44 <sup>bcd</sup>	817.49 <sup>i</sup>	2.32 <sup>efgh</sup>	11.73 <sup>cdef</sup>	202.22 <sup>fg</sup>	3.65 <sup>i</sup>	4.98 <sup>fghij</sup>	0.95 <sup>gh</sup>
Rato Anadi	71.11 <sup>h</sup>	786.37 <sup>i</sup>	3.00 <sup>a</sup>	9.16 <sup>i</sup>	146.78 <sup>k</sup>	4.69 <sup>fgh</sup>	4.75 <sup>hij</sup>	1.51 <sup>de</sup>
Lamcho								
Dalle Masino	97.33 <sup>a</sup>	1042.92 <sup>cd</sup>	2.10 <sup>hij</sup>	12.33 <sup>cde</sup>	216.78 <sup>abc</sup>	5.13 <sup>def</sup>	5.45 <sup>cdef</sup>	1.04 <sup>gh</sup>
Piyale	90.22 <sup>def</sup>	975.84 <sup>def</sup>	2.26 <sup>fghi</sup>	10.92 <sup>fg</sup>	198.89 <sup>gh</sup>	5.16 <sup>def</sup>	5.21 <sup>efgh</sup>	1.65 <sup>d</sup>
Jhumka	92.00 <sup>cde</sup>	1299.35 <sup>a</sup>	2.53 <sup>cde</sup>	12.95 <sup>bc</sup>	196.00 <sup>ghi</sup>	8.38 <sup>b</sup>	5.45 <sup>cdef</sup>	2.29 <sup>c</sup>
Kalo patle	84.89 <sup>g</sup>	1233.87 <sup>ab</sup>	2.59 <sup>cd</sup>	10.64 <sup>fgh</sup>	181.89 <sup>j</sup>	8.90 <sup>a</sup>	4.57 <sup>ij</sup>	2.82 <sup>b</sup>
Rato Anadi	88.89 <sup>ef</sup>	853.61 <sup>hi</sup>	2.40 <sup>defg</sup>	11.11 <sup>efg</sup>	193.56 <sup>hi</sup>	3.38 <sup>i</sup>	5.59 <sup>bcde</sup>	1.05 <sup>gh</sup>
Dalle								
Parmali	94.22 <sup>abc</sup>	1028.18 <sup>cd</sup>	2.40 <sup>defg</sup>	12.62 <sup>cd</sup>	204.33 <sup>efg</sup>	5.61 <sup>cd</sup>	5.07 <sup>fghi</sup>	1.57 <sup>d</sup>
Darmali	94.67 <sup>abc</sup>	870.62 <sup>ghi</sup>	2.26 <sup>fghi</sup>	12.44 <sup>cde</sup>	207.44 <sup>def</sup>	4.31 <sup>gh</sup>	4.76 <sup>hij</sup>	1.17 <sup>gh</sup>
Juhari	96.44 <sup>a</sup>	936.50 <sup>efg</sup>	2.23 <sup>ghi</sup>	12.87 <sup>bc</sup>	211.67 <sup>cde</sup>	4.83 <sup>fg</sup>	4.83 <sup>hij</sup>	1.25 <sup>efg</sup>
Kalo	88.00 <sup>f</sup>	852.90 <sup>hi</sup>	2.42 <sup>defg</sup>	11.71 <sup>cdef</sup>	188.78 <sup>ij</sup>	4.35 <sup>gh</sup>	5.34 <sup>efg</sup>	0.86 <sup>h</sup>
Jhinuwa								
Pahale	94.22 <sup>abc</sup>	1033.42 <sup>cd</sup>	1.84 <sup>kl</sup>	10.19 <sup>ghi</sup>	215.78 <sup>abcd</sup>	5.05 <sup>ef</sup>	5.87 <sup>bc</sup>	1.09 <sup>gh</sup>
Chiniya	97.33 <sup>a</sup>	1073.92 <sup>c</sup>	2.02 <sup>jk</sup>	11.78 <sup>cdef</sup>	218.89 <sup>abc</sup>	5.52 <sup>cde</sup>	5.36 <sup>defg</sup>	2.31 <sup>c</sup>
Pahelo	96.00 <sup>ab</sup>	847.52 <sup>hi</sup>	2.26 <sup>fghi</sup>	12.77 <sup>bc</sup>	210.33 <sup>cdef</sup>	4.19 <sup>h</sup>	4.57 <sup>j</sup>	1.12 <sup>gh</sup>
Mansaar								
Dalle	92.44 <sup>bcd</sup>	910.09 <sup>fgh</sup>	2.72 <sup>bc</sup>	14.08 <sup>ab</sup>	192.67 <sup>hi</sup>	4.64 <sup>fgh</sup>	4.93 <sup>ghij</sup>	1.09 <sup>gh</sup>
F-test	***	***	***	***	***	***	***	***
LSD	3.08	73.35	0.19	1.20	7.70	0.47	0.43	0.29
<b>2. Moisture</b>								
Control	95.00 <sup>a</sup>	1172.90 <sup>b</sup>	1.75 <sup>c</sup>	13.45 <sup>a</sup>	219.70 <sup>a</sup>	6.12 <sup>a</sup>	6.26 <sup>b</sup>	0.99 <sup>b</sup>
10% PEG	95.27 <sup>a</sup>	1329.35 <sup>a</sup>	1.96 <sup>b</sup>	11.09 <sup>b</sup>	215.60 <sup>b</sup>	6.16 <sup>a</sup>	7.83 <sup>a</sup>	0.80 <sup>c</sup>
20% PEG	82.00 <sup>b</sup>	443.65 <sup>c</sup>	3.22 <sup>a</sup>	9.91 <sup>c</sup>	160.78 <sup>c</sup>	3.63 <sup>b</sup>	1.67 <sup>c</sup>	2.67 <sup>a</sup>
F-test	***	***	***	***	***	***	***	***
LSD	1.19	28.41	0.07	0.46	2.98	0.18	0.17	0.11
<b>Interaction</b>								
Mean	90.75	981.97	2.31	11.48	198.69	5.31	5.26	1.49
CV%	3.64	8.00	8.77	11.18	4.15	9.62	8.72	21.12

GP: Germination Percentage; VI: Vigor Index; MGT: Mean Germination Time; CVG: Coefficient of Velocity of Germination; GI: Germination Index; RL: Root Length; SL: Shoot Length; R:S: Root-Shoot Ratio; CV: Coefficient of Variation; \*, \*\* and \*\*\* represents significance at  $p \leq 0.05$ ,  $p \leq 0.01$  and  $p \leq 0.001$  respectively; LSD: Least Significant Difference at 5% level of significance; Means in columns not sharing the same letters are significantly different according to LSD test ( $p \leq 0.05$ )

The Figure 1 showed that germination percentage was reduced under higher level application of PEG6000. It was observed that decline in germination percentage with decreasing water potential might be due to low hydraulic conductivity of the environment, where PEG 6000 makes water unavailable to seeds, affecting the imbibition processes of the seed which is fundamental for germination (Lobato *et al.*, 2009).

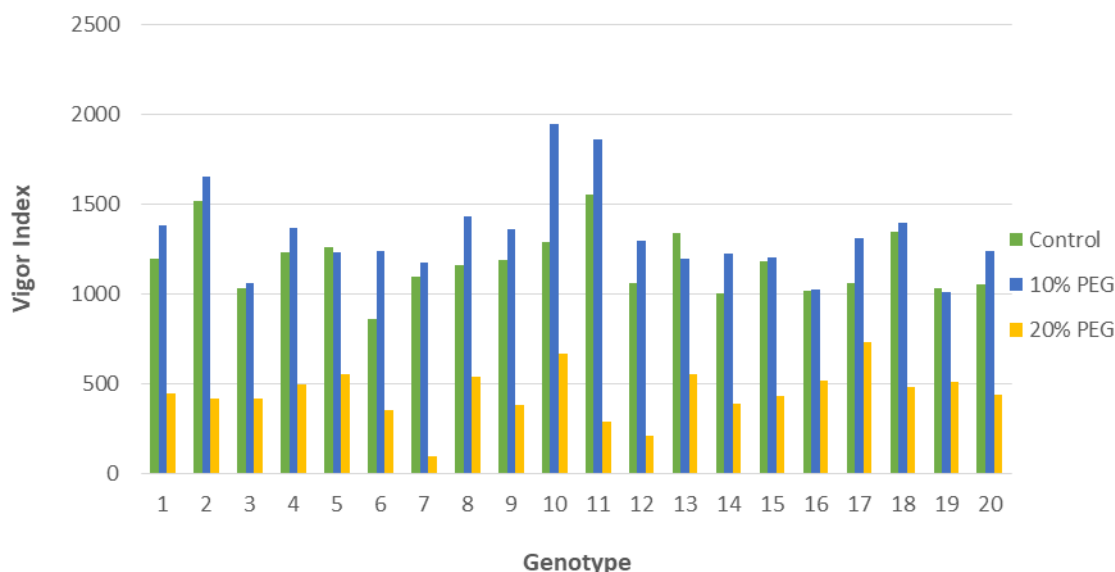
This larger reduction with PEG solution could be attributed to high viscosity, where solubility and diffusion of oxygen were reduced compared to control. The gradient of water potential between dry seeds and pure water (0.0 bars) decreases rapidly with the addition of any soluble substances like PEG. Since, The water potential is the summation of osmotic and turgor pressure, the water potential of the seed also decreases (Islam *et al.*, 2018). The decrease in water potential gradient between seed and media causes disruption in the process of imbibition and tissue hydration on rice seeds ultimately lowering the germination percentage. Results of the current study corroborate with (Radhouane, 2007), (Govindaraj *et al.*, 2010) and (Basha *et al.*, 2015).



**Figure 1: Effect of different osmotic stress induced by PEG (6000) on Germination Percentage**

#### Effect of water stress on vigor index

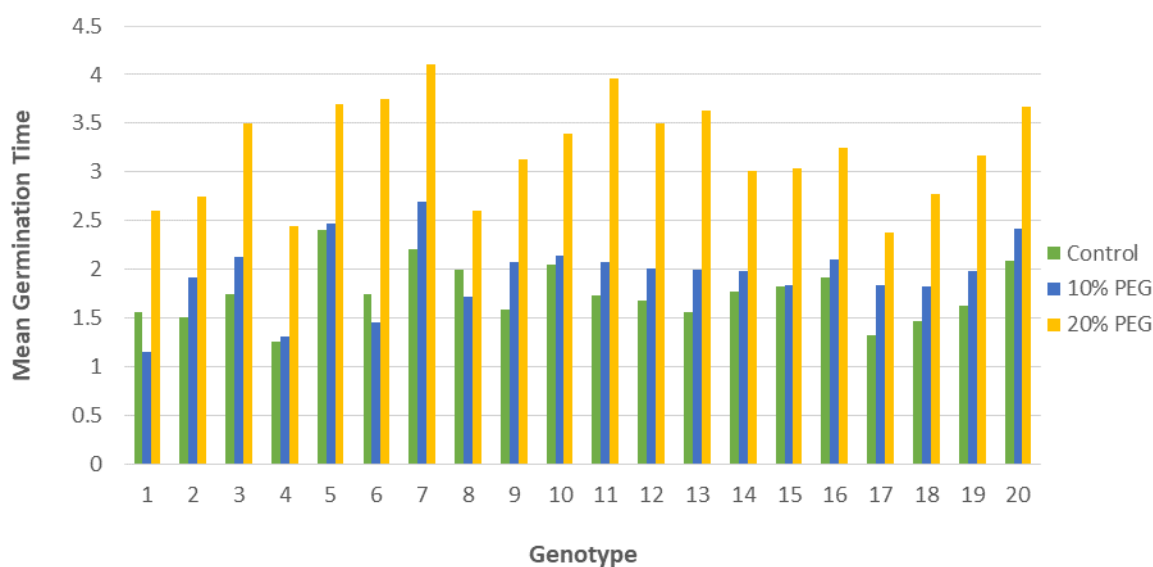
The maximum index of 1299.35 was shown by the genotype Jhumka and the minimum index was shown by genotypes Kartike (817.489) and Rato Anadi Lamcho (786.375) statistically at par. Comparison of mean showed that PEG concentration up to 10% increased the vigor index of rice compared to control but further increase of concentration to 20% decreased the vigor index significantly (Table 3). The Figure 2 showed that vigor index is lower at higher level of PEG6000. Similar results of the experiment were showed by (Tang *et al.*, 2019) where seed germination was increased under 10% PEG treatment compared with control and 20% PEG treatment. Significant decrease in seed vigor index of rice genotypes at severe osmotic stress (20% PEG) induced by increasing PEG concentrations was probably due to decreasing trend in shoot and root lengths. Lower index values represent drought sensitivity and higher index values represent drought tolerance.



**Figure 2: Effect of different osmotic stress induced by PEG (6000) on Vigor Index**

### Effect of water stress on mean germination time

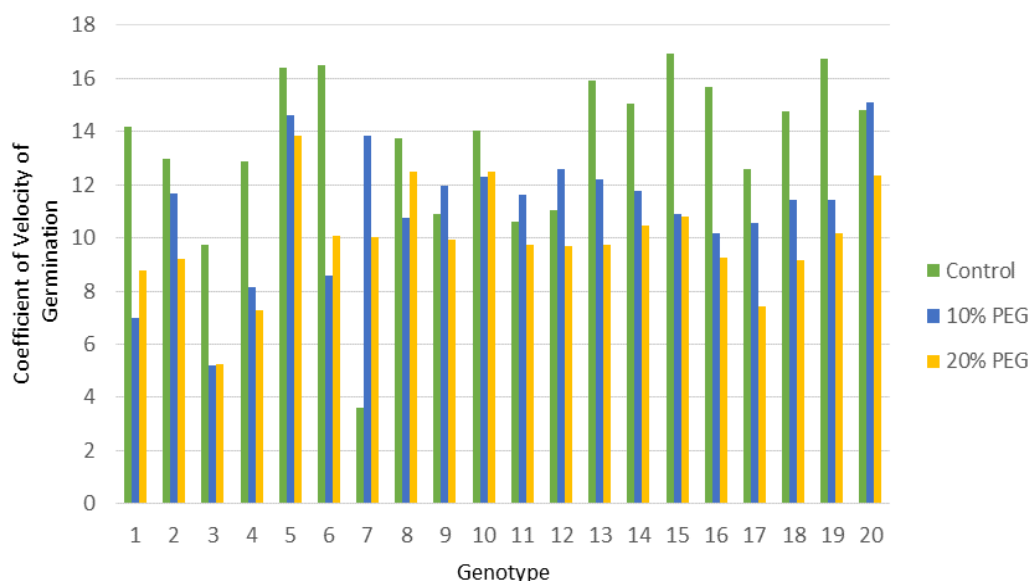
The minimum mean germination time was shown by the genotype Maanamure (1.67 days) and Aapjhutte (1.77 days) which were statistically at par. Rato Anadi Lamcho showed the maximum mean germination time of 3 days. The highest mean germination time of 3.22 days was taken by 20% PEG while control took the mean germination time of only 1.75 days (Table 3). Under water stress conditions, delay in completion of germination is a common response, because seeds require more time to absorb sufficient amount of water, which is vital for the act of initiation of germination (Sukifto *et al.*, 2020). This justifies our result where the mean germination time increased with the increase in PEG concentration (Figure 3). Soil moisture deficit stress extends the average amount of time required for seed germination (Queiroz *et al.*, 2019).



**Figure 3: Effect of different osmotic stress induced by PEG (6000) on Mean Germination Time**

### Effect of water stress on coefficient of velocity of germination

The results showed that coefficient of velocity of germination decreased with the increase in PEG concentrations. Control (13.45) showed the highest while PEG 20% (9.91) showed the lowest coefficient of velocity of germination (Table 3). Genotype Gaure showed the maximum coefficient of velocity of germination of 14.95 and the minimum coefficient of velocity of 6.74 was shown by the genotype Lekali Basmati. The shorter the germination time the greater is the coefficient of velocity of germination (Figure 4).

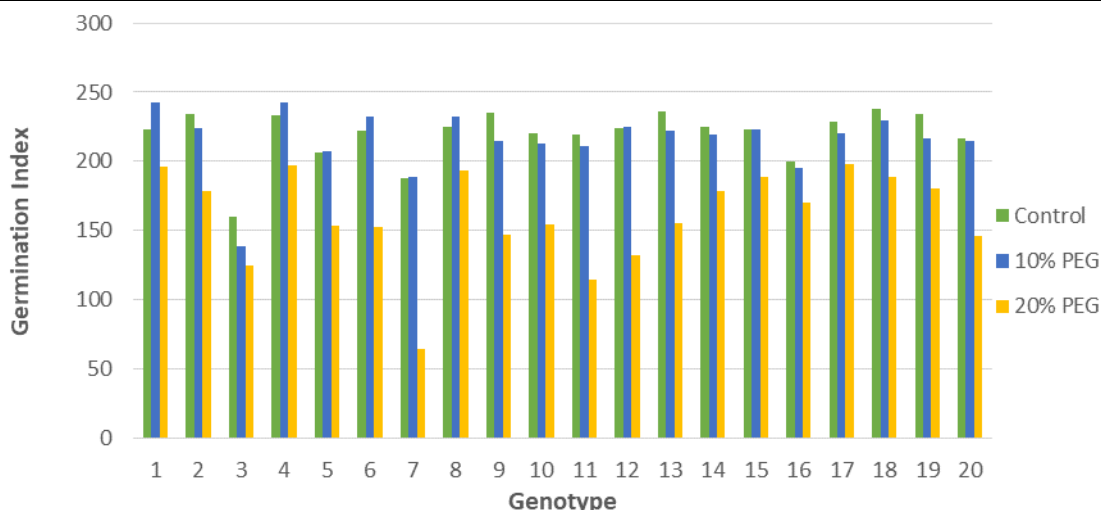


**Figure 4: Effect of different osmotic stress induced by PEG (6000) on Coefficient of Velocity of Germination**

### Effect of water stress on germination index

Genotype Maanamure showed the highest germination index of 224.33 while Lekali Basmati (141.44) and 'Rato Anadi Lamcho (146.78) were statistically at par and showed the lowest germination index. The maximum germination index of 219.7 was recorded in the control whereas minimum germination index of 160.78 was observed in 20% PEG (Figure 5). Germinated seedling under moisture deficit environment shows a reduction in seedling vigor and germination index (Tang *et al.*, 2019).

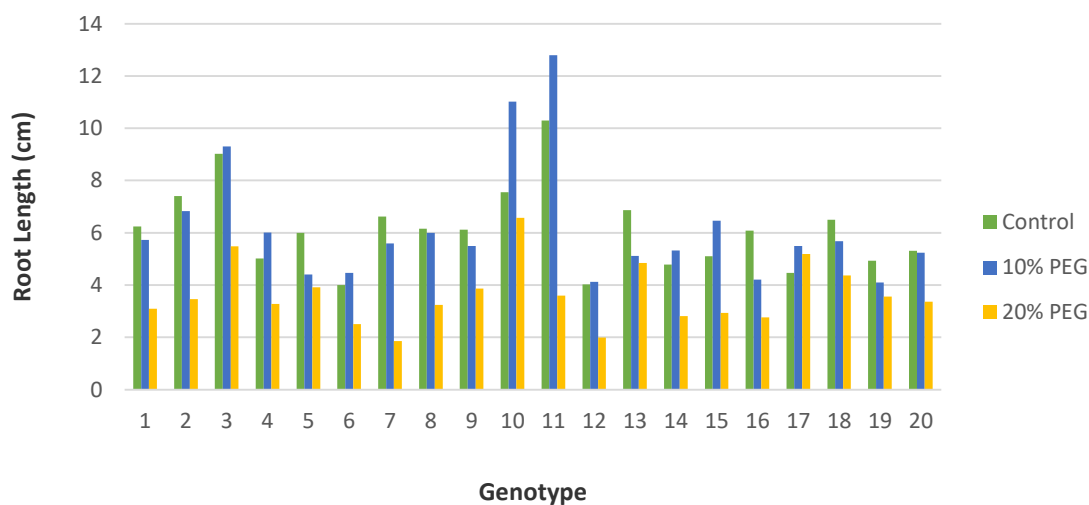




**Figure 5: Effect of different osmotic stress induced by PEG (6000) on Germination Index**

### Effect of water stress on root length

The maximum root length was recorded in the genotype Kalo Patle (8.9cm) whereas Pahelo Mansaar (4.19cm) recorded the minimum root length. Comparison of means showed that the osmotic stress at control and 10% PEG were statistically at par. However the highest root length was observed in 10% PEG. The root length decreased subsequently at 20% PEG condition (Figure 6). The result reflects on adaptive response involving an increase in root length to reach deeper water. Similar observation was reported by (Almaghrabi, 2012). However, for severe stress in the seed germination experiment the root length was reduced drastically. Fraser *et al.* (1990) concluded that the reduction in the root length under severe drought stress may due to an impediment of cell division and elongation leading to Kind tuberization. This tuberization and the lignifications of the root system allow the conditions to become favorable again. Drought stress creates an impact on root cell development, which would likely impair nutrient uptake as well as having detrimental effects on photosynthesis, essential for biomass accumulation and therefore on shoot and root elongation.

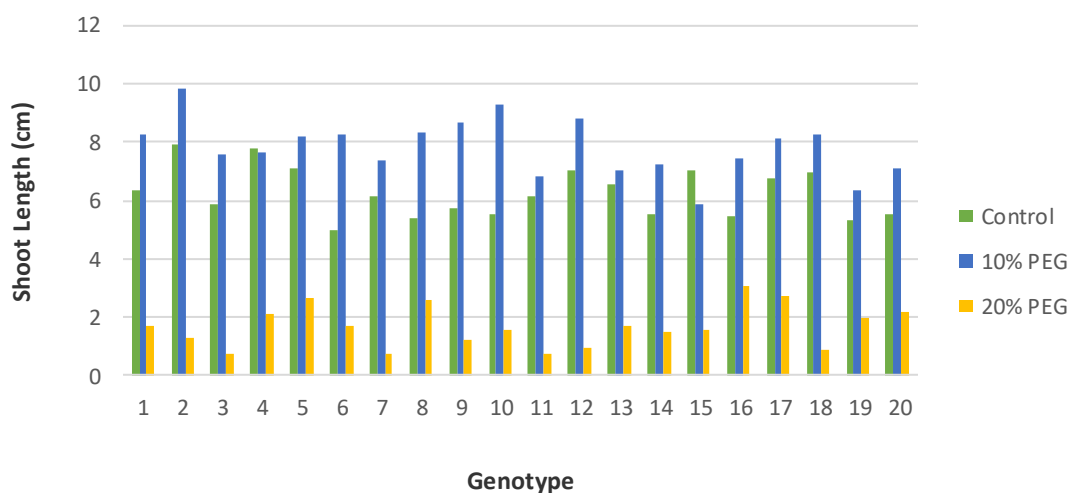


**Figure 6: Effect of different osmotic stress induced by PEG (6000) on Root Length**

Long roots was reported as a component trait for drought tolerance by Govindaraj *et al.* (2010) and Piwowarczyk *et al.* (2014) as they play a direct role with high penetration ability and have large xylem vessel radii and lower axial resistance to water flux aiding in greater water acquisition. According to Kim *et al.* (2001) and Pandey & Shukla (2015), a root system with longer root length at deeper layer is useful in extracting water in upland conditions. Early and rapid elongation of root is an important indication of drought tolerance

### Effect of water stress on shoot length

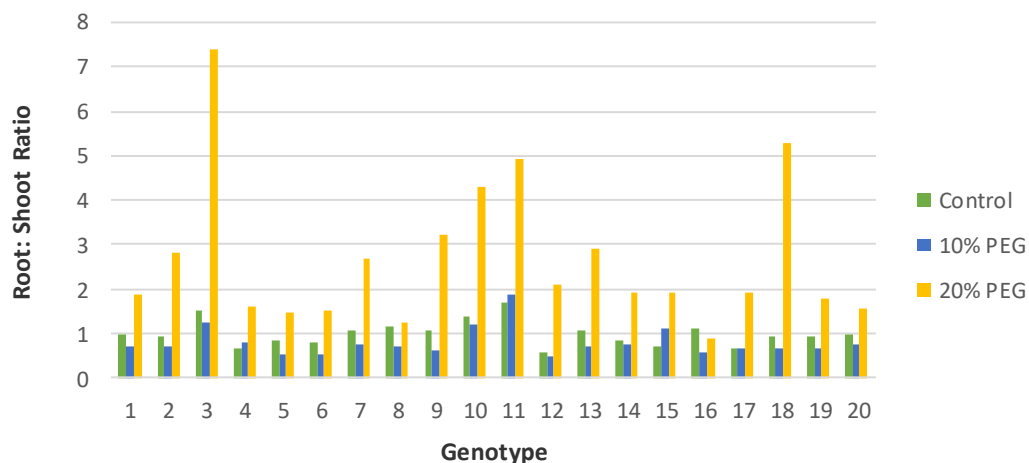
Highest shoot length was recorded in the genotype Kaathe (6.37cm) whereas Pahelo Mansaar (4.57cm) recorded the lowest shoot length (Table 3). The result of the experiment showed that shoot length decreased abruptly with the increase in drought stress. However, a slight increase in shoot length was observed at 10% PEG as compared to control. It corroborates with the findings of (Govindaraj *et al.*, 2010), (Basha *et al.*, 2015) and (Pandey & Shukla, 2015). Figure 7 showed the shoot length is reduced by application of PEG6000. The shoot length was decreased with an increase in external water stress. Though root length is more affected by drought than shoot length, the effect of drought is exhibited mostly on the shoot as well as aerial parts of the plant, which will bear most economic parts of the crops. Hence, the shoot parameters will also help the breeder while selecting the superior genotypes against drought.



**Figure 7: Effect of different osmotic stress induced by PEG (6000) on Shoot Length**

### Effect of water stress on root-shoot ratio

Maximum root-shoot ratio was shown by the genotype Lekali Basmati (3.4) whereas genotype Kalo Jhinuwa showed the minimum root-shoot ratio (Table 3). The root-shoot ratio was increased in the current study indicating that all the tested genotypes tended to increase their root length under low moisture availability (Figure 8). However, the increased root length could not compensate the damage caused by low water availability to shoot growth. Under mild water deficit, the root growth usually maintains while shoot growth is inhibited. This is because of the facts that, adjustment like, re-establishment of water potential gradient through osmotic alteration and increase in loosening ability of the cell wall, permit roots to resume growth under low water potential. In contrast, there is no such regulation in leaves, leading to marked growth inhibition (Hsiao & Xu, 2000).



**Figure 8: Effect of different osmotic stress induced by PEG (6000) on Root-Shoot Ratio**

When water deficiency occurs, root growth is favored over shoot growth. Root to shoot ratio also plays a major role in selecting drought tolerant lines. Genotypes with high root/shoot ratio under drought are much preferred. High root to shoot ratio has been reported as a component trait for drought avoidance (Govindaraj *et al.*, 2010; Radhouane, 2007; Xu *et al.*, 2015). The studies of Xu *et al.* (2015) revealed that under drought conditions the plasticity of the plants allow increased allocation of several primary metabolites to roots while decreasing allocation to shoots.

## CONCLUSION

There were different responses for all the early growth traits studied on 20 rice landraces under 0%, 10% and 20% PEG conditions. Comparison of mean performance revealed increasing levels of drought stress to have high limiting effect on different early growth parameters studied. Genotype Manamure demonstrated enhanced Germination Index with the fastest Mean Germination Time whereas genotype Rato Anadi Lamcho showed an inferior performance with the lowest Vigor Index, Germination Index and the slowest Mean Germination Time. The interactive effect of genotypes and moisture levels has shown genotype Pahele to have maximum values for Germination Percentage, Vigor Index, Germination Index, Shoot Length and the fastest Mean Germination Time, under 20% PEG condition. Thus, Manamure and Pahele can be considered relatively drought tolerant genotypes among the 20 landraces. Also, the application of PEG 6000 would be a simple, rapid and cost-effective method of screening the early growth traits of large set of rice landraces for drought tolerance.

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### Authors' Contribution

R. Karanjit designed and performed the experiment, recorded and analysed data and wrote the main manuscript. A. Khakural helped in analysing data and editing the manuscript. B.P. Kandel supervised the research.

### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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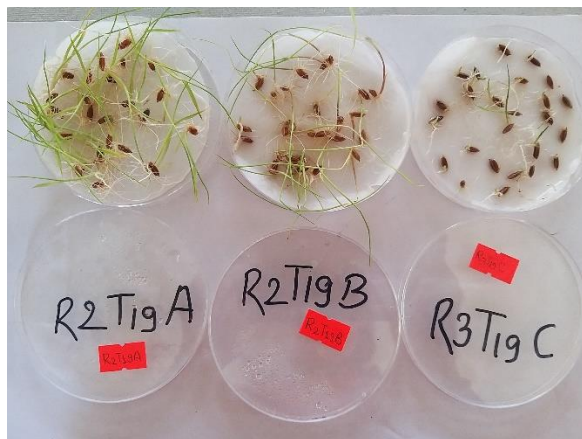
## SUPPLEMENTARY MATERIALS



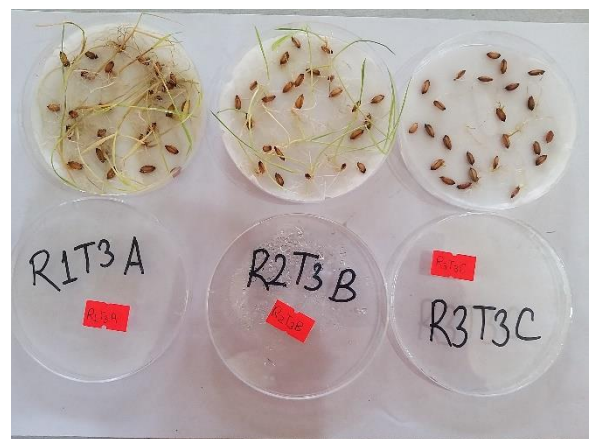
**Figure: Experimental setup**



**Figure: Different concentrations of PEG 6000**



**Figure: Variation in germination of relatively drought tolerant genotype *Pahelo Mansaar* under different levels of drought stress**



**Figure: Variation in germination of relatively drought sensitive genotype *Lekali Basmati* under different levels of drought stress**