

Review Article

Evaluation of Upland Rice Response to Water Stress Using Polyethylene Glycol (PEG - 6000) at Germination and Early Seedling Stage

ABSTRACT

Water stress is one of the most important crop growth limiting factors which leads to low crop productivity and yield instability. Water stress affects crop growth and development, especially during the germination and seedling period. The experiment was conducted in the growth chamber at the seed physiology laboratory of the Department of Seed, Crop and Horticultural Sciences, University of Eldoret to evaluate sixteen upland rice varieties to water stress tolerance at germination and early seedling growth stage. These varieties were tested against four levels of water stress imposed by Polyethylene glycol 6000 (PEG - 6000) at 0%, 5%, 10% and 15% concentration. The layout of the experiment was complete randomized design (CRD) with three replications. Osmotic stress induced by PEG levels significantly reduced plant growth parameters. The result showed that the germination percentage, germination index, relative seedling height (%), seedling dry weight, seedling fresh weight, seedling length, seedling vigor index and mean germination time of all tested rice varieties were found decreasing trends with increasing the levels of PEG from 0 to 15% concentration levels. Among all the sixteen varieties, NERICA rice varieties followed by MWUR, White rice, Kpatawee, and Komboka had an outstanding performance in terms of germination percentage, germination index, seedling height, seedling dry weight, root length and relative dry weight under water induced stress by using PEG compared to other varieties. Therefore, these varieties could be useful in breeding programs and can be cultivated in arid and semi-arid environment or where water shortage is a regular constraint.

Key words: Water osmotic stress, Upland rice response varieties, Polyethylene glycol 6000, germination and early seedling stage growth

Comment [h1]: You have to add statistical analysis results (probability) to show difference between means under PEG concentrations for each parameter and each variety. See comments in table 2, results

Comment [h2]: Here, you can consider the obtained results with 15% PEG only

1. Introduction

Water stress is one of the major constraints to rice production and yield stability in rainfed upland ecology and estimates indicate that 70% of the yield losses are attributed to abiotic stress, especially drought [16]. Growth stages, age, plant species and drought severity and duration are the key factors that influence rice plant, the resistance mechanism to drought varies among plant species [10].

Rice (*Oryza sativa* L.) is one of the most important human food crop in the world, feeding more people than any other crop. It plays a predominant role by providing 50–80% of the daily calories [6]. Rice is considered to be a drought vulnerable crop as it exhibits serious harmful effects when exposed to water stress at critical growth stages, especially at the reproductive stage [11]. As a result, drought stress affects about 50% of rice production in the world [15].

Seed germination and early seedling growth are the most critical stages of drought stress in many plant species including rice [30]. Water availability and movement into seeds are very important to promote germination, initial root growth, shoot elongation and therefore at the establishment of a uniform stand. There has been reports of water stress affecting seed germination and early seedling growth that are potentially the most crucial stages of rice production [1]. It has also been found that drought stress impairs seed germination [1] and seedling height of rice [22]. However, the sensitivity of rice to drought stress varies with timing, duration, and severity of water unavailability, variety and the growth stage of rice [22].

Polyethylene glycol (PEG) 6000 is a metabolically inactive compound often used to induce uniform drought stress at early germination and seedling growth stages to study the effects of water stress in different plant groups [11] and [19]. Polyethylene glycol (PEG) 6000 is also a non-ionic water polymer, uncharged, and is widely used to simulate water stress in plants [3].

Drought stress causes various physiological changes in plants that may include, reduction in photosynthetic rate, transpiration rate, stomatal conductance, pigment degradation and relative water content that decreases water use efficiency and growth reduction [8]. Plants' response to water stress is much complex which includes the adaptation of various mechanisms when they encounter drought stress at various growth stages [8].

Hence, the selection of varieties under water stress conditions through rapid and uniform germination is crucial during germination and early seedling establishment [24]. Therefore, germination and seedling growth traits and their response to water stress can be useful for the selection of drought-tolerance varieties [29].

2.MATERIALS AND METHODS

2.1 Planting materials

Sixteen upland rice varieties were selected for the study, seven varieties were selected from Liberia and nine were from Kenya (Table 1).

Comment [h3]: Why this choice?

Table 1: List of rice varieties and their sources used in the study

No.	Variety Name	Sources	Origin
1	Dourado	Kenya	Commercial
2	Jaowo	Liberia	Local
3	Komboka	Kenya	Commercial
4	Kpatawee	Liberia	Local
5	L - 22	Liberia	Local
6	LAC-23	Liberia	Local
7	MWUR	Kenya	Commercial
8	Nerica 11	Kenya	Africa Rice
9	Nerica 1	Kenya	Africa rice
10	Nerica 10	Kenya	Africa rice
11	Nerica 14	Liberia	Africa rice
12	Nerica 2	Kenya	Africa rice
13	Nerica 3	Kenya	Africa rice
14	Nerica 4	Kenya	Africa rice
15	White rice	Liberia	Local
16	Red Youmo	Liberia	Local

2.1.2EXPERIMENTAL SITE

The experiment was conducted at the University of Eldoret crop physiology laboratory in the growth chamber at 25°C with a relative of 80±1% at the department of Seed, Crop and Horticultural Sciences.

2.1.3EXPERIMENTAL DESIGN AND TREATMENTS

The germination test experiment was set up in a Completely Randomized Design (CRD) with three replications with sixteen upland rice varieties and four water stress treatments, namely 0% T1- Control, T2 PEG 5%, T3 PEG 10%, and T4 15% Polyethylene glycol (PEG-6000; Sigma Chemicals). During screening, water deficit stress was artificially induced by desired strengths of polyethylene glycol (PEG-6000; Sigma Chemical). Polyethylene glycol has been used to simulate water stress effects in plants [25]. The different water levels were obtained by dissolving polyethylene glycol 6000 (PEG) in distilled water. Distilled water was used as a control (0 MPa) and osmotic potentials -0.3, -0.6, -0.9 and -1.2 MPa was created by adding PEG 6000 at 0, 5, 10 and 15 g per 100 ml distilled water. Seeds were sterilized with 5% sodium hypochlorite for 5 minutes and washed thoroughly with distilled water. Germination assessment was conducted by evenly distributing the seeds in a 10 cm diameter sterilized Petri dish with two layers of what man No.1 filter paper. Each petri dish was moistened with 10 ml distilled water or uniform amounts of desired osmotic solutions to mimic water stress. Fifty seeds of each variety were set up in a Petri dish and was treated with respective treatment solutions and distilled water. The number of germinated seed was recorded at 24 hours' interval. The seedling height and seedling dry weights were measured on the 14th day. Seeds were considered germinated when both plumule and radicles extended to more than 2 mm from the seeds.

2.1.4 Data Collection and Analysis

Ten seedlings were selected randomly and height was measured. The lengths of seedling were measured with a ruler. Data on germination and seedling characteristics for each treatment were compared with the control for determining the drought tolerant rice varieties. Data on seedling fresh weight (SFW) was determined after 21 days. Shoot dry weight (SDW) was determined after drying the seedlings at 70°C for 48 hours.

The germination index, germination percentage, Mean germination time, seedling vigor index and relative seedling height were calculated after the final germination using the following equations:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds planted}} \times 100$$

$$\text{Germination index \%} = \frac{\text{Germination percentage in each treatment}}{\text{Germination percentage in the control}} \times 100$$

[7]

Mean germination time = $n_1 \times d_1 + n_2 \times d_2 + n_3 \times d_3 + \dots$ / total number of days

Where, n= number of germinated seed, d = number of days

The relative seedling height (RSH) was calculated using the following equation:

$$\text{RSH (\%)} = \frac{\text{Plant height in each treatment}}{\text{Plant height under control}} \times 100$$

Seedling Vigor index (SVI) = germination percent x seedling height.

All the data collected were summarized in Microsoft excel and subjected to analysis of variance (ANOVA) using GENSTAT 14th-edition (VSN International Limited, 2011). Treatment means for the different parameters were separated using Fisher's least significant difference (LSD) procedure at 5 % level of significance.

3. RESULTS

3.1 Germination Percentage and Germination Index

Increasing concentration of PEG-6000 ~~seed germination reduced~~ ~~reduced seed germination and~~ ~~hgermination index of each variety and~~. Different PEG concentrations had a substantial effect ($P < .001$) ~~on percent seed germination of~~ ~~according~~ different rice varieties as shown in (Table 1).

Table 2: Influence of different levels of PEG 6000 on germination (%) and germination Index of upland rice varieties

Germination %					Germination index %					
Variety	PEG concentrations				Probability mean	PEG concentrations				Mean
	0%	5%	10%	15%		0%	5%	10%	15%	
MWUR	85.7 ^{ab}	84.7 ^{abc}	76.7 ^{abc}	74.7 ^{abc}	80.5	100 ^a	95.1 ^{ab}	91.2 ^{abc}	88.3 ^{abcd}	93.7
Dourado	86.0 ^{ab}	67.3 ^{cde}	64.7 ^{gh}	58.7 ^{hij}	69.2	100 ^a	67.7 ^{def}	78.3 ^{bcd}	74.3 ^{cde}	70.6
L- 22	76.0 ^{abc}	57.3 ^{ijk}	45.3 ^{kl}	28.0 ^{lm}	51.7	100 ^a	64.0 ^{efg}	59.3 ^{ghi}	38.2 ^{ij}	53.8
Komboka	87.0 ^{ab}	69.0 ^{bcd}	74.7 ^{abcd}	61.3 ^{ghi}	73.0	100 ^a	86.6 ^{abcd}	80.1 ^{bcd}	71.1 ^{def}	79.3
LAC 23	88.0 ^{abc}	84.3 ^{abc}	77.3 ^{abcd}	65.3 ^{efg}	78.7	100 ^a	90.8 ^{abc}	84.1 ^{abc}	77.8 ^{bcd}	88.2
White rice	90.3 ^{ab}	84.0 ^{abc}	77.0 ^{abcd}	75.0 ^{abc}	81.6	100 ^a	93.0 ^{abcd}	85.3 ^{abc}	83.1 ^{abcd}	87.1
Kpatawee	90.7 ^{ab}	78.7 ^{abc}	78.3 ^{abcd}	29.3 ^{lm}	69.2	100 ^a	86.8 ^{abc}	86.0 ^{abc}	32.2 ^j	68.3
Red youmo	88.0 ^{abc}	79.3 ^{abc}	72.0 ^{abcd}	61.3 ^{kl}	75.1	100 ^a	90.1 ^{abc}	82.0 ^{abcd}	69.5 ^{def}	80.5
NERICA 10	89.3 ^{abc}	82.7 ^{abc}	79.2 ^{abc}	69.3 ^{abc}	80.1	100 ^a	92.6 ^{abc}	88.7 ^{abcd}	78.1 ^{bcd}	86.5
NERICA 14	80.3 ^{abc}	73.3 ^{abc}	73.3 ^{abcd}	25.4 ^m	63.1	100 ^a	93.8 ^{abc}	87.8 ^{bcd}	36.2 ^{ij}	79.5
Jaowo	84.5 ^{ab}	84.0 ^{abcd}	80.1 ^{abc}	49.1 ^{jk}	74.4	100 ^a	95.2 ^a	90.4 ^{abc}	59.6 ^{fgh}	86.6
NERICA 11	87.7 ^{abc}	82.0 ^{ab}	82.0 ^{abc}	80.0 ^{abc}	82.9	100 ^a	94.2 ^{abc}	91.8 ^a	91.6 ^a	93.2
NERICA 4	91.7 ^a	83.3 ^{abc}	81.3 ^{abc}	81 ^{abc}	84.3	100 ^a	88.7 ^{abc}	90.9 ^{abcd}	78.5 ^{bcd}	86.0
NERICA 1	91.7 ^a	85.3 ^{abc}	80.7 ^{abc}	78.0 ^{abc}	83.9	100 ^a	93.1 ^{abc}	88.1 ^{abc}	85.1 ^{abcd}	88.8
NERICA 2	89.7 ^{abc}	82.7 ^{abc}	79.3 ^{abc}	50.3 ^{jk}	75.5	100 ^a	92.2 ^{abc}	88.4 ^{abc}	56.6 ^{hi}	79.1
NERICA 3	89.7 ^{abc}	81.3 ^{abc}	66.5 ^{def}	69.0 ^{abc}	76.6	100 ^a	90.7 ^{abc}	74.4 ^{cdef}	77.4 ^{bcd}	80.8

Comment [h4]: 2 tables following the data : see table 2 and table 3 below in comments

Comment [h5]: To show difference between means under PEG concentrations for each parameter and each variety

Comment [h6]: Note here the probability from the comparison between germination percentages at different PEG concentrations for MWUR variety

Comment [h7]: Add here a line for probability from the comparison between germination percentages at each PEG concentration for all varieties.

LSD_{0.05} Variety = 8.8***, Treatment = 4.4***, Variety x Treatment = 17.6**

Variety = 10.99***, Treatment = 5.50**, Variety x Treatment = 21.99**

Mean values in column of each treatment with the same letter(s) are not significantly different at the threshold of 0.05 ($P < .05$).

Table 2: Influence of different levels of PEG 6000 on germination (%) and germination Index for each upland rice varieties

Germination %					Germination index %					
Variety	PEG concentrations				Probability	PEG concentrations				Probability
	0%	5%	10%	15%		0%	5%	10%	15%	
MWUR	85.7 ^{ab}	84.7 ^{abc}	76.7 ^{abc}	74.7 ^{abc}	80.5	100 ^a	95.1 ^{ab}	91.2 ^{abc}	88.3 ^{abcd}	93.7
Dourado	86.0 ^{ab}	67.3 ^{cde}	64.7 ^{gh}	58.7 ^{hij}	69.2	100 ^a	67.7 ^{def}	78.3 ^{bcd}	74.3 ^{cde}	70.6
L- 22	76.0 ^{abc}	57.3 ^{ijk}	45.3 ^{kl}	28.0 ^{lm}	51.7	100 ^a	64.0 ^{efg}	59.3 ^{ghi}	38.2 ^{ij}	53.8
Komboka	87.0 ^{ab}	69.0 ^{bcd}	74.7 ^{abcd}	61.3 ^{ghi}	73.0	100 ^a	86.6 ^{abcd}	80.1 ^{bcd}	71.1 ^{def}	79.3
LAC 23	88.0 ^{abc}	84.3 ^{abc}	77.3 ^{abcd}	65.3 ^{efg}	78.7	100 ^a	90.8 ^{abc}	84.1 ^{abc}	77.8 ^{bcd}	88.2
White rice	90.3 ^{ab}	84.0 ^{abc}	77.0 ^{abcd}	75.0 ^{abc}	81.6	100 ^a	93.0 ^{abcd}	85.3 ^{abc}	83.1 ^{abcd}	87.1

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Comment [h8]: To show difference between means under PEG concentrations for germination percentage and for each variety

Comment [h9]: To show difference between means under PEG concentrations for Germination index and for each variety

Comment [h10]: Note here the probability from the comparison between germination percentages at different PEG concentrations for MWUR variety

Kpatawee	90.7 ^{ab}	78.7 ^{abc}	78.3 ^{abcd}	29.3 ^{lm}	69.2	100 ^a	86.8 ^{abc}	86.0 ^{abc}	32.2 ^j	68.3
Red youmo	88.0 ^{abc}	79.3 ^{abc}	72.0 ^{abcd}	61.3 ^{kl}	75.1	100 ^a	90.1 ^{abc}	82.0 ^{abcd}	69.5 ^{def}	80.5
NERICA 10	89.3 ^{abc}	82.7 ^{abc}	79.2 ^{abc}	69.3 ^{abc}	80.1	100 ^a	92.6 ^{abc}	88.7 ^{abcd}	78.1 ^{bcd}	86.5
NERICA 14	80.3 ^{abc}	73.3 ^{abc}	73.3 ^{abcd}	25.4 ^m	63.1	100 ^a	93.8 ^{abc}	87.8 ^{bcd}	36.2 ^{ij}	79.5
Jaowo	84.5 ^{ab}	84.0 ^{abcd}	80.1 ^{abc}	49.1 ^{jk}	74.4	100 ^a	95.2 ^a	90.4 ^{abc}	59.6 ^{fgh}	86.6
NERICA 11	87.7 ^{abc}	82.0 ^{ab}	82.0 ^{abc}	80.0 ^{abc}	82.9	100 ^a	94.2 ^{abc}	91.8 ^a	91.6 ^a	93.2
NERICA 4	91.7 ^a	83.3 ^{abc}	81.3 ^{abc}	81 ^{abc}	84.3	100 ^a	88.7 ^{abc}	90.9 ^{abcd}	78.5 ^{bcd}	86.0
NERICA 1	91.7 ^a	85.3 ^{abc}	80.7 ^{abc}	78.0 ^{abc}	83.9	100 ^a	93.1 ^{abc}	88.1 ^{abc}	85.1 ^{abcd}	88.8
NERICA 2	89.7 ^{abc}	82.7 ^{abc}	79.3 ^{abc}	50.3 ^{jk}	75.5	100 ^a	92.2 ^{abc}	88.4 ^{abc}	56.6 ^{hi}	79.1
NERICA 3	89.7 ^{abc}	81.3 ^{abc}	66.5 ^{def}	69.0 ^{abc}	76.6	100 ^a	90.7 ^{abc}	74.4 ^{cdef}	77.4 ^{bcde}	80.8
LSD_{0.05} Variety = 8.8***						Variety = 10.99***				
Treatment = 4.4***, Variety xTreatment = 17.6**						Treatment= 5.50**,VarietyxTreatment= 21.99**				

Mean values in column of each treatment with the same letter(s) are not significantly different at the threshold of 0.05

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Note varieties for which the germination percentage or germination index are near to 0%PEG condition and those for which the percentage is between 50% and the 0%PEG condition

Table 3: Influence of each concentration of PEG 6000 on germination (%) and germination index of upland rice varieties

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Variety	Germination %					Germination index %				
	PEG concentrations					PEG concentrations				
	0%	5%	10%	15%	Mean	0%	5%	10%	15%	Mean
MWUR	85.7 ^{ab}	84.7 ^{abc}	76.7 ^{abc}	74.7 ^{abc}		100 ^a	95.1 ^{ab}	91.2 ^{abc}	88.3 ^{abcd}	
Dourado	86.0 ^{ab}	67.3 ^{cde}	64.7 ^{fgh}	58.7 ^{hij}		100 ^a	67.7 ^{def}	78.3 ^{bcd}	74.3 ^{cde}	
L- 22	76.0 ^{abc}	57.3 ^{ijk}	45.3 ^{kl}	28.0 ^{lm}		100 ^a	64.0 ^{efg}	59.3 ^{ghi}	38.2 ^{ij}	
Komboka	87.0 ^{ab}	69.0 ^{bcd}	74.7 ^{abcd}	61.3 ^{ghi}		100 ^a	86.6 ^{abcd}	80.1 ^{bcd}	71.1 ^{def}	
LAC 23	88.0 ^{abc}	84.3 ^{abc}	77.3 ^{abcd}	65.3 ^{efg}		100 ^a	90.8 ^{abc}	84.1 ^{abc}	77.8 ^{bcd}	
White rice	90.3 ^{ab}	84.0 ^{abc}	77.0 ^{abcd}	75.0 ^{abc}		100 ^a	93.0 ^{abcd}	85.3 ^{abc}	83.1 ^{abcd}	
Kpatawee	90.7 ^{ab}	78.7 ^{abc}	78.3 ^{abcd}	29.3 ^{lm}		100 ^a	86.8 ^{abc}	86.0 ^{abc}	32.2 ^j	
Red youmo	88.0 ^{abc}	79.3 ^{abc}	72.0 ^{abcd}	61.3 ^{kl}		100 ^a	90.1 ^{abc}	82.0 ^{abcd}	69.5 ^{def}	
NERICA 10	89.3 ^{abc}	82.7 ^{abc}	79.2 ^{abc}	69.3 ^{abc}		100 ^a	92.6 ^{abc}	88.7 ^{abcd}	78.1 ^{bcd}	
NERICA 14	80.3 ^{abc}	73.3 ^{abc}	73.3 ^{abcd}	25.4 ^m		100 ^a	93.8 ^{abc}	87.8 ^{bcd}	36.2 ^{ij}	
Jaowo	84.5 ^{ab}	84.0 ^{abcd}	80.1 ^{abc}	49.1 ^{jk}		100 ^a	95.2 ^a	90.4 ^{abc}	59.6 ^{fgh}	
NERICA 11	87.7 ^{abc}	82.0 ^{ab}	82.0 ^{abc}	80.0 ^{abc}		100 ^a	94.2 ^{abc}	91.8 ^a	91.6 ^a	
NERICA 4	91.7 ^a	83.3 ^{abc}	81.3 ^{abc}	81 ^{abc}		100 ^a	88.7 ^{abc}	90.9 ^{abcd}	78.5 ^{bcd}	
NERICA 1	91.7 ^a	85.3 ^{abc}	80.7 ^{abc}	78.0 ^{abc}		100 ^a	93.1 ^{abc}	88.1 ^{abc}	85.1 ^{abcd}	
NERICA 2	89.7 ^{abc}	82.7 ^{abc}	79.3 ^{abc}	50.3 ^{jk}		100 ^a	92.2 ^{abc}	88.4 ^{abc}	56.6 ^{hi}	
NERICA 3	89.7 ^{abc}	81.3 ^{abc}	66.5 ^{def}	69.0 ^{abc}		100 ^a	90.7 ^{abc}	74.4 ^{cdef}	77.4 ^{bcde}	
LSD_{0.05} Variety = 8.8***						Variety = 10.99***				
Treatment = 4.4***, Variety xTreatment = 17.6**						Treatment= 5.50**,VarietyxTreatment= 21.99**				

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Mean values in column of each treatment with the same letter(s) are not significantly different at the threshold of 0.05

Comment [h13]: Add here an upper line for probability from the comparison between germination percentages / germination index at each PEG concentration for all varieties.

The highest germination percentage at 5% PEG level was found in MWUR, LAC-23, White rice, Kpatawee, Red youmo, NERICA 1, 2, 3, 4, 10, 11, 14 and Jaowo while Komboka, Dourado and L-22 recorded lowest germination percentage. At 10% PEG concentration, all the varieties had similar germination percentage except L-22, Dourado and NERICA 3 which produced the lowest germination percentage according to the control (0%). For the 15% PEG concentration, MWUR, White rice, NERICA 1, 3, 4, 10, 11 produced the maximum germination percentage.

Results indicated that the germination index was significantly affected by the osmotic potential among the varieties and their interaction effects of the four concentrations of PEG 6000 was also significant ($P < .001$) (Table 1). Underwater stress conditions, the highest germination index was recorded in MWUR, White rice, Kpatawee, Red youmo, NERICA 1, 2, 3, 4, 10, 14, 11, Komboka and Jaowo at 5% PEG concentration, and L-22 and Dourado produced the lowest germination index. However, at 10% PEG concentration, the germination index of all the varieties were not statistically different except L-22, Dourado, Komboka and NERICA 3 which had the lowest germination index among the varieties (Table 1). Under 15% PEG concentration, MWUR, White rice, NERICA 1 and 11 produced the maximum Seed germination index followed by Dourado, Komboka, Jaowo, NERICA 2, 3, 4, 10, and Red youmo, while NERICA 14, Jaowo and L-22, recorded the lowest germination index in relation to the control (Table 1).

3.2 Root Length and Relative Seedling Height

The data on root length of rice crops affected by different PEG stress levels and their interaction presented in (Table 2) was highly essential ($P \leq .001$). The results on root length showed that when upland rice were subjected to 5% PEG concentration level, there was no statistically difference among the varieties in relation to the control. However, at 10% PEG level, the maximum root length was recorded in NERICA 1, 2, 3, 4, 10, 11, 14, Dourado and White rice while the lowest root length was observed in Red youmo, MWUR, Komboka, LAC-23, L-22 and Kpatawee. Under the 15% PEG concentration level, the longest root length was recorded in NERICA 1, 2, 10, 11 and white rice while the shortest root length was found in MWUR, Komboka and LAC-23 (Table 2).

Table 3: Effects of water Stress and Different Levels of PEG 6000 on Root Length and Relative Seedling Height (%) of Upland Rice Varieties

Comment [h14]: Idem, separate into 2 tables

Root length (cm)						Relative seedling height (%)				
PEG concentrations						PEG concentrations				
Variety	0%	5%	10%	15%	Mean	0%	5%	10%	15%	Mean
MWUR	7.4 ^{abc}	5.9 ^{abc}	3.4 ^{efg}	0.1 ^t	4.2	100 ^a	60.1 ^{efg}	38.5 38.5 ^{klm}	19.5 ^{grst}	69.9
Dourado	8.3 ^{abc}	6.10 ^{abc}	5.1 ^{abc}	4.2 ^{cde}	5.9	100 ^a	81.8 ^{abcd}	62.4 ^{def}	36.9 ^{klm}	70.3
L-22	7.2 ^{abc}	4.0 ^{def}	2.6 ^{hij}	2.3 ^{ijk}	4.0	100 ^a	25.3 ^{opq}	22.5 ^{opqr}	13.7 ^{rst}	40.4
Komboka	7.1 ^{abc}	5.7 ^{abc}	3.2 ^{efg}	0.1 ^l	4.0	100 ^a	45.1 ^{ijk}	43.3 ^{kl}	8.3 st	49.2
LAC-23	6.5 ^{abc}	6.3 ^{abc}	3.5 ^{efg}	0.4 ^{kl}	4.2	100 ^a	52.5 ^{ghij}	35.0 ^{lmn}	19.5 ^{grst}	51.8
White rice	8.4 ^{abc}	6.8 ^{abc}	5.9 ^{abc}	5.3 ^{abc}	6.6	100 ^a	81.8 ^{abcd}	52.6 ^{ghi}	21.9 ^{pqrs}	64.1
Kpatawee	8.3 ^{abc}	6.7 ^{abc}	4.9 ^{bcd}	2.6 ^{hij}	5.6	100 ^a	80.5 ^{abcd}	59.4 ^{efg}	19.4 ^{grs}	54.8
Red youmo	8.7 ^{abc}	8.3 ^{abc}	4.1 ^{cde}	2.8 ^{ghi}	5.1	100 ^a	90.2 ^{abcd}	70.8 ^{abc}	49.6 ^{ijk}	77.7
NERICA 10	11.1 ^{abc}	11.10 ^a	9.1 ^{abc}	8.8 ^{abc}	10	100 ^a	72.1 ^{abc}	73.4 ^{abcd}	71.7 ^{abc}	79.3
NERICA 14	11.8 ^{ab}	7.9 ^{abc}	7.5 ^{abc}	3.5 ^{efg}	7.7	100 ^a	74.2 ^{abcd}	65.0 ^{cdef}	5.4 ^t	61.3
Jaowo	9.4 ^{abc}	8.1 ^{abc}	7.4 ^{abc}	4.6 ^{cde}	7.4	100 ^a	89.4 ^{ab}	81.5 ^{abc}	54.3 ^{efg}	81.2
NERICA 11	10.9 ^{abc}	9.8 ^{abc}	9.2 ^{abc}	7.1 ^{abc}	9.3	100 ^a	79.5 ^{abcd}	59.5 ^{efg}	53.8 ^{gh}	73.2
NERICA 4	7.5 ^{abc}	7.0 ^{abc}	6.4 ^{abc}	4.5 ^{cde}	6.4	100 ^a	80.5 ^{abcd}	41.6 ^{klm}	38.7 ^{klm}	65.2
NERICA 1	10.2 ^{abc}	9.5 ^{abc}	9.2 ^{abc}	8.0 ^{abc}	9.2	100 ^a	93.0 ^{abc}	82.6 ^{abcd}	41.9 ^{klm}	79.4
NERICA 2	10.3 ^{abc}	7.5 ^{abc}	6.1 ^{abc}	5.10 ^{abc}	7.3	100 ^a	65.5 ^{cdef}	51.0 ^{hij}	28.7 ^{mno}	61.3
NERICA 3	10.4 ^{abc}	10.4 ^{abc}	10.1 ^{abc}	0.8 ^{kl}	7.9	100 ^a	81.6 ^{abcd}	56.3 ^{fghi}	34.4 ^{lmn}	68.1
LSD_{0.05}Variety= 2.75***						Variety = 11.78***				
Treatment =1.38**, VarietyxTreatment =5.50***						Treatment= 5.89**, Varietyx Treatment=23.55*				

Figure having common letter(s) in a column do not differ significantly at 5% level of significance

When water stress along with PEG treatments was imposed, Red Youmo, Jaowo, Kpatawee, Dourado, White rice and NERICA 1, 3, 4, 10, 11, 14 recorded the tallest seedling height percent while MWUR, L-22, Komboka, LAC-23 and NERICA 2 produced the shortest relative seedling height at 5% PEG level. At 10% PEG concentration, the highest relative seedling height percent was observed in Red youmo, Jaowo, NERICA 1, 10 followed by Dourado and NERICA 14 while L-22 produced the shortest seedling height percent (Table 2). For the 15% PEG concentration, NERICA 10 recorded the highest seedling height percent followed by NERICA 11 and Jaowo while NERICA 14 produced the shortest seedling height percent.

3.3 Fresh Shoot Weight and Dry Weight

The results on Table 3 revealed that, the highest fresh shoot weight at 5% PEG concentration level was observed in White rice (2.3g), NERICA14 (2.2g) and NERICA 1 (2.1g) rice varieties followed by NERICA 2, 3, 4, 4, 10, Red youmo, LAC-23, MWUR and Dourado whereas the lowest was found in Jaowo, Komboka and L- 22.

Table 4: Effect of Concentration of PEG 6000 on the Fresh Shoot Weight and Dry Shoot Weight of Upland Rice Varieties

Comment [h15]: Idem separate into 2 tables

Fresh shoot weight (gram)						Dry shoot weight (gram)				
PEG concentrations						PEG concentrations				
Variety	0%	5%	10%	15%	Mean	0%	5%	10%	15%	Mean
MWUR	2.7 ^{bcd}	1.8 ^{hij}	1.4 ^{klm}	1.2 ^{qrs}	1.8	0.4 ^{lm}	0.34 ^{opq}	0.3 ^{tu}	0.28 ^{stu}	0.3
Dourado	2.5 ^{bcd}	1.10 ^{ghi}	1.7 ^{hij}	1.5 ^{klm}	1.7	0.6 ^{efg}	0.59 ^{cdef}	0.56 ^{efg}	0.4 ^{nop}	0.5
L- 22	2.3 ^{cde}	1.4 ^{klm}	1.1 ^{stu}	1.1 ^{uv}	1.5	0.57 ^{def}	0.40 ^{mn}	0.16 ^{xy}	0.3 ^{uv}	0.4
Komboka	1.8 ^{hij}	1.4 ^{klm}	1.3 ^{opq}	0.6 ^x	1.3	0.18 ^{wx}	0.13 ^y	0.27 ^{uv}	0.32 ^{pqr}	0.2
LAC-23	2.0 ^{gh}	1.8 ^{hij}	1.3 ^{opq}	1.0 ^{wx}	1.5	0.47 ^{hij}	0.39 ^{mn}	0.3 ^{rs}	0.29 ^{rst}	0.4
White rice	2.7 ^{bcd}	2.3 ^{cde}	1.9 ^{gh}	1.4 ^{klm}	2.1	0.63 ^{abc}	0.43 ^{lm}	0.4 ^{nop}	0.48 ^{ghi}	0.5
Kpatawee	2.9 ^{ab}	1.9 ^{hij}	1.4 ^{klm}	1.2 ^{qrs}	1.9	0.64 ^{abc}	0.45 ^{ijk}	0.4 ^{nop}	0.30 ^{rst}	0.4
Red youmo	1.9 ^{hij}	1.9 ^{hij}	1.2 ^{qrs}	1.1 ^{tuv}	1.5	0.38 ^{mn}	0.33 ^{pqr}	0.3 ^{uvw}	0.28 ^{tu}	0.3
NERICA 10	2.3 ^{cde}	1.9 ^{gh}	1.7 ^{hij}	1.6 ^{kl}	1.9	0.58 ^{def}	0.31 ^{rst}	0.26 ^{uv}	0.24 ^{vw}	0.3
NERICA 14	2.8 ^{bcd}	2.2 ^{def}	1.8 ^{hij}	1.2 ^{qrs}	2.0	0.69 ^{abc}	0.53 ^{gh}	0.3 ^{tuv}	0.27 ^{uv}	0.4
Jaowo	1.8 ^{hij}	1.5 ^{klm}	1.3 ^{pqr}	1.3 ^{pqr}	1.5	0.61 ^{abc}	0.37 ^{mn}	0.3 ^{tuv}	0.24 ^{vw}	0.4
NERICA 11	2.7 ^{bcd}	1.9 ^{gh}	1.7 ^{hij}	1.4 ^{klm}	1.9	0.68 ^{abc}	0.39 ^{mn}	0.33 ^{pq}	0.3 ^{tuv}	0.4
NERICA 4	3.3 ^a	1.8 ^{ghij}	1.5 ^{klm}	1.4 ^{klm}	2.0	0.45 ^{ijk}	0.38 ^{mn}	0.35 ^{no}	0.3 ^{vw}	0.4
NERICA 1	2.7 ^{bcd}	2.1 ^{efgh}	2.1 ^{ef}	1.7 ^{ij}	2.2	0.71 ^{ab}	0.42 ^{lm}	0.4 ^{no}	0.37 ^{mn}	0.5
NERICA 2	2.8 ^{abc}	1.9 ^{ghi}	1.7 ^{hij}	1.5 ^{klm}	2.0	0.4 ^{mno}	0.37 ^{mn}	0.72 ^a	0.56 ^{def}	0.5
NERICA 3	2.5 ^{bcd}	1.9 ^{ghi}	1.2 ^{qrs}	1.1 ^{vw}	1.7	0.58 ^{def}	0.38 ^{mn}	0.3 ^{vw}	0.28 ^{stu}	0.4
LSD_{0.05}Variety = 0.233***						Variety = 0.047**, Treatment = 0.024***				
Treatment = 0.116**, Variety x Treatment = 0.465**						Variety x Treatment = 0.095**				

Figure having similar letter(s) in a column do not differ significantly at 5% level of significance.

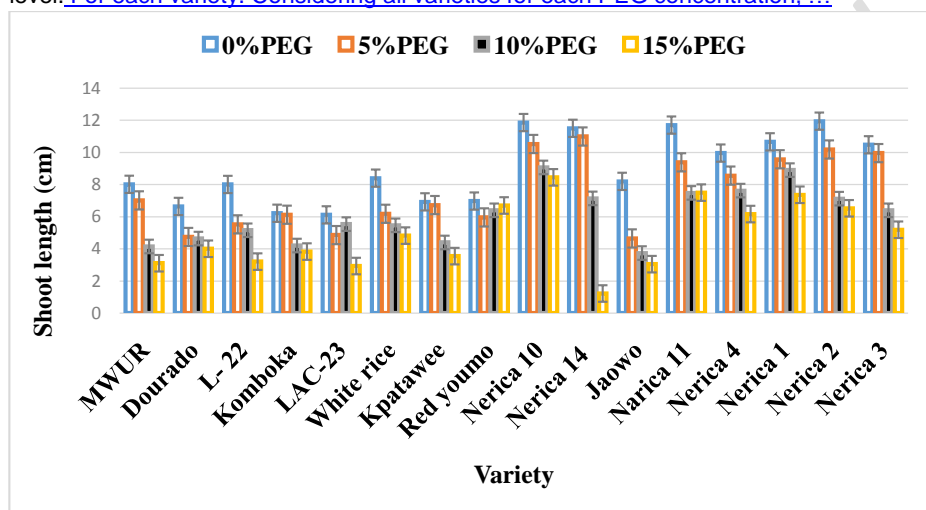
At 10% PEG concentration, the maximum shoot fresh weight was recorded in NERICA 1 (2.1g) followed by Dourado, White rice, NERICA 2, 10, 11, and NERICA 14 and the lowest was found in L-22 and NERICA 3 rice varieties (Table 3). On the other hands, NERICA 1 (1.7g) and NERICA 10 (1.6g) rice varieties performed better with the highest fresh shoot weight at 15% PEG concentration water stress level (Table 3).

The result in Table 3 showed that the effect of PEG concentration levels and water stress dry shoot weight of upland rice varieties was highly significant, the interaction between the treatments and the varieties were also significant ($P < .001$). When water stress treatments were imposed with 5% PEG concentration, Dourado and NERICA 14 recorded the highest dry shoot weight with Komboka recording the lower dry shoot weight in relation to the control (0%). At the 10% PEG drought stress, NERICA 2 (0.7g) produced the highest dry shoot weight followed by Dourado while L-22 had the lowest dry shoot weight as compared to the control (Table 3). For the 15% PEG concentration, NERICA 2 measured the highest dry shoot weight followed by Dourado and White rice.

3.4 Shoot Length (cm)

The data presented in figure 1 indicated that effects of PEG 6000 concentration at the control, 5%, 10%, 15% and water stress were found statistically significant ($P < .001$) on the shoot length of upland rice varieties, their interaction was also highly significant ($P < .001$) (Figure 1). The Shoot length of upland rice varieties exhibited diminishing trends from PEG control (0%) to 15% level.

[For each variety. Considering all varieties for each PEG concentration, ...](#)



Comment [h16]: add letters (from statistical analysis) above each histogram

Figure 1: Effect of Drought Stress and Different Levels of PEG 6000 on Shoot Length of Upland Rice Varieties.

Error Bars Represent Means \pm Standard Error of Means

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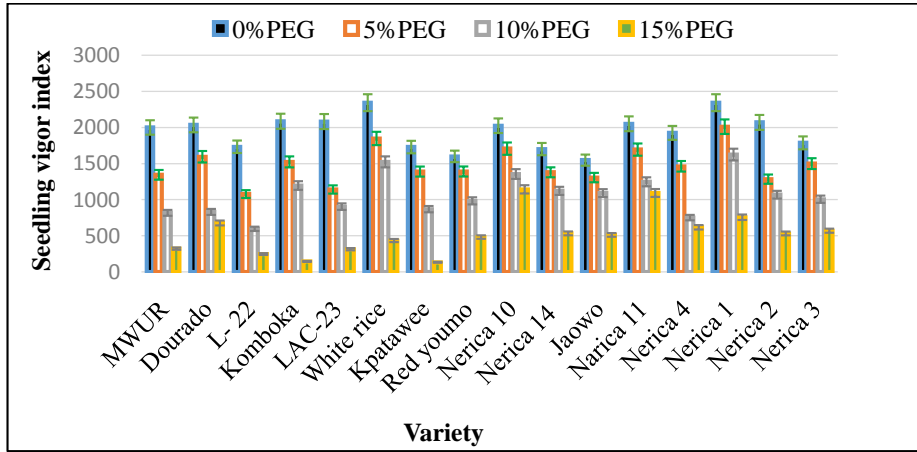
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With the 5%PEG concentration, the maximum shoot length was observed in NERICA 10 and 14 followed by NERICA 1, 2, 3, 4, 11 and the minimum was in Jaowo and Dourado. At 10%, the longest shoot height was found in NERICA 1 and 10 followed by NERICA 4, 11 and the lowest was in Jaowo. At 15% the longest shoot length was observed in NERICA 10, followed by NERICA 11 and 1 while the lowest was in NERICA 14 (Figure 1).

3.5 Seedling Vigor Index

Data presented in figure 2 regarding seedling vigor index of upland rice varieties displayed that there were significant influences ($P < .001$) of PEG levels on rice varieties and their interactions (Figure 2).

Comment [h17]: same as 3.4 section



Comment [h18]: idem figure 1

Figure 2: Effect of Different Levels of PEG 6000 on seed Vigor index of Upland Rice Varieties.

Error Bars Represent Means ± Standard Error of Means

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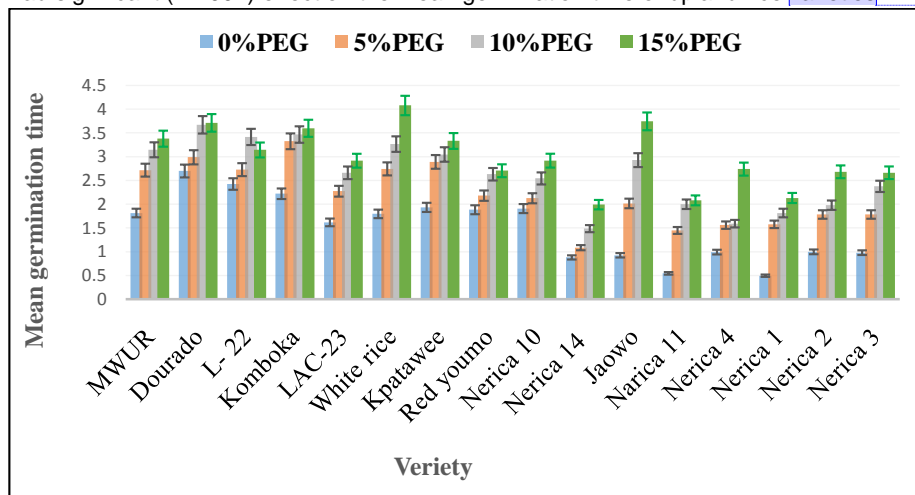
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The highest values of seedling vigor index at 5% PEG was found in NERICA 1 followed by NERICA 10, 11, white rice and lowest values were found in L-22 and LAC-23 in relation to the control. Under the 10% PEG stress conditions, NERICA 1 and white rice produced the highest values of vigor index followed by NERICA 10, 11 and Komboka while L-22 recorded the lowest among the varieties. At 15% PEG concentration levels, NERICA 10, and 11 produced the highest seedling vigor index among the varieties.

Even though the varieties showed different responses to water stress levels. Among the varieties, NERICA 1 at 5%, 10%, White rice at 10%, and 15% NERICA 10 and 11 were the least affected variety at PEG concentrations and water stress by producing the highest values of seedling vigor index. Indicating that these varieties performed better in terms seedling vigor index.

3.6 Mean Germination Time (MGT)

The results presented in Figure 3 showed that the varieties, the PEG concentration treatments had significant ($P \leq 0.001$) effect on the mean germination time of upland rice varieties.



Comment [h19]: idem 3.4

Comment [h20]: idem figure 1

Figure 3: Influence of water Stress and Different Levels of PEG 6000 on Mean Germination Time of Upland Rice Varieties.

Error Bars Represent Means \pm Standard Error of Means

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The longest mean germination time at 5% PEG concentration was recorded in all the varieties except for NERICA 14 which recorded the shortest Mean germination time followed by NERICA1, 4, and NERICA 11. Regarding the 10% PEG concentration, all the varieties had similar mean germination time except for NERICA 4, 14, which had the shortest Mean germination time followed by NERICA 1, 2, 4 and 11 as compared to the control (0%). However, in the 15% PEG concentration, NERICA 1, 11 and 14 had the shortest mean germination time followed by NERICA 2, 3, 4, 10, LAC-23, and Red youmo while L-22, Komboka, Kpatawee, Jaowo, White rice produced the longest mean germination time in comparison to the control (0 %).

4. DISCUSSION

Germination of seed is one of the most critical phases in the life cycle of plants. The effect of increasing concentration of PEG- 6000 during seed germination and the response of varieties to the increasing concentrations was measured to determine the tolerance of upland rice varieties under water stress conditions. The result depicted that drought stress greatly affects seed germination percentage, but the response intensity and adverse effect of stress depend on the variety genetic makeup and the duration of the stress (Table 1). It has been reported that drought stress adversely affects seed germination, and seedling growth [22]; [23]. Under water

stress, low water potential is a determining factor for inhibiting seed germination [28]. PEG is an osmotic agent, which play an important role in the regulation of mineral elements, hormone, protein metabolism and effects of signal transduction [27]. The main function of PEG is to slow down the moisture rate of seeds [18]. Across all the PEG concentration levels, NERICA varieties including MWUR and White rice exhibited better performance in terms germination percentage.

An increase in PEG stress levels evidently decreased the germination index of all varieties compared to their relative controls (0%). The results further indicated that some of the varieties showed superiority over other varieties in relation to germination index. The results clearly showed that at 15% PEG level, water stress greatly affects seed germination index, but the response severity and effect of stress depend on the level of PEG concentration (Table 1). Water stress is one of the major constraints for crop production in the world, understanding germination responses to water stress be may helpful to crop improvement program directed toward the generation of drought resistance upland rice varieties [5]. Water stress simulated by PEG at the germination stage affected the seed germination index, root growth, and seedling growth in rice crops [28]. In the present study, the rate of germination, and germination index decreased with the decrease of the osmotic potential of PEG 6000 solution in all varieties, which are in agreement with those reported by [28]. However, [28] concluded that moderate water stress only delayed germination while high water stress reduced the final germination rate.

The results revealed that root length reduced as the PEG concentration increased and their length for different rice varieties were significantly affected by water stress (Table 2). The results on root length in this study are same as [2] who stated that water stress leads to decrease in root length and shoot length. Among the rice cultivars NERICA 1, 2, 10, 11 and White rice exhibited relatively better root length against water stress of different level of PEG indicating that all these cultivars performed relatively better under water stress induced by PEG 6000 concentrations.

Data presented in Table 2 showed that PEG concentration treatments significantly ($P \leq 0.01$) affected relative seedling height percentage, as the PEG concentration increased, the relative Seedling height percent also decreased. There were major effects on rice varieties and their interactions ($P \leq 0.01$) (Table 2). Under water stress, it has been shown that the inhibition of radicle emergence is mainly affected because of decrease in water potential gradient between the external environment and the seed and consequently impairs seedling height [22]. Rice germination and early growth depend on the ability to absorb water from the surrounding environment. Water imbibition into the seed activated cell metabolism which produces energy to support seedling growth and development [21]. Moreover, water is important for cell elongation which needed for root elongation in plant [21]. PEG 6000 is a non-ionic molecule which commonly used to induced water stress in the plant. The previous report showed water stress induced by PEG 6000 inhibit shoot and root development in rice seedling [4]. Similarly, the shoot and root growth of upland rice varieties were inhibited by water stress produced by the application of PEG 6000 (Table 2). The inhibition effect of PEG 6000 was increasing when its concentration was increase which means less water available leading to less growth. Additionally, among the rice cultivars, NERICA 10 showed the highest seedling relative height percent followed by Jaowo and NERICA 11 when treated with PEG concentrations. The

different responses of the relative seedling height under water stress in these varieties might be caused by genetic variation among them.

The fresh shoot weights (FSW) of different upland rice varieties were highly influenced by PEG-6000 concentration levels and water stress. In all the varieties, the fresh weight decreased due to the increased PEG concentration ($P \leq 0.001$) (Table 3). At all the PEG concentration levels of fresh shoot weight, NERICA 1, 2, White rice and Dourado varieties had an outstanding performance among the varieties, Meanwhile, Dourado, NERICA 1, 2 and White rice performed better in all the PEG concentrations in terms of shoot dry weight. Osmotic stress caused low water availability to plants leading to decrease in cell division and elongation by lowering turgor pressure as well as cell growth resulting in a reduction in biomass and dry matter [18] and [17]. The fresh and dry weight of rice seedlings revealed a decrease exposed to increase PEG concentrations (Table 3).

Substantial differences were observed in shoot length between control and stress environment created by PEG 6000 of all the treatments. Among the rice varieties, NERICA 10, 14 at 5%, NERICA 1, 10 at 10%, NERICA 10 at 15% exhibited relatively better shoot length against water stress of different levels of PEG concentrations indicating that all these varieties demonstrated relatively better under water stress induced by PEG 6000 as compared to the control. [9] reported that inhibition of radical emergence is due to decrease in water potential gradient between the external environment and seed and consequently impairs seedling height under water stress conditions.

Seedling vigor index has been used as a tolerance index to evaluate the effect of drought stress on seedling growth [13]. The results revealed important variation in seedling vigor index of rice varieties under drought stress conditions. This agrees with [12], who reported that seed vigor index is most sensitive to water stress. In the case of 15% PEG concentration, NERICA 10 and 11 produced the highest seedling vigor index while Kpatawee and Komboka recorded the lowest seedling vigor index. The reduction in vigor index of seedlings under water stress conditions was reported by other researchers [32]. Seedling vigor index is an important index of seed quality, evaluates the potential for fast and uniform emergence of plants. The early vigor of seedling with good development can be used as a beneficial trait of interest for the selection of tolerant varieties [30] and others also suggested that plants with a higher vigor index could improve crop productivity and development.

Seed mean germination time is a measure of the time it takes for the seed to germinate, taking account on the days at which most seeds have germinated. The mean time taken for a maximum germination in days increased in all the rice varieties with increase of PEG-6000 concentration levels [20]. The mean germination time (MGT) was delayed by the 15% PEG concentration levels as compared with control treatments (0%) (Figure 1). A delay in the average time to germination could be detrimental for successful establishment of seedling and could exposed the seed to pest and disease attacks, and, therefore, compromising the establishment of a uniform stand. [14] reported that highly negative osmotic potential may affect the seeds water uptake, delaying germination and making it impossible. Additionally, the osmotic potential

of the external medium can affect the enzymatic reactions in the seed, therefore, the delay in germination is due to delay of enzymatic reactions [26] caused by the break of the imbibition period. The most common responses of plants to the reduction of osmotic potential are a delay in initial germination and a reduction in the rate and total germination [20].

4.1 Conclusions and Recommendations

The study concluded that among all the rice varieties tested, NERICA rice showed the greatest performance under artificial induced stress by using PEG 6000 treatments followed by MWUR, White rice and Kpatawee. Therefore, these varieties could be useful in breeding programs and can be cultivated in arid and semi-arid environment or where water scarcity is a frequent constraint.

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