EARLY TEST OF THE TOLERANCE OF UPLAND RICE TO DROUGHT IN THE GERMINATION PHASE

Comment [DK1]: The study took place in which country????

ABSTRACT

Climate change in the form of drought will reduce agricultural production, so it is necessary to develop superior drought-tolerant varieties. The study aimed to determine the drought-tolerant local upland rice cultivars at the germination stage. The research was conducted at the Seed Science and Technology Laboratory, Faculty of Agriculture, Tadulako University, Palu, from September to December 2021. A completely random design with a two-factor factorial pattern was employed in the study. Twelve cultivars made up the first factor, while two superior drought-resistant variants served as a comparator. The second factor is osmotic stress, namely P0 = control, P1 = -1 bar, P2 = -2 bar and P3 = -3 bar, so there are 56 treatment combinations repeated four times, so there are 224 experimental units. The observed variables were maximum growth potential, germination rate, radicle length, plumule length, radicle dry weight, plumule dry weight, sprout dry weight, water volume and germination rate. The results showed that drought stress through PEG – 1 bar did not affect germination. Using PEG down to -3 bar resulted in a significant reduction in germination.

Keywords: polyethene glycol (PEG-6000), drought stress, germination, upland rice local

1. Background

The international economy and agriculture have both been significantly impacted by climate change. Approximately 95% of climate change is related to drought, and it is estimated that in 2100 drought will cause a decrease in corn crop production by 20-45%, wheat by 5-50% and rice by 20-30% (Arora, 2019). Drought severely affects plant growth and development by causing physiological, metabolic and molecular changes (Zu et al., 2017;Larkunthod et al., 2018). According to Furlan et al., (2012), drought causes cell dehydration, which releases water from the cytosol to the vacuole and apoplast. Water stress occurs when the rate of transpiration exceeds the uptake and transport of water in plants. Therefore, plants will try to respond to these conditions by changing stomatal conductance, growth, osmolyte accumulation, and specific gene expression. Abscisic acid (ABA), the primary stress hormone, will accumulate and participate in physiological and biochemical processes that allow plants to survive (Purbajanti et al., 2019).

Sealing plants with better drought tolerance is especially important in dry environments. However, drought simulation that is controlled and uniformly repeated in the field cannot be easily achieved. The slow progress in developing drought-resistant cultivars also reflects the need for more specific methods to screen the large number of genotypes required in plant breeding for drought tolerance. Using natural field conditions is difficult because rainfall can eliminate water deficits. However, in vitro, drought screening methods facilitate advances in our understanding of drought-resistant/tolerant traits and the selection of drought-resistant genotypes(Muscolo et al., 2014). It further emphasized germination as a helpful criterion in screening for water stress tolerance. Khakwani et al., (2011) showed that the varieties tested and drought tolerant during in vitro germination tests were equally tolerant under field conditions. Thus, the study of the effects of drought using osmotic solutions is one of the methods for evaluating resistance during the germination phase. Exposure to polyethene glycol (PEG-6000) solutions has been effectively used to mimic drought stress with limited metabolic disturbances, such as those associated with the use of low molecular weight osmolytes that can be taken up by plants (Pradhan et al., 2015). PEG-6000 has long been used as a reliable marker to test drought-tolerant genotypes under laboratory conditions. This is because polyethene glycol acts as a non-penetrating osmotic agent, which increases solubility potential and a barrier to water absorption by the root system (Chutia & Borah, 2012). PEG-based in vitro screening for drought tolerance is a suitable method to screen germplasm sets with reasonable accuracy (Shitole & Dhumal, 2012).

Pant & Bose, (2016) conducted a study to see the effect of PEG-induced water stress using an osmotic potential of -0.3 to -0.60 MPa; up to -0.60 PEG caused the sprouts to die. Alaei et al., (2015), Swapna & Shylaraj, 2017) use an osmotic pressure of -0.03 to -1.35 MPa. Basha et al., (2015) use a 0% to 16% PEG concentration. Chutia & Borah, (2012) used an osmotic stress of 0.15 to 0.56 bar.

The research results by Nazirah et al., (2016) concluded that water stress caused a decrease in root weight, root crown ratio, leaf proline content and total leaf chlorophyll. According to Aniat-ul-Haq & Agnihotri, (2010), increased concentrations of PEG reduced water absorption by seeds resulting in decreased germination. Therefore, the decrease in the potential water gradient between seeds and the surrounding media by the effect of PEG 6000 harmed seed germination. Pradhan et al., (2015) also reported that different osmotic potential and priming duration significantly affected the germination percentage, vigour index and dry weight of seedlings. Seed-priming of various types has been observed to increase seed germination rates and seedling emergence in several studies (Pant & Bose, 2016;Srivastava et al., 2010; Chen & Arora, 2011). Therefore, this study aims to detect early drought tolerance of upland rice cultivars during germination.

2. MATERIALS AND METHODS

2.1. Place and time

The Seed Science and Technology Laboratory was the site of this study., Faculty of Agriculture, Tadulako University, Palu, from September to December 2020.

2.2. Tools and materials

Petri dishes, straw paper, scissors, germination tools, paper presses, sprayers, rulers, threads, and stationery were among the supplies utilized in this investigation.Local upland rice seeds, two drought-resistant superior kinds, purified water, and Polyethylene glycol (PEG) 6000 were the ingredients employed in this investigation.

2.3. Research procedure

This research was started by saturating the straw paper substrate with a PEG 6000 solution. Then, a paper press removes the excess water from the straw paper substrate. The straw paper used in each roll is five sheets, each with one plastic as the base. The PEG 6000 osmotic pressure level consists of four levels, namely 0 bar, -1 bar, -2 bar and -3 bar.

The PEG 6000 osmotic pressure calculation formula, according to (Kaufmann & Michel, 1973), is as follows:

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

Information:

Ψs = osmotic pressure of the solution (bar)

C = concentration of PEG 6000 in grams PEG/kg H_2O

T = room temperature (^oC)

Based on the approach of the Michel & Kaufmann formula (1973) with a room temperature of 28^oC, osmotic pressure of 0 bar is obtained, equivalent to 0 g PEG/L H_2O; -1 bar = 82.5 g PEG/L H_2O, -2 bar = 124.38 g PEG/L H_2O, -3 bar = 156.75 g PEG/L H_2O and -4 bar = 184.11 g PEG/L H_2O. The method used to germinate the seeds is the Rolled Paper Test method established in plastic (RPTmep) and then put in the seed germination tool.

2.4. Research design

This experimental study was arranged with a factorial pattern of two factors using a Completely Randomized Design (CRD). The first factor consisted of twelve cultivars of local upland rice, namely C1 = Dongan, C2 = Siang, C3 = Pulut ko, C4 = Roda, C5 = Logi, C6 = Raki, C7 = Kenari, C8 = Kalendeng, C9 = Sina didi, C10 = Sina ponding, C11 = Tagolu, C12 = Pae pulu palang, C13 = Gajah Mungkur variety and C14 = Inpago 4 variety (Gajah Mungkur and Inpago 4 are exceptional drought resistant varieties used as check/comparison varieties). The second factor consists of four osmotic pressure levels, namely P0= 0 bar, P1 = -1 bar, P2 = -2 bar, and P3 = -3 bar. Thus there were 56 treatment combinations, where each treatment was repeated four times, so in total, there were 224 experimental units. Each experimental unit used the Rolled Paper Test method established in plastic (RPTmep), where each experimental unit used 50 seeds.

2.5. Observation Parameters

Observations were made of 50 seeds in each treatment unit, such as:

Maximum growth potential (%), calculated based on the percentage of seeds that grow and carried out at the last observation

MGP=(∑sprouted seeds)/(∑planted seeds) x100%

Germination speed (GS) is calculated based on the percentage of usual sprouts per etmal (1 etmal = 24 hours) and starts on the first day until the seventh day with the following formula:

$GS=(\%KN_1)/etmal_1) +(\%KN_2/etmal_2) +(\%KN_3/etmal_3) +\cdots+(\%KN_7/etmal_7)$

%KN=Percentage of seeds germinating on a given day

Germination power (%)

DB(%)=(∑KN count I+ ∑KN count II)/(∑seed planted)

KN = normal sprouts

Plumula length (cm), measured from the base of the plumula to the tip of the plumula on the seventh day after planting.

Radicle length (cm), measured from the root's tip to the root's base on the seventh day.

The dry weight of plumula (cm) measured the average dry weight from regular sprouts by considering the growth structure of seven-day-old sprouts oven-dried at 60 0C.

Radicle dry weight (mg), measured by the roots of regular sprouts by weighing the growth structure of seven-day-old sprouts which had been oven-dried at 60 0C

Sprout dry weight (mg), total dry weight of seven-day-old plumule and radicle, which had been oven-dried at 60 0C

The root volume (ml) is determined by determining the initial volume of water to be put into the measuring cup, putting the roots into the measuring cup and then recording the increase in water volume after putting the roots into it (Munarso, 2011).

2.6. Data analysis

The data obtained from the measurement results on each observation variable are tabulated and processed using analysis of variance. To find out the effect of each treatment on the variables observed using the F-Test (Fisher-Test) at an accuracy level of 95%, and if the F-Test of each treatment shows an authentic or genuine effect, it will be followed by a test BNT at 5% level by comparing the average treatment with the check variety (2 comparisons).

3. RESULTS AND DISCUSSION

The diversity analysis showed that cultivars, PEG, and PxC interactions had a very significant effect except for cultivars for observing maximum growth potential and plumule length, which had no significant effect (Table 1). The cultivars that had no significant effect showed that the cultivars gave the same response to the observed variables under drought stress conditions. Cultivars with significant effect indicated that each cultivar responded differently to drought stress conditions. PEG had a significant effect indicating that each concentration had a different effect on the germination phase of rice plants. The significant effect interaction showed that each cultivar responded differently at each PEG concentration. The coefficient of variance in all observed variables ranges from 5.78 to 18.42, meaning that all observed variables can be analyzed for diversity (Cox et al., 1985).

Table 1. Effect of cultivars, PEG and their interactions on some of the observed variables

Souce of Diversity	MGP	GP	RL	PL	RDW	PDW	SDW	VR	GS
Treatment	**	**	**	**	**	**	**	**	**
Cultivars	ns	**	**	ns	**	**	**	**	**
PEG	**	**	**	**	**	**	**	**	**
C x P	**	**	**	**	**	**	**	**	**
CV	5.78	4.63	9.51	12.25	8.66	10.81	7.88	18.42	14.17

Information: SD=source of diversity; MGP=maximum growth potential; GP=germination power; RL=radicle length; PL=plumule length; RDW=radicle dry weight; PRW=plumule dry weight; SDW=spout dry weight, VR= volume root; GS=germination speed; CV=coefficient of variation; **=significantly; ns=non significant

3.1. The effect of PEG on the maximum growth potential and germination of local upland rice plants.

The performance of cultivars that were given stress through PEG concentrations resulted in different maximum growth potentials and germination rates (Figure 1). An increase in PEG up to -3 bar causes a decrease in the maximum growth potential and germination capacity of local upland rice.

The maximum growth potential is the ability of the seed to grow and develop in both standard and abnormal growing media. The results showed that the initial growth potential of the cultivars evaluated ranged from 91.5% (Kalendeng, Tagolu, and Gajah Mungkur varieties) to 97.50% (roda and kenari cultivars). Application of osmotic pressure of -1 bar showed no significant decrease in the maximum growth potential of cultivars/varieties (Figure 1). Giving PEG up to -3 bar caused the maximum growth potential of all cultivars/varieties to decrease by 8% (pulut ko cultivar) and 22.28% (logi cultivar), while other cultivars/varieties decreased by 30.57% (roda) to 81.03% (kenari). The decrease in the maximum growth potential of the inpago-4 and Gajah Mungkur

Comment [DK2]: Authors should specify Figure 1. For example Figure 1A: maximum growth potential and Figure 1B: germination rate

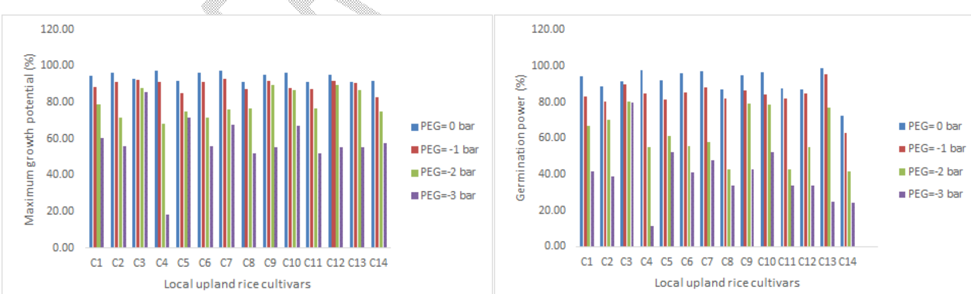

Figure 1. Response of cultivars given drought stress conditions through PEG on the maximum growth potential and germination of local upland rice

Comment [DK3]: Authors should make a caption: A: maximum growth, B: germination rate

varieties was 39.34 and 37.50%, respectively. The response of roda cultivars was feeble when given PEG up to -3 bar for maximum growth potential. The pulut ko cultivar has the highest value, so it is considered the most tolerant if given PEG up to -3 bar for maximum growth potential. Dongan, Siang, Raki, Kalendeng, Sina Didi, Tagolu, and Pae Pulu Siang are cultivars that look relatively the same as the control varieties with maximum growth potential (Table 2). These cultivars have good tolerance after pulut ko.

Germination capacity (DB) describes the percentage of seeds that can generally germinate under certain conditions. The germination rate of the cultivars/varieties used in the study was quite good, ranging from 83.05% (page 4) to 97.50% (wheels). Giving PEG -1 bar caused a slight decrease in germination, but using osmotic pressure up to -3 bar caused a decrease in the germination of 13.21% (pulut ko) and 88.21% (roda). Application of PEG-6000 down to -3 bar decreased germination capacity by 70.5% (Gajah Mungkur) and 74.62 (Inpago-4). Pulut ko is the cultivar with the highest germination rate and is different from the control variety, and is more tolerant to the osmotic pressure of up to -3 bar. The control varieties had better germination performance than the tested cultivars (Table 2).

Using PEG-6000 down to -3 bar causes maximum growth potential and decreases germination. This is because the more osmotic solution in the germination medium causes the water potential of the media to decrease so that the water content in the seed decreases. Kadhimi et al., (2016), the content of PEG-6000 in seed germination medium can reduce the availability of water that seeds can absorb during germination. This causes the seeds to experience osmotic stress, which can affect sprouts' growth; seeds that cannot adapt to this osmotic stress cannot grow properly or abnormally. The addition of PEG-6000 can affect the accumulation of solutes in cells, affecting the physiological function of cells (Ekowati & Widijastuti, 2018). These conditions cause maximum growth potential and decreased seed germination.

Table 2. The average value of the control test between each cultivar and the control variety given PEG up to -3 bar for maximum growth potential, germination, radicle length, plumula length, and radicle dry weight

Treatment	Maximum growth potential		Germinati oncapacit y		Radicle length		Plumul e length		Radicle dry weight	
C1P4	60.50 ns	*	41.50 *	*	5.43 *	*	1.77 *	*	30.83 *	*
C2P4	56.00 ns	ns	39.00 *	*	1.00 *	*	0.95 *	*	57.00 *	ns
C3P4	85.50 *	*	79.50 *	*	4.92 ns	*	2.20 *	*	39.50 *	*
C4P4	18.50 *	*	11.50 *	*	4.87 ns	*	0.68 *	ns	21.33 *	*
C5P4	71.50 *	*	52.00 *	*	8.36 *	*	1.97 *	*	59.08 *	ns
C6P4	56.00 ns	ns	41.00 *	*	6.29 *	*	1.98 *	*	27.63 *	*
C7P4	67.50 *	*	47.50 *	*	7.14 *	*	1.64 *	*	52.20 ns	*
C8P4	51.75 ns	ns	33.50 *	*	0.98 *	*	0.93 ns	*	44.00 *	*
C9P4	55.50 ns	ns	42.50 *	*	5.98 *	*	1.07 *	*	76.63 *	*
C10P4	67.00 *	*	52.00 *	*	6.44 *	*	2.66 *	*	42.10 *	*
C11P4	51.75 ns	ns	33.50 *	*	1.00 *	*	0.98 *	*	64.00 *	*
C12P4	55.50 ns	ns	34.00 *	*	1.00 *	*	0.73 ns	ns	58.00 *	ns
Varietas 1	55.50		25.00		4.75		0.80		51.00	
Varietas 2	54.00		24.50		3.25		0.68		56.50	
BNT 5%	5.01		2.28		0.52		0.14		3.49	

Description: ns = not significantly different; * = significantly different

3.2. *Effect of PEG on radicle and plumule length of local upland rice plants*

The results showed that the radicle length at 0 bar PEG (control treatment) ranged from 4.31 cm (kalendeng cultivar) to 12.67 cm (roda cultivar) (figure 2). Using PEG up to -3 bar caused the radicle length to decrease by 28.79% (Logi cultivar) to 86.38% (Pae Pulu Siang cultivar). Logi cultivars had the highest radicle length values, followed by kenari and sina ponding, and were better than the superior varieties used as controls. The plumule length of several cultivars in the control treatment ranged from 3.19 cm (sina didi cultivar) to 6.59 cm (sina ponding cultivar). The use of PEG osmotic pressure of up to -3 bar caused a decrease in plumula length from 59.61% (sina ponding) to 92.93% (Pae Pulu Palang cultivar) (Figure 2). Pulut ko and sina ponding cultivars had higher plumula lengths than control varieties (Table 2). The difference in the amount of reduction due to the use of PEG osmotic pressure of up to -3 bar on the length of the radicle and the length of the plumula is due to genetic factors in each cultivar. According to Sokoto & Muhammad, (2014), employing PEG up to a certain percentage will result in challenges for the radicle and plumule's appearance because of alterations in the gradient of the water potential between the seed's interior and the surrounding environment. The length of the radicle and plumule will decrease due to cell division and display under water stress conditions (Sobahan et al., 2022).

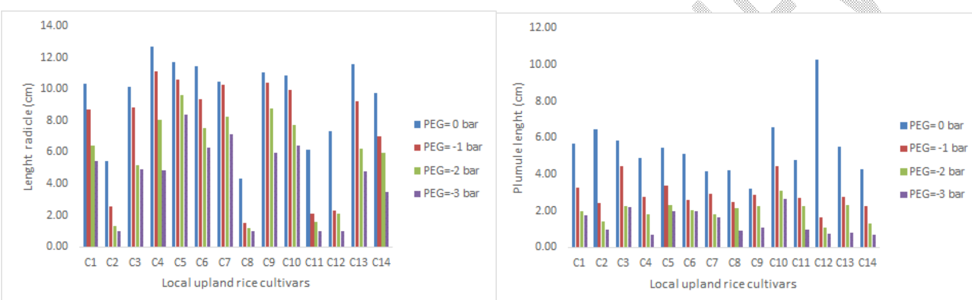

Figure 2. Response of cultivars subjected to drought-stressed conditions through PEG on radicle length and plumule length of local upland rice

Comment [DK4]: Authors should also separate Figure 2 into Figure 2A and Figure 2B

3.3. Effect of PEG on radicle and plumule dry weight of local upland rice plants

The results showed that the radicle's dry weight without using PEG ranged from 63.43 mg (raki cultivar) to 288 mg (pae pulu palang cultivar). PEG application up to -3 bar caused a decrease in radicle dry weight from 47.74% (logi cultivar) to 82.21% (roda cultivar). Sina didi and Tagolu were the cultivars with the highest radicle dry weight values, followed by Tagolu. Both cultivars were more tolerant than the varieties used as comparisons. Plumule dry weight without PEG ranged from 109 mg (raki cultivar) to 273 mg (roda cultivar). The dry weight of plumula withoutPEG produced a dry weight of 109 mg (raki cultivar) to 288 mg (pae pulu palang cultivar). Using PEG up to -3 bar caused plumula dry weight to decrease in the range of 52.51% (raki cultivar) to 93.04% (Pae Pulu Palang cultivar). The results of the 5% BNT test between cultivars and control varieties on radicle dry weight showed that the Dongan, Siang, Pulut ko, Roda, Raki, Kalendeng, and Tagolu cultivars were not different from the comparison one. Dongan, Roda, Raki, Sina Didi, and Tagolu were cultivars that looked the same as the second control (Table 3). The cultivars that did not differ from the control variety had the same tolerance as the control variety. The cultivar sina ponding had the highest dry weight of plumula, followed by kenari and logi. The three cultivars had better drought tolerance than the control and were significantly different.

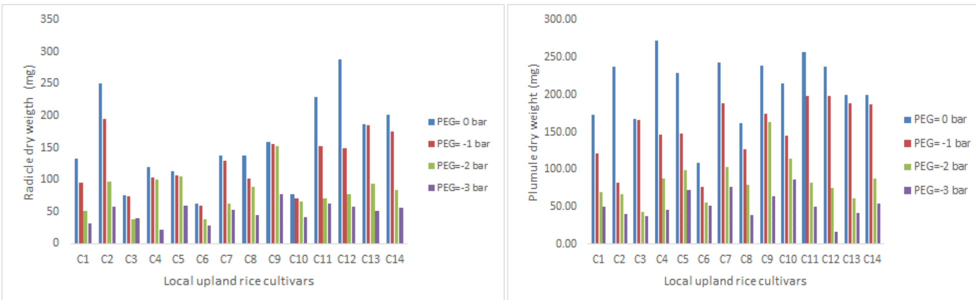

Figure 3. Response of cultivars subjected to drought-stressed conditions through PEG on the dry weight of radicle and plumula of local upland rice.

Comment [DK5]: Authors should also separate Figure 3 into Figure 3A and Figure 3B

The dry weight of a plant reflects its strength to survive. Osmotic stress causes low water availability for plants. The availability of water then causes a decrease in cell division and elongation. This condition causes a decrease in turgor pressure and cell growth, resulting in a decrease in biomass and a reduction in dry mass (Farooq et al., 2015; Sagar et al., 2020).

Table 3. The average value of the control test between each cultivar and the control variety has given PEG up to -3 bar for plumula dry weight, dry seedling weight, water volume and germination rate

Perlakuan	Plumula dry weight		Dry seedling weight		Water Volume		Germination rate	
C1P4	50.20 ns	ns	81.03 *	*	0.08 *	ns	4.66 *	*
C2P4	39.50 ns	*	95.75 ns	*	0.08 *	ns	5.78 ns	*
C3P4	37.68 ns	*	77.18 *	*	0.11 *	*	3.99 *	*
C4P4	45.94 ns	ns	67.26 *	*	0.08 *	ns	6.46 ns	ns
C5P4	71.95 *	*	131.03 *	*	0.15 *	*	5.18 *	*
C6P4	51.83 ns	ns	79.45 ns	*	0.08 *	ns	6.30 ns	*
C7P4	76.48 *	*	128.68 *	*	0.15 *	*	6.75 ns	ns
C8P4	38.50 ns	*	82.50 ns	*	0.08 *	*	4.15 *	*
C9P4	64.25 *	ns	140.88 *	*	0.13 *	*	5.73 ns	*
C10P4	86.83 *	*	128.93 *	*	0.10 *	ns	4.97 *	*
C11P4	50.50 ns	ns	114.88 *	ns	0.05 ns	*	5.45 *	*
C12P4	16.50 *	*	77.75 *	*	0.10 *	ns	7.10 *	ns
Varieties 1	41.00		92.25		0.05		6.28	
Varieties 2	54.50		113.88		0.09		7.00	
BNT 5%	5.01		2.28		0.52		0.14	

Description: ns = not significantly different; * = significantly different

3.4. Effect of PEG on the dry weight of sprouts and water volume of local upland rice plants

According to the findings, there were differences between cultivars in both the dry weight and water content of sprouts. Without the use of PEG, the dry weight of the sprouts ranged from 172.55 mg (raki cultivar) to 527 mg (pae pulu palang) (Figure 4). The use of PEG up to -3 bar resulted in a decrease in the lowest seedling weight of 53.96% (raki) and the highest, 85.25% (pae pulu palang), while the controls were respectively 77.45% and 71.69% (Figure 4). Siang, raki, Kalendeng, and Tagolu were cultivars that performed better than the others and did not differ from the control varieties (Table 2). The volume of water ranges from 0.13 ml (raki) to 0.65 ml (pae pulu palang) without PEG. Applying PEG up to -3 bar caused a decrease in water volume from 40% (pulut ko and raki) to 85.71% (siang), while the control variety decreased by 77.45% and 71.69%. According to Qayyum et al., (2021), the dry weight of the roots and shoots of wheat cultivar seedlings decreased with increased PEG. Osmotic stress results in limited water availability for plants, which lowers cell development and turgor pressure and ultimately lowers biomass.

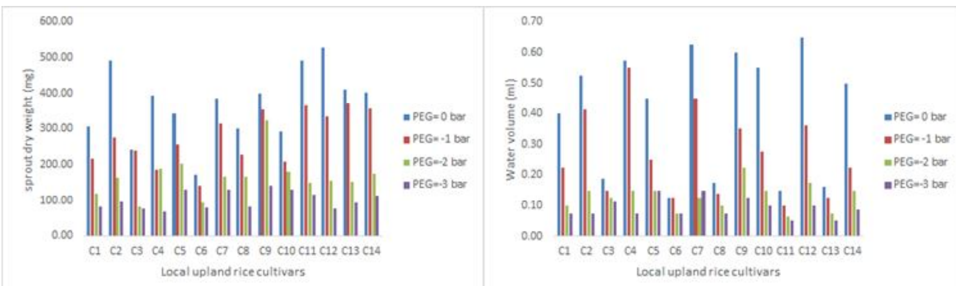

Figure 4. Response of cultivars subjected to drought stress conditions through PEG on sprout dry weight and water volume of local upland rice.

Comment [DK6]: Authors should also separate Figure 4 into Figure 4A and Figure 4B

3.5. Effect of PEG on the germination rate of local upland rice

The results showed that without PEG, the germination rate was 2.56 (day) to 4.33 days (Inpago-4). Without PEG application, cultivars/varieties germinate for 3 to 4 days. Application of PRG up to -3 bar caused slow germination of cultivars/varieties, namely -39.48% to -131.55% (Figure 5). This means that using PEG for up to -3 days causes the seeds to germinate on the fourth day (pulut ko) to seven days (Gajah Mungkur). PEG stress up to -3 bar caused some cultivars to germinate faster than control varieties, such as pulut ko and roda, which germinated on the fourth day and performed better than the control. Delay in seed germination caused by PEG administration up to -3 bar has caused metabolic disturbances such as slower hydrolysis of storage compounds in the endosperm or cotyledons and slower transport of the hydrolyzed material to the axis of the developing embryo under drought conditions (Sobahan et al., 2022).

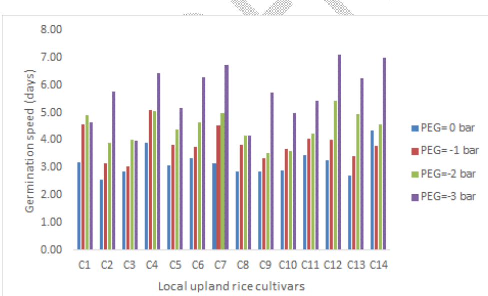

Figure 5. Response of cultivars subjected to drought stress conditions through PEG on the germination rate of local upland rice

4. CONCLUSION

Generally, -1 bar drought stress has not resulted in a significantly different decrease. Application of PEG up to -3 bar resulted in a decrease in the maximum growth potential of 8% to 81.03%, germination rate of 13.21% to 88.21%, 28.79% -86.38% for radicle length, plumula length 59.61 to 92 .93%, radicle dry weight 47.74% to 82.21%, 52.51% to 93.04% for plumula dry weight, sprout dry weight 53.96% - 85.25%, 40% to 85.71 % for water volume and germination rate -39.48% to -131.55%. Using PEG up to -3 bar caused a significant decrease in the germination of local upland rice. None of the cultivars showed tolerance for all the observed variables. Pulut ko is a cultivar with drought stress tolerance, followed by logi and sina ponding.

References

Alaei, S., Khibary, Z. M., & Rad, A. A. (2015). *Effect of Different Concentration of Salt and PEG Solution on Dracocephalum moldavica Seed Germination and Seedling Early Growth*. *7*(1), 1755–1759.

Aniat-ul-Haq, R., & Agnihotri, R. (2010). Effect of osmotic stress (PEG) on germination and seedling survival of lentil (Lens culinaris Medik.). *Research Journal of Agricultural Sciences*, *1*(3), 201–204. http://www.rjas.info/wp-content/uploads/2010/08/Effect-of-Osmotic-Stress-PEG-on-Germination-and-Seedling-Survival.pdf

Arora, N. K. (2019). Impact of climate change on agriculture production and its sustainable solutions. *Environmental Sustainability*, *2*(2), 95–96. https://doi.org/10.1007/s42398-019-00078-w

Basha, P. O., Sudarsanam, G., Reddy, M. M. S., & Sankar, N. S. (2015). Research Article Effect of Peg Induced Water Stress on Germination and Seedling Development of Tomato Germplasm. *Int J Rec Sci Research*, *6*(May), 4044–4049.

Chen, K., & Arora, R. (2011). Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in Spinach (Spinacia oleracea). *Plant Science*, *180*(2), 212–220. https://doi.org/10.1016/j.plantsci.2010.08.007

Chutia, J., & Borah, S. P. (2012). Water Stress Effects on Leaf Growth and Chlorophyll Content but Not the Grain Yield in Traditional Rice (<i>Oryza sativa</i> Linn.) Genotypes of Assam, India II. Protein and Proline Status in Seedlings under PEG Induced Water Stress. *American Journal of Plant Sciences*, *03*(07), 971–980. https://doi.org/10.4236/ajps.2012.37115

Cox, D. F., Gomez, K. A., & Gomez, A. A. (1985). Statistical Procedures for Agricultural Research. *Journal of the American Statistical Association*, *80*(390), 486. https://doi.org/10.2307/2287932

Ekowati, N. Y., & Widijastuti, R. (2018). Uji Ketahanan Cekaman Kekeringan Menggunakan Polyethylene Glycol (Peg) 6000 Pada Padi Lokal Dan Non Lokal Di Kabupaten Merauke. *Prosiding SINTESIS (Seminar Nasional Sains, Teknologi Dan Analisis)*, *1*(1), 47–53.

Farooq, M., Hussain, M., Wakeel, A., & Siddique, K. H. M. (2015). Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agronomy for Sustainable Development*, *35*(2), 461–481. https://doi.org/10.1007/s13593-015-0287-0

Furlan, A., Llanes, A., Luna, V., & Castro, S. (2012). Physiological and Biochemical Responses to Drought Stress and Subsequent Rehydration in the Symbiotic Association Peanut-Bradyrhizobium sp. . *ISRN Agronomy*, *2012*, 1–8. https://doi.org/10.5402/2012/318083

Kadhimi, A. A., Zain, C. R. C. M., Alhasnawi, A. N., Isahak, A., Ashraf, M. F., Mohamad, A., Doni, F., & Yusoff, W. M. W. (2016). Effect of irradiation and polyethylene glycol on drought tolerance of MR269 genotype rice (Oryza sativa L.). *Asian Journal of Crop Science*, *8*(2), 52–59. https://doi.org/10.3923/ajcs.2016.52.59

Kaufmann, M., & Michel, B. (1973). The Osmotic Potential of Polyethylene Glycol 6000. *Plant*

Physiology, *51*, 914–916.
Khakwani, A. A., Dennett, M. D., & Munir, M. (2011). Drought tolerance screening of wheat varieties by inducing water stress conditions. *Songklanakarin Journal of Science and Technology*, *33*(2), 135–142.
Larkunthod, P., Nounjan, N., Siangliw, J. L., Toojinda, T., Sanitchon, J., Jongdee, B., & Theerakulpisut, P. (2018). Physiological responses under drought stress of improved drought- Tolerant rice lines and their parents. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *46*(2), 679–687. https://doi.org/10.15835/nbha46211188
Munarso, Y. P. (2011). Keragaan Padi Hibrida pada Sistem Pengairan Intermittent dan Tergenang. *Jurnal Penelitian Pertanian Tanaman Pangan*, *30*(3), 145–153.
Muscolo, A., Sidari, M., Anastasi, U., Santonoceto, C., & Maggio, A. (2014). Effect of PEG-induced drought stress on seed germination of four lentil genotypes. *Journal of Plant Interactions*, *9*(1), 354–363. https://doi.org/10.1080/17429145.2013.835880
Nazirah, L., Purba, E., Hanum, C., & Rauf, A. (2016). Characterization of Tolerant Upland Rice to Drought on Rooting and Physiology. *Journal of Agriculture and Life Sciences*, *3*(2), 43–49.
Pant, B., & Bose, B. (2016). Mitigation of the influence of PEG-6000 imposed water stress on germination of halo primed rice seeds. *International Journal of Agriculture, Environment and Biotechnology*, *9*(2), 275. https://doi.org/10.5958/2230-732x.2016.00036.x
Pradhan, N., Prakash, P., Manimurugan, C., kumar tiwari, S., Sharma, R. p., & Singh, P. . (2015). Screening of tomato genotypes using osmopriming with PEG 6000 under salinity stress. *Research in Environment and Life Sciences*, *8*(2), 245–250.
Purbajanti, E. D., Kusmiyati, F., Fuskhah, E., Rosyida, R., Adinurani, P. G., & Vincēviča-Gaile, Z. (2019). Selection for drought-resistant rice (Oryza sativa L.) using polyethylene glycol. *IOP Conference Series: Earth and Environmental Science*, *293*(1). https://doi.org/10.1088/1755-1315/293/1/012014
Qayyum, A., Al Ayoubi, S., Sher, A., Bibi, Y., Ahmad, S., Shen, Z., & Jenks, M. A. (2021). Improvement in drought tolerance in bread wheat is related to an improvement in osmolyte production, antioxidant enzyme activities, and gaseous exchange. *Saudi Journal of Biological Sciences*, *28*(9), 5238–5249. https://doi.org/10.1016/j.sjbs.2021.05.040
Sagar, A., Rauf, F., Mia, M., Shabi, T., Rahman, T., & Hossain, A. (2020). Polyethylene Glycol (PEG) Induced Drought Stress on Five Rice Genotypes at Early Seedling Stage. *Journal of Bangladesh Agricultural University*, *0*, 1. https://doi.org/10.5455/jbau.102585
Shitole, S. M., & K.N. Dhumal. (2012). EFFECT OF WATER STRESS BY POLYETHYLENE GLYCOL 6000 AND SODIUM CHLORIDE ON SEED GERMINATION AND SEEDLING GROWTH OF CASSIA ANGUSTIFOLIA. *International Journal of Pharmaceutical Scineces and Research*, *3*(02), 528–531.
Sobahan, M. A., Akter, N., & Rana, M. M. (2022). Polyethylene glycol mediated drought stress impacts on germination, growth and accumulation of proline in rice (Oryza sativa L.). *SAARC Journal of Agriculture*, *20*(1), 107–119. https://doi.org/10.3329/sja.v20i1.60544
Sokoto, M. B., & Muhammad, A. (2014). Response of Rice Varieties to Water Stress in Sokoto, Sudan Savannah, Nigeria. *Journal of Biosciences and Medicines*, *02*(01), 68–74. https://doi.org/10.4236/jbm.2014.21008
Srivastava, A. K., Lokhande, V. H., Patade, V. Y., Suprasanna, P., Sjahril, R., & D'Souza, S. F. (2010). Comparative evaluation of hydro-, chemo-, and hormonal-priming methods for imparting salt and PEG stress tolerance in Indian mustard (Brassica juncea L.). *Acta Physiologiae Plantarum*, *32*(6), 1135–1144. https://doi.org/10.1007/s11738-010-0505-y
Swapna, S., & Shylaraj, K. S. (2017). Screening for Osmotic Stress Responses in Rice Varieties under Drought Condition. *Rice Science*, *24*(5), 253–263. https://doi.org/10.1016/j.rsci.2017.04.004
Zu, X., Lu, Y., Wang, Q., Chu, P., Miao, W., Wang, H., & La, H. (2017). A new method for

evaluating the drought tolerance of upland rice cultivars. *Crop Journal*, *5*(6), 488–498. https://doi.org/10.1016/j.cj.2017.05.002