International Journal of Agriculture System
Vol. 9 Issue 1, June 2021
Nationally Accredited Journal Decree No. 48a/E/KPT/2017
P-ISSN: 2337-9782, E-ISSN: 2580-6815. **DOI: 10.20956/ijas.v9i1.2558**

Effect of Osmo-Priming with Polyethylene Glycol 6000 (PEG-6000) on Rice Seed (*Oryza sativa* L.) Germination and Seedling Growth Under Drought Stress

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How to Cite: AR. Trisnawaty A.R., R. Asra, N.J. Panga and R. Sjahril (2020). Effect of Osmo-Priming with Polyethylene Glycol 6000 (PEG-6000) on Rice Seed (*Oryza sativa* L.) Germination and Seedling Growth Under Drought Stress. *Int. J. Agr. Syst. 9*(1): 40-50

ABSTRACT

One of the efforts to increase rice production is by extensive farming through the utilization of marginal lands affected by drought. The present study aims to examine the influence of seed priming with PEG 6000 on rice seed germination and growth of rice seedlings in drought conditions and to group the rice varieties tested based on their tolerance to drought, and determine priming formulations for early adaptation of rice seeds that can be used by farmers and industry. The research was organized in the form of a Split Plot Design with Randomized Group Design. The Main Plot is a rice variety, consisting of 10 levels, i.e., Inpari 31, Inpari 33, Inpari 36, Inpari 39, Inpari 40, Inpago 8, Inpago 10, Mekongga, Rindang 1 and Rindang 2. While the Split Plot is a concentration of PEG, which consists of 5 levels, i.e., without soaking as negative control concentration 0 g L^{-1} PEG as positive control, and 50, 100, and 150 g L^{-1} PEG-6000. Each of these treatments is repeated three times, so there are 150 observation units. Results showed that among the 10 varieties tested, there were 2 less tolerant varieties with scores of 5-10 (Inpari 31 and Inpari 39), 5 varieties are quite tolerant with a score of 11-16 (Inpari 33, Inpari 36, Inpari 40, Inpagi 10 and Mekongga) and 3 varieties are very tolerant with a score of 17-22 (Rindang 1, Rindang 2 and Inpago 8) with priming concentrations of 100 g L^{-1} PEG-6000 for 36 hours.

Keywords:
Abiotic Stress; Drought; Polyethylene Glycol; Rice; Seed Priming

1. Introduction

The cause of the decline in rice production in Indonesia is the deterioration of the physical properties of the soil which reduces the land productivity. One of the ways to increase rice production is by extensive agriculture, utilizing marginal lands that are affected by drought. Therefore, the use of technologies for dry land agriculture to produce rice as food resources needs to be increased.

The problem with dry land agriculture is the limited capacity of groundwater to meet the needs of plants. This is known as drought stress. Drought stress problems can be overcome in two ways, i.e., by changing the environment so that stress can be minimized and improving plant genotypes so that they are resistant to drought stress (Hussain et al., 2004). Further effects due to drought's dryness on rice plants are 1) reduced germination rate; 2) reduced plant height and the number of tillers; 3) poor root growth; 4) increased seed sterility; 5) less grain weight and total protein content per 1000 seeds due to excessive Na absorption, and 6) reduced biological N_2 fixation and delayed soil mineralization.

Plants that experience drought stress shows an effect that is initiated by the slow growth of shoots and leaves caused by a decrease in leaf turgor potential, followed by a decrease in the cell wall and protein synthesis. The decrease in tissue water potential causes slow cell division and the activity of enzymes such as nitrate reductase begins to decrease. The stomata begin to close followed by a decrease in CO_2 transpiration and assimilation, and an accumulation of abscisic acid. If the stress continues and the water potential continues to decrease, respiration also decreases, the translocation of photosynthate and cytokinin in leaves become larger. The activity of several hydrolysis enzymes increases and ion transport slows down and eventually, the accumulation of protein and CO_2 becomes very low or zero (Gunadi & Djunaidy, 2019).

Related to these stressful environmental conditions, plants have a mechanism that allows them to respond to environmental changes slowly (Shao et al., 2006; Bruce et al., 2007; Wu et al., 2007) . The existence of a stress tolerance response mechanism is a symptom of "priming". Priming is the process by which stress imprints are created, which is a form of memory that occurs due to stresses in plants.

Priming in plants is described as one of the activations of various defense responses that are faster and stronger against abiotic stress by reopening the "memory" so that plants are more resistant to repeated stresses (Conrath et al., 2006). This means that plants can form a form of memory which is termed stress improvement, as a biochemical or genetic modification in plants that occurs after stress exposure, which results in plant response to stress is different from before (Bruce et al., 2007). The best part of the plant for priming is the imbibition and metabolic stages before seed germination. Research on the tolerance of seed germination to growing stress is a seed management technique under osmotic conditions (Osmo-conditioning) which is also associated with priming (Syaiful et al., 2014).

Several studies on priming have provided hope, but there is much more information that must be extracted before this practical seed technology is routinely implemented. Seed priming with various treatments, such as hydro-priming, halo-priming, osmo-priming, and various other treatments has been carried out by several researchers on various types of plants in both laboratory and field tests. Elkheir et al., (2016) and Abdallah et al., (2016) examined the effect of PEG-8000 priming on the growth and yield of rice plants. Abdallah et al. (2016), reported the effect of osmo-priming on the germination and growth of seeds with polyethylene glycol (PEG-8000) for 3 days at 25°C and air-dried for 3 days before planting, can increase germination. However, PEG-8000 priming results with a concentration of more than 200 g L^{-1} can cause a decrease in the percentage of germination. Sun et al., (2010) tested Gangyou 527 (indica hybrid rice) which was osmo-primed in 20% PEG-6000 solution and showed satisfactory results on germination percentage under drought stress, and confirmed effect of PEG priming on enhancing drought tolerance in germination stage.

The fact of the benefits and important role of this priming technique in the development of plants that are tolerant to environmental stresses also gives hope for the development of rice on marginal lands with drought stress scattered in several regions in Indonesia.

Based on the description above, it becomes rationale that the development of drought-resistant rice is a very important study to address drought which is a serious problem in agriculture land in general, and national food security programs currently and in the future. One way to develop rice that can adapt to drought stress is by providing induced drought tolerance from an early age in the germination phase through seed invigoration using the seed priming method.

2. Materials and Methods

2.1. Place and time

This research was conducted at the laboratory and the green house of Plant Bioscience and Reproduction Biotechnology, Department of Agronomy, Faculty of Agriculture, Hasanuddin University, Makassar which took place from March to August 2020.

2.2. Material and equipment

Materials used in this study were comprised of 10 high-quality rice seed varieties from superior national varieties (Inpari 31, Inpari 33, Inpari 36, Inpari 39, Inpari 40, Inpago 8, Inpago 10, Mekongga, Rindang 1, and Rindang 2), which represent rice varieties which are commonly planted by farmers in irrigated and water fed paddy field in upland rice. To simulate drought stress, we used PEG 6000 and distilled water. NPK (16:16:16) fertilizer and pesticides were used for growth and pest and disease maintenance. While the tools and equipment used in this study included Petri dishes (∅15 cm), plastic pots (∅10 cm), plastic trays containers for priming, small shovels and hoes, measuring meter, cameras, paper sticker labels, and writing instruments.

2.3. Experiment Method

The research was conducted in three stages. The first stage was a preliminary test which was carried out to determine the average soaking duration of the seeds for all varieties tested. Rice seeds are primed separately in distilled water in Petri dishes accordingly. Periodically they are observed until the emergence of the seed radicle as an early sign of germination. The moment this radicle appears is then recorded as an indication to determine the soaking duration (hours) in subsequent experiments.

2.4. *Observation Parameters*

Experimental observations include:

1. The periodic curing time of the seeds until the time before these radicles appeared was recorded as a guide to determine the curing time (hours).
2. Germination, the percentage of sprouts at the end of the observation (%) is measured by the following equation: (Sadjad et al, 1999).

$$\% G = \frac{\Sigma KN}{\Sigma TB} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots (1)$$

Remarks:
% G : Percentage of germination
Σ KN : The number of normal sprouts that grew until the end of the observation
Σ TB : Total number of seeds germinated

3. Seed height (cm), measured from the base of the stem to the tip of the leaf.
4. The number of leaves (leaf) counts the total number of leaves that grow at the end of the observation.
5. Leaf length (cm), measured from the base to the tip of the leaf
6. Seed dry weight (g), measured by weighing the seed dry weight after oven drying for 3 days at 85°C.

2.5. Data Analysis

The research data were analyzed statistically using analysis of variance (ANOVA). A further test is carried out if the F test shows significant or very significant differences. Determination of the drought tolerance level of the 10 tested varieties and a good priming formulation for early adaptation was carried out through the data scoring method. Observation data from 10 tested varieties in the form of germination capacity, seed height, leaf length, number of leaves, root length, and seedling dry weight were grouped into three criteria classes based on the scoring results, such as very tolerant, tolerant, and less tolerant.

Determination of the level of tolerance of each variety to drought stress based on observation parameters was carried out by the scoring method. Each observed parameter of the seed growth component will be given a score, and then added up to obtain the level of association. The final result of the scoring system is a grouping of seed adaptation rates and the determination of a good priming formulation, which is calculated by the following equation:

$$S_n = (X_n - X_1)/SD + S_1 \qquad (2)$$

Remarks:
S_n = data n^{th} score
X_n = Value of the n^{th} data
SD = Standard Deviation
X_1 = Lowest data value
S_1 = Lowest data score

3. Results and Discussion

3.1. Priming Period

Rice seeds were soaked in distilled water where they were periodically observed until before the radicles appeared, but the seeds had swollen and then recorded as a guide to determine the duration of soaking time (hours) in the next experiment. The results obtained indicated that the proper soaking time was 36 hours because soaking for more than 36 hours caused the seed radicles to appear and continue growing, and this condition did not allow the seeds to be stored again. The seed condition after priming can be seen in Figure 1.

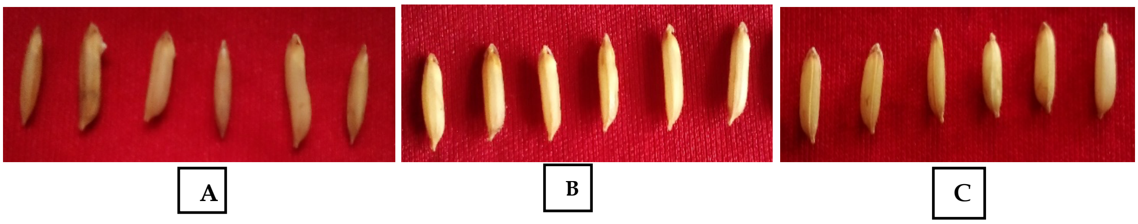


Figure 1. Seed condition after priming (A. 40 hours; B. 38 hours; C. 36 hours)

The results of the experiment showed that after priming for 36 hours, the plumules began to emerge and cracked the seed coat. This condition is not recommended for seeds that are still going to be stored so that the soaking time of 36 hours is then used as a parameter of the length of soaking time. This results corroborate the research of Abdallah et al. (2016), where they found that the longest rice seed immersion time in osmo-priming conditions is around 30-36 hours.

3.2. *Germination Rate*

Germination testing needs to be done to determine the viability of seeds, or the ability of seeds to grow into seeds in optimum environmental conditions. The results of the germination test on 10 rice varieties tested at various concentrations of priming agents are presented in Table 1 and Figure 2 below.

Table 1. Percentage of seed germination (%) in various priming treatments

Varieties	Priming					Average	CV LSD $_{0.01}$
	Control	PEG 0 g L^{-1}	PEG 50 g L^{-1}	PEG 100 g L^{-1}	PEG 150 g L^{-1}		
Inpari 31	51.33	52.33	56.67	93.67	68.00	64.40 ^{de}	
Inpari 33	75.33	76.67	83.00	85.33	83.00	80.67 ^{bcd}	
Inpari 36	72.33	81.00	84.67	93.00	87.00	83.60 ^{abc}	
Inpari 39	48.67	51.00	53.33	96.33	59.00	61.67 ^{e}	
Inpari 40	80.33	81.00	82.33	95.00	92.00	86.13 ^{abc}	16.45
Inpago 8	88.67	92.00	92.00	92.00	92.67	91.47 ^{ab}	
Inpago 10	58.67	66.67	73.33	92.00	73.67	72.87 ^{cde}	
Mekongga	75.67	79.33	80.33	87.00	82.33	80.93 ^{bc}	
Rindang 1	96.33	96.33	97.67	99.00	98.33	97.53 ^{a}	
Rindang 2	87.00	93.00	93.33	96.00	93.67	92.60 ^{ab}	
Average	73.43 ^{c}	76.93 ^{bc}	79.67 ^{bc}	92.93 ^{a}	82.97 ^{b}		
CV LSD $_{0.01}$			8.48				

Remarks: Coefficient of diversity V = 15%; P = 9%. The numbers followed by the same letter in the column are not significantly different at the LSD $_{\alpha=0.01}$ level

Table 1 shows that of the 10 tested rice varieties, three varieties had the highest germination with a percentage of germination above 90% (i.e., Rindang 1, Rindang 2, and Inpago 8). As for the priming treatment with PEG-6000 with a concentration of 100 g L^{-1} gave the highest germination value (92.93%) compared to other treatments. This indicate that the seed priming treatment using PEG-6000 at a concentration of 100 g L^{-1} was able to increase germination due to the improvement in metabolic processes during the imbibition process, which caused the metabolites produced to increase and then stimulate germination (Heydariyan et al., 2014). In other words, the growth of seeds primed with PEG-6000 was faster and simultaneously, and uniform. The results showed that there was an improvement in the viability and vigor of the seeds as indicated by physiological indications i.e., improved germination performance and increased germination. Priming is a pre-sowing treatment in which seeds are partly hydrated such that there is an initiation of germination process with no observable radicle emergence. An increase in germination rate, greater germination uniformity, vigorous growth, early flowering, and higher yield can be observed with the use of primed seeds (Aryal et al., 2020). Purba et al., (2013) stated that the deterioration of the seeds during the storage period was caused by the higher moisture content of the seeds. This results in a faster respiration rate so that more CO_2 and heat are produced. This physiological activity can be suppressed through the ideal storage water content so that the germination of the seeds can be maintained until the time for the seeds to be germinated.

The average germination rate of rice seeds in the initial test (control) was 73.43%. This was categorized as low germination capacity, after priming the germination increased to 92.93% in the 100 g L^{-1} PEG priming treatment. Seed germination can reflect a seed strength. Giving various concentrations of PEG will have an impact on air imbibition and oxygen diffusion. The process of entering water into the seed by imbibition is the entry of water through the seed coat. This is due to the osmotic potential, between high to low potential.

The treatment of PEG concentration has an impact on the osmotic potential, so that the speed of air diffusion occurs faster. However, presenting high concentrations of PEG can be detrimental to seeds. In accordance with (Sofinoris, 2009) statement that Polyethylene Glycol is soluble in air and causes a decrease in air potential. The magnitude of the decrease in water is very important at the concentration of reducing the molecular weight of PEG. This situation is used to simulate the reduction of air potential. The water potential in a medium containing PEG can be used to mimic the magnitude of the air potential. This is very good for reducing the metabolic process of seeds, so that food reserves in the endosperm are not depleted, and during the germination phase the seeds can produce high germination (Fazilla et al., 2014). Elkheir *et al*. (2016); and Abdallah et al. (2016) examined the effect of PEG-8000 priming on the growth and yield of rice plants. Priming results of PEG-8000 with a concentration of more than 200 g L^{-1} can cause a decrease in the percentage of germination.

3.3. Drought Test at Seedling Level

Table 2 shows the results of the analysis of variance on the treatment of varieties and the concentration of PEG solution as priming treatment. The interaction between the two resulted in a significant difference between the variety treatment and the priming treatment which is shown through the observation parameters of seedling height, leaf length, root length, and seedling dry weight significant differences in the parameter number of leaves in rice seedlings aged 30 DAS. Seed priming using PEG 100 g L^{-1} showed a better growth rate in the Rindang 1 variety on the parameters of seed weight, leaf length, and root length, and Rindang 2 variety in the number of leaves dry weight, compared to other priming treatments. The results showed that three varieties had the best growth in drought testing, namely the Rindang 1, Rindang 2, and Inpago 8 varieties. Basically, the genotypes of these three varieties are varieties that are quite tolerant of drought based on the description of the variety issued by the Sasmita et al., (2020), then given a priming treatment to cause the varieties to be more tolerant of the drought conditions tested. The growth of rice seeds primed using PEG-6000 with a concentration of 100 g L^{-1} showed the best growth and affected increasing the adaptive tolerance of rice compared to other treatments.

Table 2. Growth of seedlings 30 days after sowing from 10 rice varieties after seed priming and grown in 150 g L^{-1} PEG-6000 solution as a simulation of drought conditions

Treatments		Plant height (cm)	Leaf length (cm)	Number of leaf (sheet)	Root length (cm)	Seed dry weight (g)
Varieties (V)						
Inpari 31		19.90 bc	13.12 bc	3.67 a	5.16 ab	0.048 bc
Inpari 33		21.50 bc	14.03 bc	3.97 a	5.25 ab	0.049 bc
Inpari 36		21.89 bc	15.55 abc	3.59 a	5.51 ab	0.046 bc
Inpari 39		16.19 c	10.63 c	3.29 a	3.32 b	0.038 c
Inpari 40		23.00 abc	15.33 abc	3.82 a	6.61 ab	0.073 abc
Inpago 8		26.03 abc	17.47 abc	3.84 a	6.32 ab	0.079 ab
Inpago 10		20.77 bc	13.82 bc	3.45 a	5.67 ab	0.062 bc
Mekongga		21.85 bc	14.45 bc	3.91 a	6.58 ab	0.064 abc
Rindang 1		31.02 a	20.22 a	3.82 a	7.81 ab	0.075 abc
Rindang 2		28.12 abc	18.33 ab	4.22 a	7.77 ab	0.100 a
Priming (P)						
Non Priming		16.27 c	10.54 b	3.32 a	3.32 b	0.041 b
PEG 0 g L^{-1}		20.93 bc	13.42 b	3.67 a	3.67 ab	0.057 ab
PEG 50 g L^{-1}		22.14 abc	14.36 b	3.76 a	3.76 ab	0.070 ab
PEG 100 g L^{-1}		28.27 a	19.11 a	4.08 a	4.08 a	0.082 a
PEG 150 g L^{-1}		27.52 ab	19.05 a	3.97 a	3.97 a	0.067 a
Coefficient of diversity (%)	V	25	25	20	38	38
	P	23	22	17	35	31
Comparative value	V	8.27	5.3	1.05	3.32	0.04
	P	6.92	4.37	0.83	2.83	0.03

Remark: The numbers followed by the same letter in the column are not significantly different at the LSD $_{\alpha=0.01}$ level

Plant dry weight is the accumulation of organic compounds resulting from plant synthesis from inorganic compounds derived from water and carbon dioxide through photochemical processes and reduction of important compounds, translocations that reflect seed activity during growth. (Fazilla et al., 2014) reported that priming seeds using an osmotic solution with PEG significantly affected the growth rate after storage.

Priming of seeds has several advantages both directly and indirectly. The immediate advantages of priming seeds on all crops include faster, better, more uniform sprouts growth, less embroidery, more vigor, better final tolerance, faster flowering, earlier harvest, and higher yields. While indirect benefits such as sowing seeds earlier, harvesting some plants earlier, and increasing the ability to use fertilizers due to the risk of neglected crop reduction (Arif et al., 2014). Priming seeds can reduce germination time and increase the uniformity of sprouts growth (Sarwar & Ali, 2007).

2.4. Selection of Treatments for Drought Adaptation

Determination of the drought tolerance level of the tested varieties, as well as the good priming formulation of the treatments tested, was carried out by determining the score value from the observed growth components. The scores obtained from each observation parameter are then added and grouped into 3 classes based on their tolerance level to drought stress through frequency distribution. The scores for the observed growth components in the varieties and priming treatments tested are presented in Tables 3 and 4 as well as in Figure 2 below.

Table 3. Scoring of 10 rice varieties based on growth components after drought simulation

Treatment	Germination (%)		Plant height (cm)		Leaf length (cm)		Root length (cm)		Seed dry weight (g)		Total Score
	Value	Score	Value	Score	Value	Score	Value	Score	Value	Score	
Inpari 31	64,40	1	19,90	2	13,12	2	3,67	2	5,16	2	10
Inpari 33	80,67	3	21,50	2	14,03	2	3,97	4	5,25	2	13
Inpari 36	83,60	3	21,89	2	15,55	3	3,59	2	5,51	3	13
Inpari 39	61,67	1	16,19	1	10,63	1	3,29	1	3,32	1	5
Inpari 40	86,13	3	23,00	3	15,33	3	3,82	3	6,61	3	15
Inpago 8	91,47	4	26,03	3	17,47	3	3,84	3	6,32	3	17
Inpago 10	72,87	2	20,77	2	13,82	2	3,45	2	5,67	3	11
Mekongga	80,93	3	21,85	2	14,45	2	3,91	3	6,58	3	14
Rindang 1	97,53	4	31,02	4	20,22	4	3,82	3	7,81	4	20
Rindang 2	92,60	4	28,12	4	18,33	4	4,22	4	7,77	4	20
Average value	81,19		23,03		15,30		3,76		6,00		13,69
Minimum value	61,67		16,19		10,63		3,29		3,32		5,00
Maximum Value	97,53		31,02		20,22		4,22		7,81		20,30
Standard of Deviation	11,86		4,29		2,77		0,27		1,33		4,65

Table 4. Results after selection based on the scoring value for the criteria of less tolerant, tolerant, and very tolerant, with the cluster method

Less Tolerant (5-10)	Inpari 31 dan Inpari 39
Tolerant (11-16)	Inpari 33, Inpari 36, Inpari 40, Inpago 10, Mekongga
Very Tolerant (17-22)	Rindang 1, Rindang 2, Inpago 8

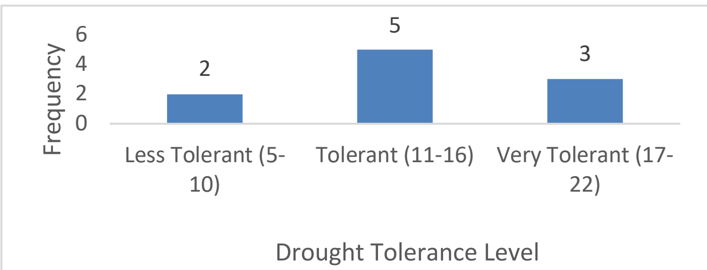
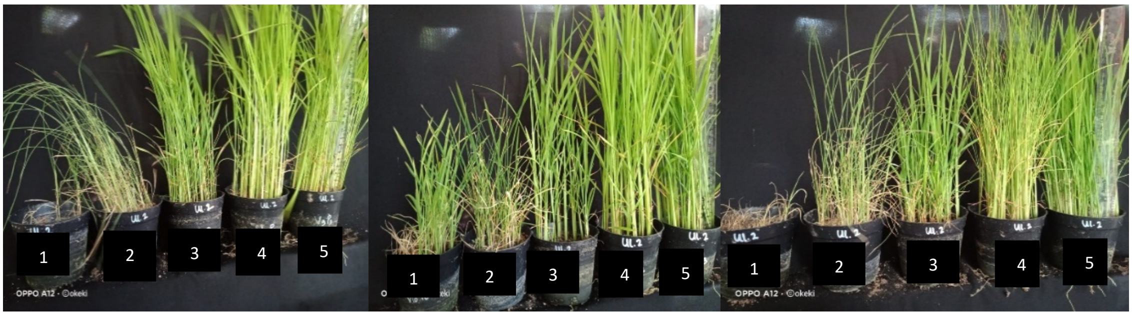


Figure 2. Graphic of classification of drought tolerance varieties based on the scoring data

Based on the data on the results of scoring and selection of varieties based on their level of tolerance to drought stress after trimming (Tables 3, 4 and Graph 2), it can be seen that of the 10 varieties that were primed and tested in drought stress conditions, there were two less tolerant varieties with a score of 5-10 (Inpari 31 and Inpari 39). There are five tolerant varieties with a score of 11-16 (Inpari 33, Inpari 36, Inpari 40, Inpari 10, and Mekongga) and three very tolerant varieties with a score of 17-22 namely Rindang 1, Rindang 2, and Inpago 8.



a) Rindang 1 b) Rindang 2 c) Inpago 8

Figure 3. The conditions for the growth of rice seedlings from three very tolerant varieties that have been primed for the drought testing. Labels: (1), Control; (2), PEG 0 g L^{-1}; (3), PEG 50 g L^{-1}; (4), PEG 100 g L^{-1}; (5), PEG 150 g L^{-1}.

The lack of varieties that can survive drought simulation testing until the harvest period and production is due to the influence of the level of tolerance of plants to high drought stress which causes plants to grow abnormally with early signs of drought stress, including narrow and curled leaves, and eventually causing the plant to become dry caused by lack of water. The symptoms that this cause looks like symptoms of chlorosis. The ability to regulate osmotic pressure in plant cells is an important mechanism in overcoming stress intolerant plants (Panga et al., 2019). Priming can also reduce the effects of temperature and groundwater stress as well as pests and diseases, sprouts grow simultaneously and germination times are faster, increase germination capacity, root and canopy length (Wojtyla et al., 2016; Akbar et al., 2018; Ruttanaruangboworn et al., 2017).

4. Conclusion

Priming treatment using PEG 6000 for 36 hours with a concentration of 100 g L^{-1} at the nursery stage was able to increase the adaptation of Rindang 1, Rindang 2, and Inpago 8 rice varieties under drought stress conditions.

Acknowledgment

We would like to thank the Republic of Indonesia Ministry of Research, Technology and Higher Education (RISTEKDIKTI) which financed this research under the Penelitian Dosen Pemula (PDP) Program (Contract Number: 636 KTR/II.3.AU/ F/Rek.UMSR/2020, dated on June 5th, 2020 and to all our colleagues and students who have helped this research.

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