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Selection for drought-resistant rice (*Oryza sativa* L.) using polyethylene glycol

E D Purbajanti[1,*], F Kusmiyati[1], E Fuskhah[1], R Rosyida[1], P G Adinurani[2] and Z Vincēviča-Gaile [3]

[1]Agriculture Department, Faculty of Animal and Agricultural Sciences, Diponegoro University, Jalan Prof Sudharto SH, Tembalang, Semarang 50275, Indonesia.
[2]Faculty of Agriculture, Merdeka University of Madiun, Jl. Serayu No.79, Pandean, Madiun 63133, Indonesia
[3]Plant Cell Culture, Departement of Biotechnology Engineering, Kulliyyah of Engineering International Islamic University Malaysia, P.O. Box 10, Kuala Lumpur 50728, Malaysia

*Corresponding author: endang.purbajanti@live.undip.ac.id

Abstract. The study was expected to provide information poly ethylene glycol (PEG) concentration that can be used for plant breeding base rice (*Oryza sativa* L.) plant. The research was conducted covering two experimentals stage, were (i). Tests of dry stress resistance of six cultivars of rice using PEG 6000, and (ii). Emerged test seeds of six rice cultivars that have been subjected to dry stress with PEG 6000. The treatments were designed based on a complete randomized design of two factors. The first factor used six cultivars of rice were cv.Mekongga, cv. Way Apo Buru, cv. Sidenuk, cv. Pepe, cv. Ciherang and cv. IR64. and the second factor was dry stress [(0, 5, 10 and 15) % of PEG] with replicates three times. The higher concentration of PEG lowered growth rate (GR), emergence rate (ER), germination energy (GE) and the relative germination rate on all cultivars of rice. Germinate the longer time, appropriate concentrations of PEG. Test of pre-emergence on soil giving the mixed response on all cultivars of rice and PEG level. Cv. Mekongga, cv. Sidenuk, cv. Pepe and cv. Ciherang have the ability to grow on soil test. Canopy and root length highest on cv. IR64. Cv. Sidenuk popularity score can be classified moderate tolerance, while cv. Pepe that was sensitive to drought stress.
Keywords: Breeding drought resistant, early selection of rice, initial selection, tolerance mechanisms, water stress

1. Introduction

Water stress occurs when transpiration rate exceeds the absorption and transport of water in plants [1]. Drought causes cellular dehydration as a consequence the release of water from the cytosol to the vacuole and apoplast. The response of plants to water stress include changes in stomatal conductance, growth, accumulation of osmolyte, and the expression of specific genes. In this process, abscisic acid (ABA) is a major stress hormone because of the rapid accumulation under conditions of stress and participate in physiological and biochemical processes that allow plants to survive [2].

Drought stress induces a reduction in plant growth and the development in rice [3]. Drought triggers a variety of plant responses, ranging from cell metabolism to changes in the rate of growth and yield. Understanding the biochemical and molecular responses to drought are important to the mechanism of plant resistance to water limited conditions [4]. Drought stress effect on photosynthesis

and assimilate translocation which excessive drought stress resulted in dead plants. Some plants respond to drought stress in various ways, namely tolerance mechanisms, and avoidance [5, 6]. Water is the limiting abiotic stress which causes a decrease in crop production. Drought is a condition that created difficult completed the normal physiological function by lowering the water potential of plants and turgor. Plants become dry due to dehydration or tolerant to dehydration. Plants deal with stress reaction varies depending dried plants and growth stage. Plants to try being in a stressful situation by way of intervention physiology and biochemistry. Water stress is the principle cause dryness resulting water deficit and inhibit plant growth by influencing physiology and biochemical processes [7].

Poly ethylene glycol (PEG) is a chemical used for the selection of plants against dry stress [8–10]. Tolerance of plants can be observed in the vegetative growth of the plant, namely the percentage of dry matter yield reduction, susceptibility index and a header-root ratio [Bayuelo-Jaminez in 11]. Rice crop stunted growth due to water deficits result in reduced photosynthesis, reduced long charging period which results in lower seed grain yield of rice [Yang in 12]. Selection mechanisms against stress tolerant crops dried studied by [13] on the plant *Impatiens capensis* Meerb., i.e. by observing the high water use efficiency, stomatal conductance and flowering time

Breeding drought resistant rice plants takes a long time. The use of PEG expected to be used for the initial selection of drought resistant rice on the seed level. The study was expected to provide information PEG concentration that can be used for basic breeding rice plants. Based on the above, in this research, giving the effect of PEG on germination of rice plants for early selection of rice.

2. Materials and methods
The research was conducted in Physiology and Plant Breeding Laboratory, Diponegoro University, Indonesia. The research used six cultivars of rice, cv. Mekongga (UJ1), cv. Way Apo Buru (UJ2), cv.Sidenuk (UJ3), cv. Pepe (UJ4), cv. Ciherang (UJ5) and cv.IR 64 (UJ6) from March to May 2016. The research was conducted covering two experimentals stage, were (i) Tests of dry stress resistance of six cultivars of rice using PEG 6000, and (ii) Emerged test seeds of six rice cultivars that have been subjected to dry stress with PEG 6000.

2.1. Stage I
Tests of dry stress resistance of six cultivars of rice using PEG 6000. The treatments were designed based on a complete randomized design of two factors. The first factor was the six cultivars of rice and the second factor was dry stress (P1 = 0 % PEG, P2 = 5 % PEG, P3 = 10 % PEG, P4 = 15 % PEG) with replicates three times. A total of 50 grains were added to the PEG solution on tissue paper in a petri disc. The calculation of germination begins after the appearance of 5 mm buds. Germination is observed for up to 10 d. The variable observed in equation (1) to equation (4):

$$\text{Germination rate (GR)} = \frac{\text{the number of germination seeds}}{\text{the number of seeds that germinated}} \times 100\,\% \tag{1}$$

$$\text{Emergence Rate} = \frac{\text{seedling count}}{\text{days to first count}} + \ldots\ldots\ldots + \frac{\text{seedling count}}{\text{days to final count}} \times 100\,\% \tag{2}$$

$$\text{Relative germination rate} = \frac{\text{number of germination seeds were subjected to stress}}{\text{the number of which is not exposed to stress}} \times 100\,\% \tag{3}$$

$$\text{Germination energy} = \frac{\text{the higher number of seeds germinated}}{\text{the number of seeds that germinated}} \times 100\,\% \tag{4}$$

2.2. Stage II
The emerging test of rice seed soil is carried out on a soil mixture (loam) and cow manure with a ratio of 1:1 (v/v). The experiment was arranged in a complete randomized design of two factors. The first factor was the six cultivars of rice and the second factor was dry stress (P1 = 0 % PEG, P2 = 5 %

PEG, P3 = 10 % PEG, P4 = 15 % PEG) with replication three time. A total of 50 grains of rice is a unit of observation. The ground emerging test is observed until the tenth day. The observed parameters are (i) Germinating day is the day at which sprouts begin to appear. (ii) Pre emergence (PE) is the number of seeds that appear to the ground until the tenth day. (iii) Emergence (Em) is the number of seeds that appear to the ground (grown) divided by the amount of planted multiplied by 100 %. (iv) The header is the length of the plant canopy (plant height). (v) Root is the root length of the plant.

Drought resistent index, measured according to Bayuelo-Jaminez [in 11].

$$DRI = (1 - drought\ stress)/no\ stress \tag{5}$$

Score of yield loss according to Soepandi [in 14], which 0 % to 4.99 % very tolerant (score 5), 5 % to 14.99 % tolerant (score 4), 15 % to 34.99 % moderat (score 2), 35 % to 49.99 % sensitive (score 1) and yield loss > 50 % was very sensitive (score 0).

3. Data analyses
All data collected then analyzed of variance and followed by DMRT (the Duncan's Multiple Range Test) [15] Furthermore yield loss measured for determination of rice selection.

4. Result and discussion
ANOVA showed the interaction between cultivars of rice with PEG concentration significantly affect GR, ER, GE, and RGR. The interaction between cultivars of rice with PEG concentration showed a significant effect on PE, Em, shoot length and root length. Table 1 showed that in all cultivars of rice, increasing the concentration of PEG resulted in lower GR, ER, GE, and RGR. Increased concentrations of PEG result in decreased of GR cv. Mekongga by 23 % to 66 %, ER fell by 14 % to 74 %, GE fell by 16 % to73 %, and RGR down 23 % to 66 %. On this kind of cv.Way Apo Buru, increasing PEG concentration result decreasing GR by 21 % to 49 %, ER < GE and RGR by 49 % to 72 %, 52 % to 75 %, 20 % to 49 %, respectively. On this kind of cv. Sidenuk increased concentration of PEG lowered GR by 16 % to 49 %, ER down 34 % to 64 %, GE fell by 30 % to 61 %, and RGR down 16 % to 49 % At cv. Pepe, increasing the concentration of PEG lowered GR 47 % to 67 %, ER down 47 % to 68 %, GE fell by 43 % to 68 %, and RGR down 27 % to 54 %. Concentrations of PEG increased result GR, ER, GE and RGR in cv. Ciherang decreased by 31 % to 56 %, 27 % to 71 %, 14 % to 68 % and 34 % to 54 %, respectively. Cv. IR64 has a value of GR, ER, GE, RGR down due to increasing concentrations of PEG respectively 26 % to 50 %, 33 % to 62 %, 30 % to 59 % and 26 % to 50 %.

Table 1. Test of the rice seedling treated with doses of PEG.

Treatment Rice cultivars	PEG (%)	GR	ER	GE	RGR
Mekongga	0	92.67[a]	20.03[ab]	268.6[a]	100. 0[a]
Mekongga	5	71.33[cd]	17.13[b]	226.0[bc]	77.0[bcde]
Mekongga	10	68.67[cd]	13.33[c]	182.6[de]	74.3[cde]
Mekongga	15	31.33[i]	5.2[g]	73.3[hi]	33.8[i]
Way Apo B.	0	73.33[c]	20.5[a]	180.67[de]	100.0[a]
Way Apo B.	5	58.0[eg]	10.4[d]	86.0[h]	79.5[bcd]
Way Apo B.	15	37.33[h]	5.7[fg]	46.0[i]	51.0[gh]
Sidenuk	0	82.67[b]	17.3[b]	234.0[abc]	100.0[a]
Sidenuk	5	69.33[cde]	11.4[cd]	164.0[ef]	83.8[bc]
Sidenuk	10	70.67[cd]	11.7[cd]	168.0[ef]	85.6[b]
Sidenuk	15	42.0[h]	6.1[fg]	89.3[h]	50.6[gh]
Pepe	0	91.33[ab]	19.4[ab]	262.0[ab]	100.0[a]
Pepe	5	66.67[cdef]	10.3[cd]	148.6[ef]	72.9[de]
Pepe	10	55.33[g]	8.8[def]	136.0[fg]	60.6[fg]
Pepe	15	42.0[h]	6.3[fg]	84.0[h]	46.0[h]

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Table 1 (Continue).

Treatment		GR	ER	GE	RGR
Rice cultivars	PEG (%)				
Ciherang	10	64.0[cdefg]	11.0[cd]	157.3[ef]	60.6[fg]
Ciherang	15	39.33[hi]	5.3[g]	78.6[hi]	46.0[h]
IR 64	0	88.67[ab]	17.2[b]	235.3[abc]	100.0[a]
IR 64	5	66.0[cdef]	11.5[cd]	164.0[ef]	74.2[cde]
IR 64	10	46.0[h]	7.1[efg]	104.0[gh]	51.9[gh]
IR 64	15	44.0[h]	6.5[efg]	96.0[h]	49.7[h]

Note: Figures followed by different letters indicate significantly different at the level of 5 % $P < 0.05$). GR: Germinatiom Rate; ER: Emergence Rate; GE: Germination Energy; RGR: Relative Germination Rate.

Plants tolerate to drought stress in a way that supports growth hormone activation and deactivate the hormone inhibitor. Mechanism carbohydrates play a role in the regulation of protein synthesis of sugar being the role of plant cell division. The salicylate acid controls the 35 types of proteins [16]. Salicylic acid plays a role in the physiological processes of plant development to stress. Exogen salicylic acid can increase growth by water deficit conditions [17]. PEG solution is used to control the potential of water in studying seed germination. PEG reduces the water potential of the osmotic solution because it reduces the availability of oxygen for the seed to germinate [Hardegree in 18].

Table 2. Application of six cultivar rice with PEG level for soil emergence tests.

Treatment		PE	Em	Shoot	Root
Rice cultivar	PEG (%)				
Mekongga	0	40.6[abcd]	87.8[abcde]	5.2[b]	3.0[a]
Mekongga	5	35.6[bc]	100[a]	2.3[g]	2.2[c]
Mekongga	10	34.3[cd]	100[a]	1.9[h]	1.6[g]
Mekongga	15	13.0[h]	82.7[cdef]	1.2[i]	1.5[gh]
Way Apo B.	0	35.0[c]	95.5[ab]	5.4[b]	3.0[a]
Way Apo B.	5	27.6[ef]	95.4[ab]	3.8[cd]	2.4[cd]
Way Apo B.	10	31.3[cde]	98.9[a]	1.9[g]	1.8[f]
Way Apo B.	15	16.6[gh]	89.2[abcde]	1.3[i]	1.6[g]
Sidenuk	0	36.0[bc]	92.7[abcd]	5.4[b]	3.0[a]
Sidenuk	5	31.3[cde]	95.0[abc]	3.8[cd]	2.4[cd]
Sidenuk	10	34.6[cd]	89.7[abcde]	1.9[g]	1.8[f]
Sidenuk	15	17.0[gh]	74.7[f]	1.3[i]	1.6[g]
Pepe	0	42.3[a]	92.7[abcd]	5.4[b]	3.0[a]
Pepe	5	31.6[cde]	95.0[abc]	3.6[d]	2.3[de]
Pepe	10	25.0[f]	89.7[abcde]	2.3[g]	1.5[gh]
Pepe	15	15.6[gh]	100.0[a]	1.2[i]	1.1[i]
Ciherang	0	44.0[a]	97.7[ab]	5.4[b]	3.0[a]
Ciherang	5	29.3[def]	97.9[ab]	3.8[cd]	2.7[b]
Ciherang	10	30.6[cde]	95.9[ab]	2.6[f]	1.8[f]
Ciherang	15	19.6[g]	100.0[a]	1.3[i]	1.0[i]
IR 64	0	40.3[ab]	91.0[abcde]	5.7[a]	3.0[a]
IR64	5	28.3[ef]	85.7[cdef]	4.0[e]	2.5[c]
IR 64	10	18.6[g]	81.2[def]	2.9[e]	2.5[c]
IR 64	15	17.3[gh]	81.2[def]	1.8[h]	1.4[h]

Figures followed by different letters indicate significantly different at the level of 5% ($P < 0.05$). PE: Pre Emergence; Em: Emergence

Table 2 show that cv. Mekongga seen that with increasing concentrations of PEG affected PE lowered by 12 % to 67 %, shoot length decreased 57 % to77 %, and root length decreased by 27 % to 50 %. The decline in PE, shoot length and root length also occurs in cv. Way Apo Buru due to the concentration of PEG increases, respectively by 21 % to 52 %, 30 % to 76 % and 20 % to 47 %. Cv. Sidenuk decreased PE, shoot length and root length by 13 % to 52 %, 30 % to 76 % and 20 % to 47 % due to the concentration of PEG increased. The concentration of PEG increased affected PE lowered by 25 % to 63 %, shoot length down 23 % to77 %, and root length decreased by 23 % to

60 % on cv. Pepe. PE on cv. Ciherang decreased by 33 % to 55 %, shoot length down 30 % to75 %, and root length decreased by 10 % to 66 % due to the concentration of PEG increased from 0 % to 15 %. PE on cv. IR64 decreased by 30 % to 57 %, shoot length down 30 % to 68 %, and root length decreased by 16 % to 53 % due to the concentration of PEG increased from 0 % to 15 %.

Interaction between rice cultivars and PEG significantly ($P < 0.05$) affect to PE. The PE of cv. Mekongga no different with cv. Ciherang, cv. Sidenuk and cv. Pepe. Seeds basically contain elements to germinate when environmental conditions favor. Test the soil sprouts Em appear relatively normal on the grown of cv.Mekongga, cv. Way Apo Buru, cv. Sidenuk, cv. Ciherang and cv. IR64 at the level to 15 % . Results sprouts declines occurred in cv. IR64 on all levels of PEG. Cv. Mekongga, cv. Way Apo Buru and cv. Ciherang no significant difference in test Em. The test Em of cv. IR64 generates the lowest value when compared to other cultivars. In general, PE, root length and header length decreases with increasing concentrations of PEG. Emergence turned out to have an irregular pattern. Emergence evaluability not always the same as when to germinate. The ratio canopy roots in rice plants with the provision of PEG decreased when compared with no PEG [19]. Cv. IR64 produce root length and highest canopy. The roots of the long drought tolerance can help to draw water from the deep soil, the results of research occur in wheat cultivars [20]. Polyethylene glycol (PEG) affects the physiological and chemical changes of the seeds because it causes the seeds lack of water for germination. Some methods for resistance to stress with chemicals can be done with PEG 6000 on germination media can cause stress-resistant plants. Plants exhibit rooting system in solution and do not show poisoning due to PEG 6000 [21]. Drought stress occurs in the canopy and roots of cv. Mekongga and cv. Ciherang. Experiment of [19] explained that dryness is a condition where normal physiological functions thereby decreasing turgor pressure adjustment. Drought stress inhibits root growth canopy due to water supply and turgor pressure which was not in accordance with the needs of growing crops.

Table 3. Drought resistent index and score of GR, ER, GE, RGR, PE, Em, shoot and root of rice seedling.

Cultivar	GR	ER	GE	RGR	PE	Em	Shoot	Root	Average DRI
Mekongga	38.37	40.64	40.20	38.3	31.94	1.93	65.38	41.11	
	(S-1)	(S-1)	(S-1)	(S-1)	M-2	VT-5	(VS-0)	(S-1)	1.5 (M)
Way Apo	27.88	58.37	61.62	27.6	28.10	1.05	56.79	35.56	
	M-2	(VS-0)	(VS-0)	M-2	M-2	VT-5	(VS-0)	(S-1)	1.5 (M)
	M-2	(S-1)	(S-1)	M-2	M-2	T-4	(VS-0)	(S-1)	1.63 (M)
Pepe	56.36	56.36	53.10	40.17	43.10	-2.37	56.17	44.44	
	(VS-0)	(VS-0)	(VS-0)	(S-1)	(S-1)	VT-5	(VS-0)	(S-1)	1.0 (S)
Ciherang	38.77	45.90	39.62	42.33	39.77	-0.14	52.47	38.89	
	(S-1)	(S-1)	(S-1)	(S-1)	S-1	VT-5	(VS-0)	(S-1)	1.38 (S)
IR64	41.36	51.36	48.43	41.4	46.90	9.12	49.12	28.89	
	(S-1)	(VS-0)	(S-1)	(S-1)	(S-1)	T-4	(S-1)	M-2	1.38 (S)

Description : Very tolerant (V), score 5; Tolerant (T), score 4; Moderat (M), score 2; Sensitive (S), score 1; Very sensitive (VS), score 0.

Cv. IR64 produce roots header high when compared to other cultivars. The data in table 3 known decreasing results of each variable GR, ER, GE, RGR, PE, Em, shoot and root endured by cultivars of rice. The highest score achieved by cv. Sidenuk (1.63) and the lowest was cv. Pepe (1.0). Cv. Sidenuk popularity score can be classified Moderate (has a moderate tolerance), while cv. Pepe was sensitive to drought stress.

5. Conclusion
Different drought response in all cultivars of rice. The higher the concentration of PEG lowered GR ER GE and RGR on all cultivars of rice. Germinate the longer time, appropriate concentrations of PEG. Test for pre-emergence on the soil appears giving a mixed response to all types of rice and PEG level. Cultivar Mekongga, cv. Sidenuk, cv. Pepe and cv. Ciherang have good ability pre-emerging test. Canopy and root length highest on cv. IR64. Cultivar Sidenuk popularity score can be classified Moderate (has a moderate tolerance), while cv. Pepe rice that was sensitive to drought stress.

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