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In Vitro Selection of Drought Stress Rice (*Oryza sativa L*) Varieties Using PEG (*Polyethylene Glycol*)

Nurhayati[a]*, Murni Sari Rahayu[b], Irham Syaukani[c], Sri Hafnida Ritonga[d]

[a,b]*Lecturer on Department of Agrotechnology, Faculty of Agriculture, Islamic University of North Sumatera, Medan, Indonesia*

[c]*Alumni on Department of Agrotechnology, Faculty of Agriculture, Islamic University of North Sumatera, Medan, Indonesia*

[d]*Alumni on Department of Agroecotechnology, Faculty of Agriculture, University Al Washliyah, Medan, Indonesia*

[a]*Email: nurhayatijb@yahoo.co.id*

[b]*Email: murnisarirahayu@ymail.com*

[c]*Email: syaukaniirham@gmail.com*

[d]*Email: hafnidasri@yahoo.co.id*

Abstract

The research was conducted as long as February to April 2016 at Laboratory of Tissue Culture, UPT of Parent Seed Centre (BBI), Johor Building, Agriculture Office, Provincial Government of North Sumatera. The purpose of the study is to obtain in vitro drought tolerant varieties. The research design is factorial completely randomized design (DCA) with two factors and three replications. The first factor is varieties (V), consisting of four varieties namely V_1 = Batutegi, V_2 = Jatiluhur, V_3 = Sigambiri Putih, and V_4 = Sigambiri Merah. The second factor is PEG concentration of 6000 (P), which consists of four levels namely P_0 = 0%, P_1 = 8%, P_2 = 16%, and P_3 = 24%. The observed variables are plantlets height (cm), number of leaves (sheet), root length (cm), number of roots (roots) and proline content (%).

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* Corresponding author.

Jatiluhur variety is drought-tolerant because show the best growth in PEG treatment at concentration 24%. Growth of plantlets height, number of leaves, and number of roots and proline content of Jatiluhur is the highest at concentration of PEG 24% so that this variety is more tolerant than other varieties by vitro test.

Keywords: Drought Stress, PEG, rice varieties, In Vitro.

1. Introduction

Rice (*Oryza sativa* L.) is one of the staples crops which has important role in Indonesia. Rice is source of carbohydrate that widely used as energy source for Indonesian peoples or ASEAN, therefore, the availability of rice becomes very important for the Indonesian nation, including systems to increase the production.

National rice production is still depending on low land rice production and the increased production is remains the major concern. Increased the low land rice production is constantly being hard challenge. Conversion of fertile paddy fields to non-agricultural use is difficult to be avoided and continuously increasing every year. While, Indonesian population growth rate is continues to increase at around 1.9% per year in.

The impact of widespread drought on rice cultivation is one of the main problems in developing the rice crop. Rice fields area in Indonesia in 2015 is around 14,18 million hectares, an increase around 13.79 million hectares than the previous year, but losses due to drought cannot be avoided due to uncertain climate. In the period from October 2014 until July 2015, the total area of rice fields affected by drought is 57,000 hectares, and at national level there are 198 thousand hectares of drought endemic every year. In 2015, there is 20,000 hectares experiencing harvest loss due to drought (*puso*) [1].

The development of upland rice on dry land can be one solution to increase rice production in Indonesia in order to establish food security. This is due to various considerations namely the reduction of lowland rice fields, high cost to open a new rice acreage and limited irrigation water.

There is large dry land in Indonesia, namely around 60.7 million hectares or 88.6% of the total area, while the wetland is only 7.8 million hectares or 11.4%. In 2012 Indonesia has around 148 million ha (78%) of dry land and 40.20 million ha (22%) of wetlands, of the 188.20 million hectares of total land area. The data show that comparison between rice fields and the mainland is enormous [2].

Rice varieties that can grow and produce well on marginal lands (sub optimum), such as upland rice, is indispensable. Planting area of upland rice in 2011 is approximately 1.8 million hectares. The average yield of upland rice is still low (2.3 ton ha^{-1}). Therefore, contribution of upland rice role to sustain national production is relatively low [3].

The main problem faced by upland rice in marginal lands is drought stress. The solutions is produces some upland rice varieties that tolerant to drought. Upland rice varieties that tolerant to drought is having deep root system, large rooting number, larger roots diameter, and roots that can penetrate to deeper layers [1].

Problems of drought stress can be solved in two ways, namely by changing the environment to minimize the stress and improve plant genotypes that resistant to drought stress [4]. Upland rice breeding is geared to get drought-tolerant genotype as shown by its ability to survive and produce at low water potential condition [5].

The use of in vitro selection method for crop breeding has been widely used to improve the tolerant properties against biotic and abiotic factors. In vitro selection is more efficient and the results can be justified because millions of cells can be selected by using only a few bottles of culture or petridish. Meanwhile, field selection have to use hundreds plants and tested in much larger area. In addition, in vitro selection is not unduly influenced by the environment and enabling the selection at the cellular level [6].

In vitro selection method is suitable to obtain drought tolerant because it can filter group of the population (group of cells) at uniform conditions in restricted environments. One of the methods for in vitro drought stress selection is the use of osmotic stress substance such as polyethylen glycol (PEG).

The purpose of this study is to obtain in vitro rice varieties that are tolerant to drought.

2. Material and Methods

2.1. Place and Time

The research was conducted at the Laboratory of Tissue Culture, UPT. Parent Seed Center (BBI), Johor Building, Agriculture Office, Provincial Government of North Sumatra, Jln. Jend. Besar A.H. Nasution Medan , with altitude of 20 ± 25 m above sea level. This reserch was conducted in April 2016.

2.2. Materials and Tools

The crop material used in this research are upland rice seeds, consisting of four varieties namely Batutegi, Jatiluhur, Sigambiri Merah, Sigambiri Putih, and PEG (Polyethylena Glykol) 600. The composition of MS (Murashige and Skoog) medium, gelatin as compactor media and sucrose (sugar) as source of carbohydrates, $HgCl_2$ solution 0.01%, KOH, HCl 0.1 N, alcohol70%, distilled sterile, tissue, spritus, and other necessary materials.

The tools is consisted of laminar air flow cabinet (LAFC), air conditioner (AC), autoclave, oven, analytical balance, pH meter, millimeter blocks paper, glass cup, measuring cups, petridish, culture bottles, erlenmeyer flask , stirrer, aluminum foil, refrigerator, gas stove, tweezers, pipette, scalpel blades, spatulas, lamps spritus, hand sprayer, panic funnel, rags, calculators, pencils, and other tools deemed necessary.

2.3. Research design

The research design is factorial completely randomized design (DCA) with two factors and three replications. The first factor is varieties (V), consisting of four varieties namely V_1 = Batutegi, V_2 = Jatiluhur, V_3 = Sigambiri

Putih, and V_4 = Sigambiri Merah. The second factor is PEG concentration of 6000 (P), which consists of four levels namely P_0 = 0%, P_1 = 8%, P_2 = 16%, and P_3 = 24%.

The observed variables are plantlets height (cm), number of leaves (sheet), root length (cm), number of roots (roots) and proline content (%).

3. Results

3.1. Planlet Height (cm)

Results of analysis of variance showed that varieties, PEG concentration and interaction of varieties and PEG have highly significant effect on the growth of rice plantlets height. The mean of rice plantlets height is presented in Table 1.

Table 1: Mean of Planlet Height of Some Rice Varieties at 5 weeks after planting (cm) in different PEG concentration

Variety (V)	*Polyethylene Glycol* (P)				Mean
	P_0	P_1	P_2	P_3	
Batutegi (V_1)	1.23 abcde	1.15 abcd	0.77 abc	0.75 a	0.97 a
Jatiluhur (V_2)	5.21 mno	5.04 mn	3.85 k	3.90 kl	4.50 d
Sigambiri Putih (V_3)	1.92 defghi	1.67 defg	1.52 abcdef	0.77 ab	1.47 ab
Sigambiri Merah (V_4)	5.39 nop	4.57 klm	2.11 fghij	1.77 defgh	3.46 c
Mean	3.43 cd	3.11 c	2.06 ab	1.80 a	3.43

Note :

Numbers followed by not the same letter at the same treatment group is significantly different, whereas numbers followed by the same letter in the same treatment group is not significantly different at DMRT 5%.

Table 1 and Figure 1 shows the highest plantlets was obtained for Jatiluhur (V_2) varieties, namely 4.50 cm, which is significantly different from Sigambiri Merah varieties (V_4), namely 3.46 cm, Sigambiri Putih (V_3), 1.47 cm and not significantly different with Batutegi (V_1), 0.97 cm.

The highest rice plantlets is obtained at PEG treatment P0 (control), namely 3.43 cm, that is significantly different from P1 (8%), 3.11 cm, P2 (16%), 2.06 cm, and not significantly different with P3 (24%), 1.80 cm (Table 1).

Figure 2 show that increasing concentrations of PEG from 0% to 24% will decrease the high growth of rice plantlets.

The interaction of varieties and PEG has significantly effect on plantlets height. All of the varieties used in this study have the same response to the increase in PEG concentration from 0% to 24%. The varieties experiencing growth inhibition with increasing concentrations of PEG, but the inhibition is not the same among varieties. Jatiluhur experienced inhibition but still the highest among other varieties at PEG concentration of 24%. Planlet height will be more depressed with increasing concentrations of PEG from 0% to 24%. The coefficient regression is r_{V1} = -0.94; r_{V2} = -0.91; r_{v3} = -0.94 and r_{v4} = -0.96 (Figure 3).

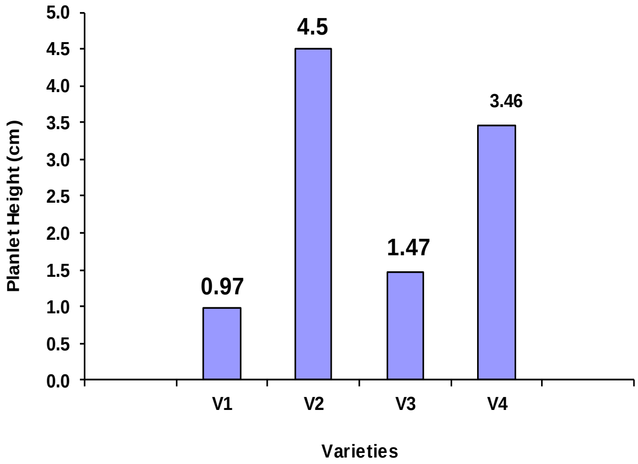

Figure 1: Planlet Height of Some Rice Varieties at 5 weeks after planting (cm)

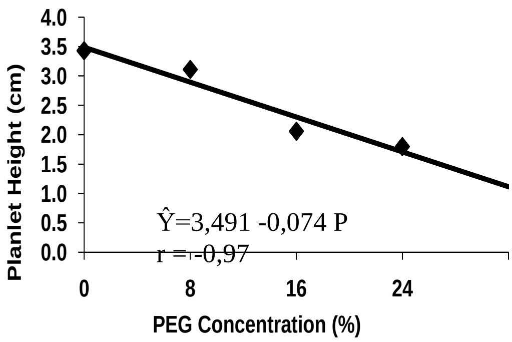

Figure 2: Relationship between rice planlet height at 5 weeks after planting (cm) and PEG concentration (%).

3.2. Number of leaves (sheet)

Results of analysis of variance showed that varieties, PEG concentration and interaction of varieties and PEG have highly significant effect on the number of rice leaves. The average of rice leaves is presented in Table 2.

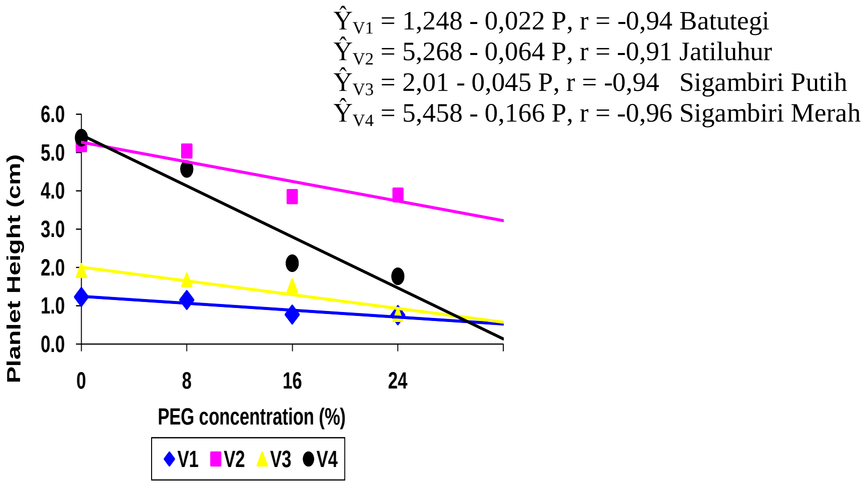

Figure 3: Interaction of some rice varieties and PEG on the Planlet Height at 5 weeks after planting

Table 1: Mean of Rice Leaves at 5 weeks after planting (cm) in varieties and PEG concentration treatment

Variety (V)	*Polyethylene Glycol* (P)				Mean
	P_0	P_1	P_2	P_3	
Batutegi (V_1)	0.80 abcd	0.84 abcde	0.71 abc	0.71 ab	0.77 a
Jatiluhur (V_2)	2.14 nop	2.00 n	1.50 k	1.52 l	1.79 d
Sigambiri Putih (V_3)	0.88 abcdefgh	0.88 abcdefg	0.88 abcdef	0.71 a	0.84 ab
Sigambiri Merah (V_4)	2.14 no	1.68 lm	1.19 ij	1.13 i	1.54 c
Mean	1.49 cd	1.35 c	1.07 ab	1.02 a	

Note : Numbers followed by not the same letter at the same treatment group is significantly different, whereas numbers followed by the same letter in the same treatment group is not significantly different at DMRT 5%..

Table 2 and Figure 4 shows the highest leaves was obtained for Jatiluhur (V_2) varieties, namely 1,79, which is significantly different from Sigambiri Merah varieties (V_4), namely 1,54, Sigambiri Putih (V_3), 0,84 and not significantly different with Batutegi (V_1), 0,77.

The highest number of leaves is obtained at PEG treatment 0% (P0), namely 1,49, that is not significantly different from P1 (8%), 1,35 and P3 (24%), 11,02 but significantly different with P_2 (16%), 1,07 (Table 2).

Figure 5 show that increasing concentrations of PEG from 0% to 24% will decrease the number of rice leaves.

The interaction of varieties and PEG has significantly effect on the number of plantlets leaves. All of the varieties used in this study have the same response to the increase in PEG concentration from 0% to 24%. The varieties experiencing growth inhibition of leaves number with increasing concentrations of PEG, but the inhibition is not the same among varieties. Jatiluhur experienced inhibition but still have the highest number of leaves among other varieties at PEG concentration of 24%. Number of Planlet leaves will be more depressed with increasing concentrations of PEG from 0% to 24%. The coefficient regression is r_{v1} = -0,79; r_{v2} = -0,93; r_{v3} = -0,77 and r_{v4} = -0,96 (Figure 6).

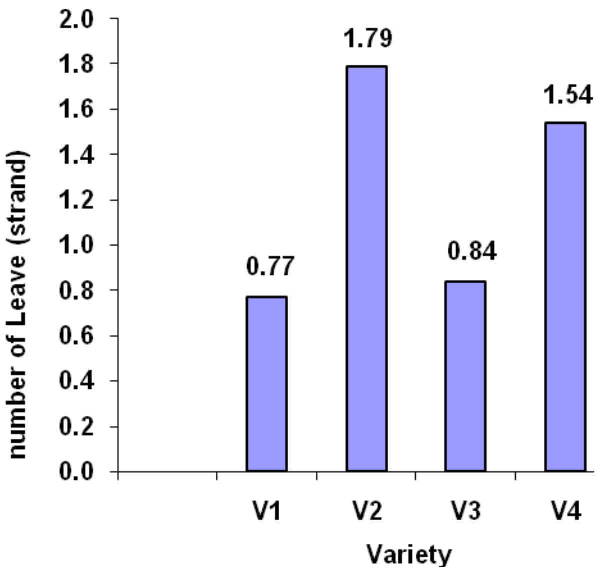

Figure 4: Number of leaves of Some Rice Varieties at 5 weeks after planting (strand)

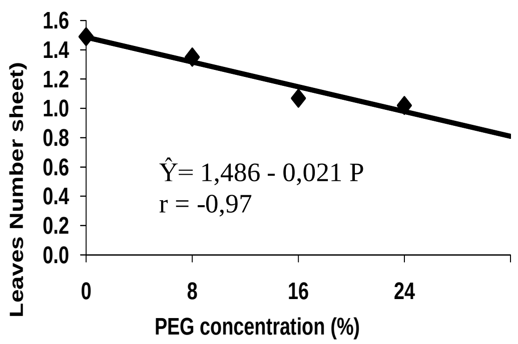

Figure 5: Relationship between rice leaves number at 5 weeks after planting and PEG concentration (%).

3.3. Root Length (cm)

Results of analysis of variance showed that varieties and PEG concentration have highly significant effect but the interaction have no significant effect on the root length of rice planlet. The mean of the root length is presented in Table 3.

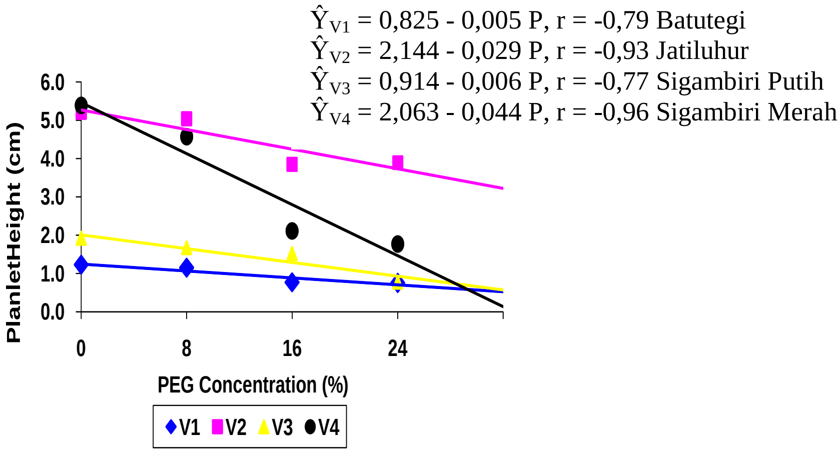

Figure 6: Interaction of varieties and PEG on the number of Rice Planlet leaves

Table 2: Mean of Planlet Root Length at 5 weeks after planting for varieties and PEG concentration treatment

Variety (V)	*Polyethylene Glycol* (P)				Mean
	P_0	P_1	P_2	P_3	
Batutegi (V_1)	1.53	1.18	0.88	1.33	1.23 ab
Jatiluhur (V_2)	2.32	2.52	2.12	2.35	2.33 c
Sigambiri Putih (V_3)	1.33	1.28	1.13	0.75	1.12 a
Sigambiri Merah (V_4)	3.29	3.16	1.61	1.69	2.44 cd
Mean	2.12 cd	2.04 c	1.43 a	1.53 ab	

Note : Numbers followed by not the same letter at the same treatment group is significantly different, whereas numbers followed by the same letter in the same treatment group is not significantly different at DMRT 5%. Table 3 and Figure 7 shows that varieties has highly significant effect on the root length. The longest root was found for Sigambiri Merah (V_4) namely 2,44 cm, but not significantly different with Jatiluhur (V_2), namely 2,33 cm, significantly different from Batutegi (V_1), 1,23 cm and not significantly different with Sigambiri Putih (V_3), 1.12 cm. PEG treatment has highly significant effect on the root length. The longest rice plantlets root is found at PEG 0% (P0), namely 2,12 cm, that is not significantly different from P1 (8%), 2,04 cm and P2 (16%), 1,43 cm and significantly different with P3 (24%), 1,53 cm (Table 3).

Figure 8 show that increasing concentrations of PEG from 0% to 24% will decrease the root length of rice plantlets. The interaction of varieties and PEG has no significant effect on root length. The longest root with PEG concentration treatment 24 % is found for Jatiluhur (V_2P_3) namely 2,35 cm, and the shortest is sigambiri putih (V_4P_3) 0,75 cm.

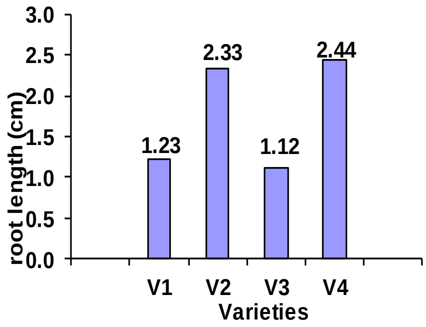

Figure 7: Length root of Planlet of Some Rice Varieties at 5 weeks after planting (cm)

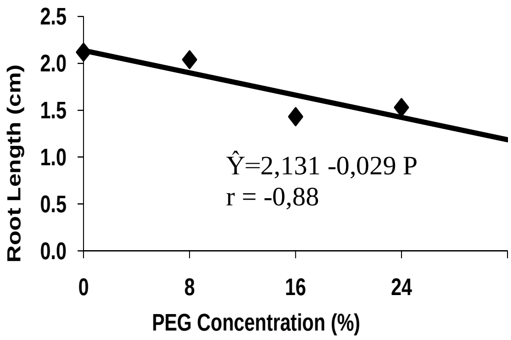

Figure 8: Relationship between rice planlet root length at 5 weeks after planting (cm) and PEG concentration (%)

3.4. Root Number (root)

Results of analysis of variance showed that varieties, PEG concentration and the interaction have highly significant effect on the root number of rice planlet. The mean of the root number is presented in Table 4.

Note : Numbers followed by not the same letter at the same treatment group is significantly different, whereas numbers followed by the same letter in the same treatment group is not significantly different at DMRT 5%.

Table 4 and Figure 9 shows that highest root number is found at Jatiluhur (V_2) namely 3,09 which is significant different to Sigambiri Merah (V_4) namely Jatiluhur (V_2) 2,36 and Sigambiri Putih (V_3), 1,01 but not significantly different to Batutegi (V_1), 0,99.

The highest number of root is found at PEG treatment 0% (P0), namely 2,53, that is not significantly different from P_1 (8%), 2,16 and P3 (24%), 1,37, but significantly different with P_2 (16%), 1,39 (Table 4).

Figure 10 show that increasing concentrations of PEG from 0% to 24% will decrease the number of rice planlet root. The interaction of varieties and PEG has significant effect on the number of plantlets root. All of the varieties used in this study have the same response to the increase in PEG concentration from 0% to 24%.

Table 4: Mean of Planlet Root Number at 5 weeks after planting for varieties and PEG concentration treatment

Variety (V)	*Polyethylene Glycol* (P)				Rataan
	P_0	P_1	P_2	P_3	
Batutegi (V_1)	1.23 abcdefgh	1.08 abcde	0.80 ab	0.86 abc	0.99 a
Jatiluhur (V_2)	4.01 op	3.55 n	2.29 k	2.50 kl	3.09 d
Sigambiri Putih (V_3)	1.20 abcdefg	1.10 abcdef	1.02 abcd	0.71 a	1.01 ab
Sigambiri merah (V_4)	3.67 no	2.92 lm	1.43 defghij	1.40 defghi	2.36 c
Rataan	2.53 cd	2.16 c	1.39 ab	1.37 a	

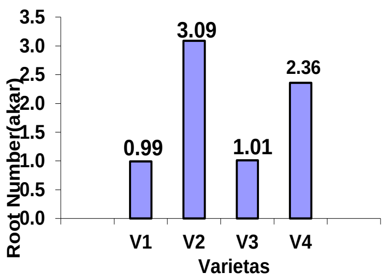

Figure 9: Root number of some rice varieties (root)

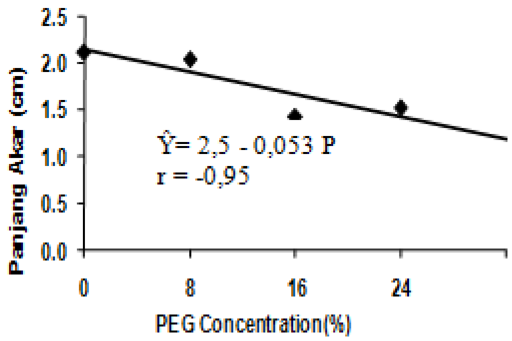

Figure 10: Relationship between root number of rice planlet at 5 weeks after planting on PEG concentration (%)

The varieties experiencing inhibition of root number with increasing concentrations of PEG, but the inhibition is not the same among varieties. Jatiluhur experienced inhibition but still have the highest number of root among other varieties at PEG concentration of 24%. Number of Planlet root will be more depressed with increasing concentrations of PEG from 0% to 24%. The coefficient regression is r_{v1} = -0,90; r_{v2} = -0,91; r_{v3} = -0,94 and r_{v4} = -0,95 (Figure 11).

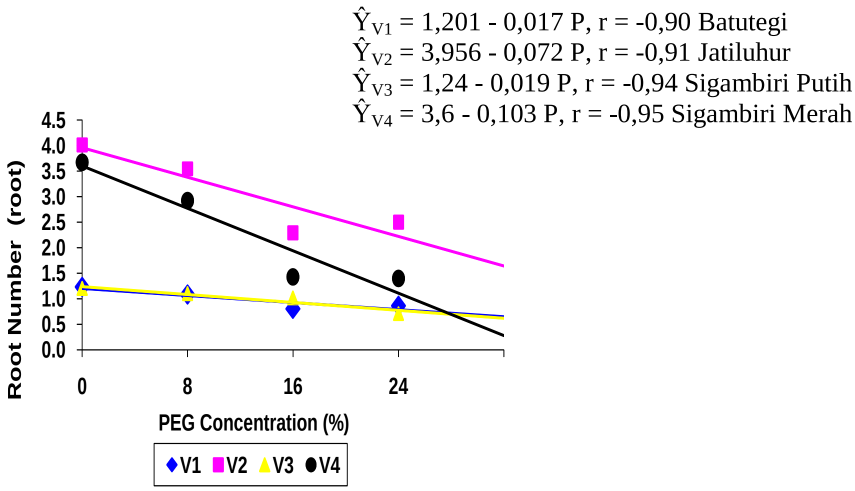

Figure 11: Interaction of some rice varieties and PEG on the number of Rice Planlet root

3.5. Proline Content (%)

Results of analysis of variance showed that varieties, PEG concentration and the interaction have highly significant effect on the proline content of rice planlet. The mean of the proline content is presented in Table 5.

Table 5: Mean of Proline Content at 5 weeks after planting for varieties and PEG concentration treatment

Variety (V)	*Polyethylene Glycol* (P)				Rataan
	P_0	P_1	P_2	P_3	
Batutegi (V_1)	18,40 m	75,08 i	175,59 g	216,49 c	121,39 c
Jatiluhur (V_2)	45,45 k	87,65 h	189,29 e	287,22 a	152,40 a
Sigambiri Putih (V_3)	18,47 m	65,89 j	179,59 f	213,01 c	119,24 d
Sigambiri Merah (V_4)	40,48 l	85,73 h	193,02 d	242,67 b	140,48 b
Rataan	30,70 d	78,59 c	184,37 b	239,85 a	

Note : Numbers followed by not the same letter at the same treatment group is significantly different, whereas numbers followed by the same letter in the same treatment group is not significantly different at DMRT 5%.

Table 5 and Figure 12 shows that the highest proline content found at Jatiluhur (V_2) namely 152,40 %, which significantly different with Sigambiri Merah (V_4) 140,48 %, Batutegi (V_1) 121,39 % and Sigambiri Putih (V_3) 119,24 %.

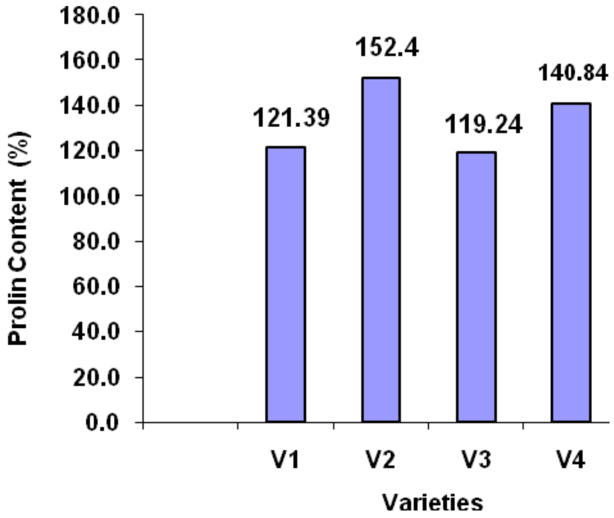

Figure 12: Proline Content of Some Rice Varieties at 5 weeks after planting

PEG treatment has significant effect on the proline content of rice planlet. The highest proline content is found at P_3 (24%) namely 239,85% which significantly different from P_2 (16%) 184,37%, P_1 (8%) 78,59% and P_0 (control) 30,70% (Table 5).

$\hat{Y}$= 23,39 + 9,165 P
r = 0,99

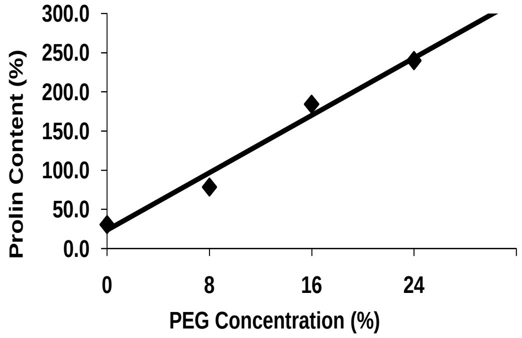

Figure 13: Relationship between proline content of rice planlet with PEG concentration (%)

Figure 13 show that increasing concentrations of PEG from 0% to 24% will increase the proline content of rice plantlets. The interaction of varieties and PEG has significantly effect on the proline content of rice plantlets. All of the varieties used in this study have the same response to the increase in PEG concentration from 0% to 24%. The varieties experiencing proline content increase with increasing concentrations of PEG, but the increase is not the same among varieties.

Jatiluhur experienced the highest proline content increase among other varieties at PEG concentration of 24%. Proline content will increase with increasing concentrations of PEG from 0% to 24%. The coefficient regression is r_{v1} = 0,98; r_{v2} = 0,98; r_{v3} = 0,98 and r_{v4} = 0,98 (Figure 14).

$\hat{Y}_{V1}$= 17,17 + 8,68 P, r = 0,98 Batutegi
$\hat{Y}_{V2}$= 28,36 + 10,33 P, r = 0,98 Jatiluhur
$\hat{Y}_{V3}$= 14,64 + 8,72 P, r = 0,98 Sigambiri Putih
$\hat{Y}_{V4}$= 33,39 + 8,92 P, r = 0,98 Sigambiri Merah

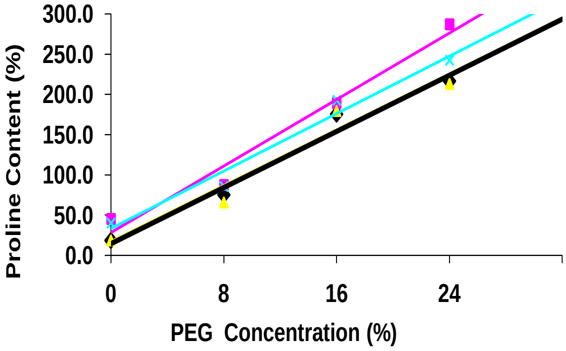

Figure 14: Interaction of some rice varieties with PEG on the Proline Content

4. Discussion

Rice varieties tested in the study are Batutegi (V1), Jatiluhur (V2), Sigambiri Putih (V3), and Sigambiri Merah (V4). Such rice varieties show very significant difference in growth in vitro. The best growth variety in this study is Jatiluhur (V2), that is show the highest plant height namely 4,50 cm, the largest number of leaves of 1,79, the highest number of roots, 3,09 strands and contains the highest proline content 152, 40%, while the longest roots found at Sigambiri merah, namely is 2,44 cm. The worst growing varieties is Batutegi. Rice varieties grown in vitro are also generating different proline contents. This is because proline content increases in the leaves that experienced stress due to the role related to resist to drought stress. The role of proline is not limited to osmotic adjustment associated with the status of water, but also neutralizing the toxic effects of NH_3 from proteins hydrolysis, as energy source and N source for the restoration of crops physiological processes after experiencing stress [6]. Application of PEG 6000 had negative impact on all observed parameters, such as plantlets height, leaf number, root length, and number of roots because the higher concentration of PEG the decline in the growth of the rice plantlets. In contrast, proline contents increased in rice plantlets with the increasing of PEG concentration. This research is using PEG 6000 treatment with concentration of 0% (P0), 8% (P1), 16% (P2), and 24% (P3). Treatment that produces the highest plantlets is the control PEG 0% (P0) namely 3.43 cm, the highest number of leaves is 1.49 strands, the longest root length is 2.12 cm, and the highest number of roots is 2.53. This happens because there is no treatment that suppresses the availability of water in the media so that the nutrients for water requirements are available for crops growth. In contrast, the lowest growth in the parameters found in treatment concentration 24% PEG (P3) with the planlet height 1.80 cm, number of leaves 1.02 strands, number of roots 1,37 and the shortest roots found at concentration of PEG 18% (P2) namely 1.43 cm. This is because the higher the concentration of PEG, the lower water availability in the media. This finding are consistent with Candra (2011) which states that the effects of polyethylene glycols 6000 application by soaking the seeds in a solution of Polyethylene Glycol 6000 with concentration of 10% w/v and 20% w/v can cause negative osmotic pressure inside the cell so that the water poorly absorbed by the seed because the water is absorbed by the seeds in small amounts. High concentration of Polyethylene Glycol 6000 leads to metabolic processes in crops is not going well, especially the nutrients transfer to the embryo is hampered due to the limited water [7].

The growth of rice plantlets on MS medium with PEG is causing a drought stress conditions. Drought stress can negatively affect various stages of plant growth and the influence can be seen morphologically, anatomically, and physiologically [8]. According to Kremer (1963), drought stress will affect all metabolic processes in plants and resulting in decreased of plant growth. Cell growth is a sensitive phase to water shortages [9]. Effendi (2008) found that increased drought stress intensity will decrease the plant height, number of tillers, and root dry weight [10]. This research showed that application of PEG increase the proline content of rice plantlets. Increasing of proline can increase crops tolerant to drought because it functions as amino acid in rice plants has changed the genetic composition which make drought tolerant. Kadir (2007) found that PEG treatment on patchouli can improve drought tolerance and also increasing the content of patchouli oil. It is also offset by increased proline activation [11].

This research showed that at the controls condition, Sigambiri Merah and Jatiluhur show the best growth, but with PEG application, the best growth is Jatiluhur varieties. With the increase in the concentration of PEG, Jatiluhur has better growth and higher proline content than Sigambiri Merah, Sigambiri Putih, and Batutegi. In other words, Jatiluhur varieties may be the most tolerant to drought. Jatiluhur varieties is an upland rice that can be grown at height of 500 m above sea level and it is recommended to dry land. In other hand, Jatiluhur is also shade-tolerant, resistant to blast disease (*Pyricularia oryzae*) and moderately resistant to hair loss. The use of PEG in vitro can be used to select drought-tolerant upland rice. Endang, and his colleagues in 2004 found that the use of PEG 6000 at a concentration of 20% can be used for selection of callus from three varieties, namely Gajah mungkur, Towuli and IR64. Mutation induction and in vitro culture could produce somaclonal variation to obtain superior character for drought tolerant and better vigor [12].

Research finding of Ballo and his colleagues in 2012 showed that the addition of PEG at concentration of 18.1% will decrease the growth rate as one indicator of the seed vigor [13]. Cahyadi and his colleagues in 2013 showed that the use of Polyethylene Glycol solution 6000 has very significant effect on early physiological characteristics of mangkawa local upland rice, in terms of germination drought tolerant [14]. Jatiluhur varieties show better roots than other varieties in this study. Each species or varieties have different roots development. In addition to the root deployment, root permeability has significant role in determining the amount of water absorbed. Varieties that fall in drought-tolerant group is characterized by roots deep, solid and strong to ensure the smooth delivery of water and nutrients from the soil to the plant [15]. Roots which is able to penetrate the base layer of pot coating wax, having length exceeding 10 cm and higher roots number is expected drought tolerant [16]. In association with roots, plants genotypes that are drought tolerant have the following properties: (1) able to develop the root system when the water is still available before the plant is experiencing drought stress so the plants can extract water from the deeper soil layer, and (2) modifying the root system to extract the water from the deepest layers. This condition occurs when there is water stress. PEG is able to control seed imbibitions and hydration. PEG is also used to test seeds to drought resistance by taking into account the drought index [17].

The use of PEG solution (BM6000 or 8000) to test the germination of rice with -0.2 and 1.2 MPa have been done at IRRI [18] for rice crops, and soybeans by Bouslama and Schapaugh (1984) with osmotic stress -6 bar (-0.6 MPa) [19]. Chutia and Borah in 2012 was using PEG 8000 (32.5%, equivalent to -13.7 bar / -1.37 MPa) to test 95 local rice varieties tolerant to drought [20]. Similarly, Lestari and Mariska (2006) was using PEG 20% for screening various rice somaclone of in vitro selection and found several drought tolerant somaclone [21].

Jatiluhur varieties have the highest proline content and the content increase by increasing the concentration of PEG. Results of in vitro selection by using PEG and root penetration ability test and allegedly tolerant to drought has produces higher proline than the control, although not under stress. Thus, the radiation treatment and in vitro selection can lead to changes in certain genes to produce drought tolerant crops and high production. In addition, the proline content can be used as physiological marker in rice crops for drought tolerant [22].

5. Conclusions

1. Jatiluhur is drought-tolerant varieties due to the best growth in concentration PEG 24%. Growth of plantlets height, number of leaves, number of roots and proline content of Jatiluhur is the highest at concentration of PEG 24% so that this variety is more tolerant than other varieties.
2. This study showed that application of PEG has negative effect on in vitro rice growth due to negative osmotic pressure inside the cell that make water is poorly absorbed by the seed and resulting drought stress condition.

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