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No. 001/ICEAS-01/FT-UNIPMA/2019

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Drought resistance selection in ponorogo local rice (oryza sativa L.) Varieties

in the International Conference on Engineering and Applied Science
"Development of Engineering and Science Toward Revolution 4.0"

Madiun, Indonesia. August 21st 2019



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To cite this article: K Jadid *et al* 2019 *J. Phys.: Conf. Ser.* **1381** 012005

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Drought resistance selection in ponorogo local rice (*oryza sativa l.*) Varieties

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Abstract. Drought is one of the main problems for the development of rice plants. The aims of this study were selecting several Ponorogo local rice cultivars that have resistance to drought stress based on germination ability in various PEG 6000 (Polyethylene Glycol 6000) concentration treatment, root penetration ability to wax layer (consisted of 60% wax and 40% vaseline) and chlorophyll content during drought stress. The germination test with PEG 6000 used a completely randomized design (CRD) factorial pattern consisting of 2 (two) treatment factors, namely cultivar and PEG concentration with 3 replications. The first treatment factor was a variety of Ponorogo local rice cultivars consisting of 5 cultivars, namely: Legowo, Mlarak 1, Malihan Ketan, Philips and Edok. The second treatment factor was PEG concentration, consisting of 4 (four) levels, namely: 0 (Control), 15, 20 and 25%. Rice seeds that passed the selection using PEG 6000 in the germination phase, were used in the root penetration ability test. Data were analyzed using variance analysis with 95% confidence level and continued with Duncan multiple distance test at 5% level. The results of the study revealed that Legowo cultivars showed the best germination in 25% PEG treatment with 15% germination percentage, 0,5 cm seminal root length, 0,1cm shoot length, seminal root length ratio per shoot length 4,2 and vigor index 0,1. At 20% PEG treatment, Edok cultivars gave the best results with 85% germination percentage, 1,7 cm seminal root length, 0,3 cm shoot length, seminal root length ratio per-shoot length 5,4 and vigor index 1,7. The highest number of roots that can penetrate the wax layer was produced by Edok cultivars averaging 19,3 strands with an average length of 25,5 cm.

1. Introduction

In order to survive in drought condition, plant have a series of mechanisms to deal with drought in terms of morphology, physiology, biochemistry, cellular and molecular[6][19]. These mechanisms include drought escape or escaping, drought avoidance, drought tolerance, and drought recovery mechanisms[5]. The mechanisms of plant resistance to drought stress appear in root and leaf characters, the ability to adjust osmotic pressure, water potential, ABA[12] content, and cell membrane stability. These characters can be used as indicators to evaluate the drought resistance in plants[19].



The development of drought resistant rice variety is one option to overcome the problem to defeat unpredicted dry season[24]. Local rice varieties are potential genetic resources to obtain the types of drought resistance rice varieties. Ponorogo is one of rice crop production center in Indonesia. Ponorogo has several local rice varieties that has been cultivated for generations, but has not been studied extensively to determine its resistance to drought[21]. Research to get drought tolerant rice types has been carried out with various experimental techniques including in vitro selection with Polyethylene glycol (PEG)[14][20], root permeability testing[22][15], and analysis of chlorophyll content[23].

Polyethylene glycol (PEG) is often used as a selection agent to identify drought-tolerant genotypes in the germination phase because of its ability to reduce the water potential in the germination media[14][20] as a simulation of hardness and thickness of the soil layer which is equivalent to 12 bar[22][16]. Root penetrability is a response to plant resistance to morphological drought.

The aim of the research was to select local Ponorogo rice cultivars through PEG treatment.

2. Material and Methods

The research was conducted at Genetic and Biotechnology Laboratory, University of Darussalam Gontor, from July until October 2018.

2.1. Material

The tools used in the study include: petri dishes, hot plates and magnetic stirers, glassware, pellets, pipettes, rulers and meters, scissors, analytical scales, pots (from 7.5cm diameter PVC pipes), electric grinders, mesh nets. The research materials used are: PEG6000, paper paper, distilled water, rice seeds, paraffin, vaseline, black tape / aquatic tape. Local rice seeds used are seeds taken directly on land through selection based on morphological differences consisting of five accessions, namely: Legowo, Mlarak 1, Malihan Ketan, Philips and Edok.

2.2 Research experimental design

The study was an experimental study using a completely randomized design (CRD) factorial pattern consisting of 2 (two) treatment factors, namely cultivar and PEG concentrations with 3 replications. The first treatment factor was the type of local Ponorogo rice cultivar consisting of 5 cultivars, namely: Legowo, Mlarak 1, Malihan Ketan, Philips and Edok. The second treatment factor is the PEG concentration, consisting of 4 levels, namely: 0% (Control), 15%, 20%, and 25%.

2.3 Methodology

2.3.1 PEG treatment. PEG treatment was done based on the described method by Lestari and Mariska (2006). The PEG concentrations were 0% (Controls), 15%, 20% and 25%. Each petridish in each treatment was filled with 20 seeds and as a control was used sterile water. The germinated seeds were planted on individual pots and root penetration test was conducted.

2.3.2 Root penetration test. The shoot that grew from PEG test were planted on individual pots which given paraffin and candle mixture (60% : 40%) as the base of the pots.

2.3.3 Data analysis. The data were analyzed using analysis of variance (ANOVA) with a confidence level of 95% and followed by a double distance test with a level of 5%. To help with data analysis, SPSS version 24 and Microsoft Excel software were used.

3. Result

3.1 Germination rate

Table 1 showed that the percentage of germination was only significantly different in the 20% PEG treatment, whereas in the control treatment, 15%, and 25% were not significantly different between cultivars. The highest percentage of germination in the control treatment was produced by cultivars Legowo (98.3%) and Mlarak 1 (98.3%). While the 15% PEG treatment, the highest percentage of germination was shown by Legowo cultivar (100%) and was not significantly different from other cultivars. In the PEG treatment, the 20% concentration level of cultivars that gave the highest percentage

of germinated seeds, Edok cultivar (85%), was not significantly different from Legowo (80%) and significantly different from Mlarak (56.7%), Malihan Ketan (35%) and Philips (75%). Whereas in the 25% PEG treatment, the highest percentage of germination was produced by Legowo cultivar (15%) and was not significantly different from other cultivars.

Table 1. Germination rate of five Ponorogo local rice varieties in PEG treatment

Variety	PEG TREATMENT			
	0 %	15 %	20 %	25 %
Legowo	98,3±2,9 ab	100,0±0,0 a	80,0±5,0 bc	15,0±5,0 f
Mlarak 1	98,3±2,9 ab	96,7±2,9 ab	56,7±22,5 d	3,3±2,9 f
Malihan Ketan	96,7±2,9 ab	91,7±2,9 abc	35,0±20,0 e	1,7±2,9 f
Philips	95,0±5,0 ab	95,0±5,0 ab	75,0±21,8 c	8,3±7,6 f
Edok	96,7±5,8 ab	98,3±2,9 ab	85,0±0,0 abc	11,7±12,6 f

3.2 Ratio of seminal root length and shoot length

The results of the ratio of seminal root length per shoot length in table 2 shows that the Legowo cultivar gives the largest ratio of root length per shoot length in control treatments, 15 and 25%, although it is not significantly different from other cultivars in the same treatment. While the highest ratio of PEG 15% was produced by Mlarak 1 cultivar and not significantly different from other cultivars. In the 20% PEG treatment, Edok cultivar gave the biggest ratio (5.4), not significantly different from Legowo (4.5), Mlarak1 (3.8) and Malihan Ketan (3.6) but significantly different compared to Philips cultivar. (3.0). Whereas the highest ratio of PEG 25% was produced by cultivars Legowo (4.2), not significantly different from Mlarak 1 (2.0) and Edok (2.1) and significantly different from cultivars Malihan Ketan (1.0) and Philips (0.8).

Table 2. Ratio of seminal root length and shoot length of five Ponorogo local rice cultivars in PEG treatment

Cultivar	PEG TREATMENT			
	0 %	15 %	20 %	25 %
Legowo	2,1±0,1 cdef	3,1±0,2 a-f	4,5±0,3 ab	4,2±2,5 abc
Mlarak 1	1,1±0,2 ef	3,2±0,1 a-f	3,8±0,8 abcd	2,0±2,0 cdef
Malihan Ketan	1,4±0,0 ef	2,4±0,3 b-f	3,6±0,5 a-e	1,0±1,7 f
Philips	1,1±0,1 ef	3,0±0,0 a-f	3,0±0,7 b-f	0,8±1,4 f
Edok	1,5±0,1 def	3,1±0,5 a-f	5,4±0,7 a	2,1±3,6 cdef

3.3 Seed index vigor

In the control treatment the best seed vigor index was owned by Legowo cultivars, Mlarak 1 and Edok, significantly different from Malihan Ketan and Philips cultivars, as shown at Table 3. At the 15% PEG concentration, the best seed vigor index was owned by the Legowo cultivar, not significantly different from the Philips and Edok cultivars, and significantly different from the Mlarak 1 and Malihan Ketan cultivars. While in the 20% PEG treatment, the best seed vigor index was owned by Edok cultivars and significantly different from Malihan Ketan. Whereas in the 25% PEG treatment, the seed vigor index

produced was not significantly different between cultivars. Based on the seed vigor index, it is known that the 20% PEG concentration is the best concentration for early screening of drought resistance in the germination phase.

Table 3. Seed index vigor of five Ponorogo local cultivar in PEG treatment

Cultivar	PEG TREATMENT			
	Kontrol	15	20	25
Legowo	6,9±0,7 a	4,2±0,4 c	1,6±0,1 e	0,1±0,0 f
Mlarak 1	6,9±0,9 a	2,9±0,6 d	0,8±0,6 ef	0,0±0,0 f
Malihan Ketan	5,8±0,4 b	1,8±0,6 e	0,2±0,2 f	0,0±0,0 f
Philips	5,2±0,5 b	3,5±0,5 cd	1,2±0,8 e	0,1±0,1 f
Edok	6,8±1,2 a	3,6±0,6 cd	1,7±0,3 e	0,1±0,1 f

3.4 Root penetration test through waxy layer

Observation of root permeability in the Legowo cultivar, showed that of the 20 plants tested that succeeded in penetrating the waxy layer as many as 19 plants. One plant that cannot penetrate the waxy layer is a plant whose seeds are germinated using 25% PEG. The highest average number of roots that can penetrate the waxy layer in the Legowo cultivar is found in plants from which the seeds are germinated with a 25% PEG. While the highest average root length that penetrates the waxy layer is a plant whose seeds are germinated using 20% PEG (Table 4).

In Mlarak 1 cultivar, 17 plants tested were all able to penetrate the waxy layer. The highest average number of roots that penetrate the waxy layer is found in plants germinated with 25% PEG. While the highest average root length that penetrates the waxy layer is a plant whose seeds are germinated using 20% PEG (Table 4).

Based on the data in table 4 Malihan Ketan cultivar shows that of the 16 plants tested were able to penetrate the waxy layer as many as 15 plants. One plant that cannot penetrate the waxy layer is a plant whose seeds are germinated at a 20% PEG solution concentration. In Malihan Ketan cultivar, plants whose seeds were germinated with 25% PEG gave the highest average number of roots while the highest average root length came from plants whose seeds were germinated with 20% PEG.

To know the ability of Philips cultivar roots to penetrate the waxy layer, Table 4 showed that from 20 plants tested, 19 plants can penetrate the waxy layer. While only 1 plant did not see its root penetrating the waxy layer, the plant whose seeds were germinated in 25% PEG solution. The highest average number of roots was shown in plants where the seed origin was germinated with 25% PEG and the highest average root length was produced by plants where the seeds were germinated using PEG20%.

While the data of Edok cultivar rice roots showed that of 20 plants tested that could penetrate the waxy layer of 18 plants, while the roots did not penetrate the waxy layer of 2 plants (each seed was added to PEG 20 and 25%). The highest average number of roots was indicated by plants germinated in PEG25% solution. While the highest average root length is produced by plants where the seeds are germinated using PEG15%. Table 4 shows that Edok cultivars have the best root permeability to the waxy coating. Edok cultivars produced the most number of roots penetrating the waxy layer, 23.6 cm (from the seed of the control treatment), 16.6 cm (PEG 15%), 14.8 cm (PEG20%), and 22.3 cm (PEG25%) with root length 26.4cm (Control), 29.5cm (PEG15%), 23.6cm (PEG20%) and 22.6cm (PEG 25%).

Table 4. The average of root number and root length that penetrated the waxy layer

Cultivar	Treatment	Tested plants	Penetrate root plants	The number of root average	The length of root average
Legowo	Kontrol	5	5	14,2	22,7
	PEG 15%	5	5	11,2	26,1
	PEG 20%	5	5	11,8	27,4
	PEG 25%	5	4	13,8	20
Mlarak 1	Kontrol	5	5	14	25,8
	PEG 15%	5	5	8,8	19,6
	PEG 20%	5	5	8,8	20,7
	PEG 25%	2	2	17,5	20,1
Malihan Ketan	Kontrol	5	5	15,8	26,6
	PEG 15%	5	5	8,2	28,5
	PEG 20%	5	4	10,3	35,4
	PEG 25%	1	1	16	31,9
Philips	Kontrol	5	5	12	24
	PEG 15%	5	5	11	22,9
	PEG 20%	5	5	6,6	25,6
	PEG 25%	5	4	17,3	22,3
Edok	Kontrol	5	5	23,6	26,4
	PEG 15%	5	5	16,6	29,5
	PEG 20%	5	4	14,8	23,6
	PEG 25%	5	4	22,3	22,6

4. Discussion

4.1 The effect of PEG treatment to seed germination

The results of the study noted that an increase in PEG concentration resulted in a decrease in the percentage of germination. The percentage of germination in PEG 0 MPa is greater than -0.25 and -0.5 MPa, even in the treatment of PEG-0.75 and -1 MPa, no sprouts were seen[2][2]. Moreover, the local Tanange upland rice germinated with PEG 6000 solution at 0 bar pressure produced the best percentage of normal germination compared to pressures of -3 and -4bar but did not differ from the treatment of giving solution at pressures -1 and -2 bar[4].

The percentage of germination decreasing occurs due to disruption of the process of imbibition and tissue hydration on rice seeds. Low water potential due to increase osmotic media levels results in plant cells not being able to take water from the medium or the amount of water absorbed by seeds decreases[2]. In addition, the germinability and viability of different seeds also affects the decrease in the percentage of germination[7].

Drought stress conditions due to PEG treatment during germination in this study also showed inhibition of seminal root growth and shoots. The Edok cultivar produced the longest seminal root at PEG20% treatment of 1.7 cm, followed by the Legowo cultivar of 1.6 cm. Whereas in the 25% PEG treatment, the longest seminal root produced by the Legowo cultivar was 0.5 cm and was followed by the Edok cultivar 0.4 cm. While the longest shoots in PEG 20 and 25% treatments were shown by Legowo cultivars, which were 0.4 cm and 0.1 cm, not significantly different from other cultivars. The increasing concentration of PEG to the media make the ratio of root length to shoot length increases. An increase in the ratio of root length to shoot length indicates tolerance to rice dryness with a decrease in shoot length. This happens because the growth of leaves is more reduced than the growth of roots[3], translocation of photosynthesis results into the root system and the presence of hormonal signals induced in roots in response to water shortages[4].

From the description above, it was concluded that the treatment of PEG 6000 in the germination phase of several local Ponorogo rice cultivars after observing on the 7th day showed a different resistance response between cultivars towards drought conditions. Based on all the observed germination parameters, it was found that the Legowo and Edok cultivars could germinate better, mean that they could be said to be more resistant to drought compared to cultivars Mlarak 1 and Philip which were somewhat resistant and Malihan Ketan which was more sensitive to drought. Several factors that can determine the ability of germination seeds, including the differences in seed size, thickness of seed coat and seed vigor[9].

Plants that have drought resistance are able to mobilize food reserves in seeds for root and plumular growth, even in conditions of water deficit. Tolerant plants respond to water deficits by optimizing physiological processes in critical phases so that plants can grow well [1]. When water stresses occur when the seeds germinate, the metabolism of the seeds is disrupted due to insufficient water needed. Therefore, only seeds that are resistant to drought are able to germinate[11][11].

4.2 The ability of Ponorogo local rice cultivars in root penetration through waxy layer

Water absorption and transport by the roots of rice plants is very important during drought conditions[10]. The ability to penetrate roots in hard soil layers (compact) is recognized as the most appropriate way in the characterization of plants for genetic improvement of plants to drought[10]. The use of a waxy combination of a paraffin and vaselin mixture has been carried out at IRRI to study the ability of roots to penetrate hard layers[19]. The hard layer is simulated from a mixture of paraffin (60%) and vaselin (40%) which are known to be equivalent to 12bar hardness[22][17].

The results of the root permeability test showed that seeds germinated with 25% PEG produced the highest average number of roots that could penetrate the waxy layer. Whereas the highest average root length comes from plants whose seeds are germinated using a 20% PEG concentration. From the observation of root permeability it is known that the Edok cultivar shows the best response. The Edok cultivar produces the highest number of roots that can penetrate the waxy layer, which is an average of 19.3 strands with an average length of 25.5 cm. Roots that are able to penetrate the basic layer of the pot form waxy layers, more than 10 cm long and relatively large in number of roots, are expected to be more tolerant of drought[8].

The form of morphological adaptation of plants to avoid drought stress is to produce longer roots to look for water sources that are relatively far from the ground surface[18]. Dense, deep roots with high root permeability will increase water absorption from the soil[13][13]. In relation to roots, genotypes of plants that have drought resistance have characteristics, including: (1) being able to develop their root systems when water is still available before experiencing drought stress so that plants can extract water from the inner soil, (2) modifying the root system so as to be able to extract water from the innermost layer of water under water stress conditions[8].

5. Conclusions

The conclusion of this research were :

1. Cultivar Legowo shows the best germination in drought stress induced by PEG 6000 in the control treatment, 15 and 25%. While Edok cultivars gave the best results in the treatment of PEG20%.

- The highest number of roots that can penetrate the waxy layer (a mixture of 60% paraffin and 40% vaseline) is produced by the Edok cultivar, which is an average of 19.3 strands with an average length of 25.5 cm.

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