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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(1): 530-539. **OPEN ACCESS**

Simulation of hybrid rice tolerance to drought stress on nutrients culture in seedling phase

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To obtain a new hybrid rice genotype, a selection needs to be undertaken. Good selection method is expected to be cheap, fast and reliably implemented in selecting genotypes simultaneously in large quantities and can separate the tolerant and susceptible genotypes. The aim of the study was to evaluate methods and variables in an early selection, to know agronomic responses of hybrid rice genotypes on the simulation of drought stress in rice fields, and to obtain hybrid rice genotypes that could potentially be developed in the rainfed lowland. The experiment was conducted in the greenhouse of Research and Development Center for Biotechnology and Genetic Resources (BB BIOGEN) Bogor. The experimental design consisted of a split plot design with PEG 6000 treatment (main plot) and genotype/varieties (subplot) with 3 replications. The plant material consisted of genotype BI485A/BP3, BI485A/BP5, BI485A/BP10, BI485A/BP12, BI485A/BP15, BI599A/BP5, BI599A/BP15, BI665A/BP6, Maro, HIPA 6, HIPA 7, HIPA 8, IR -64 and Limboto. The results showed that the method of nutrient culture with 25% (w/v) of PEG 6000 solution concentrations can detect the drought tolerant genotypes of hybrid rice in advance. Genotype BI485A/BP12, BI485A/BP15 and BI559A/BP15 were selected as drought tolerant genotypes and were potential to be developed in the rainfed lowland

Keywords: drought, rainfed lowland, hybrid rice, simulation, polyethylene glycol

INTRODUCTION

Rice is an important main staple food and consumed by more than half world of population (FAO, 2014; Widyastuti et al.,, 2017). Increase in rice demand in line with the increase in population (Dewi et al.,, 2017). In Indonesia, rice is a very important food and main staple food for most Indonesian people (Sutariati et al.,, 2017) and demanding tend to increase continuously. Many effort have been done to support the fulfillment of national rice needs, through the breeding program (Sadimantara et al.,, 2016, 2018; Kadidaa et al.,, 2017a, 2017b; Suliartini et al.,, 2018), agroforestry (Muhidin et al.,, 2013, 2018), promote local food (Muhidin et al.,, 2016) and development of upland rice (Muhidin et al.,, 2013; Syaiful et al.,, 2013; Sutariati et al.,, 2018).

Introduction of rice hybrid varieties is one alternative. Hybrid rice with an average yield potential of 15-20% is higher than the best commercial inbreed varieties (Research and Development Agency, 2007; Sing et al.,, 2009; Rumanti et al.,, 2011; Villa et al.,, 2011; Afa, 2013; Haque et al.,, 2015; Huang et al.,, 2017; Mura et al., 2017) and are expected constantly to show better yields than other preeminent varieties in drought stress conditions (Ward et al., 2013, 2014; Yuan et al., 2017).

One of the agro-ecological zones frequently used as a planting rice area is rainfed lowland (Lal et al., 2017). The quantity of water supply available during the rice growing period depends on rainfall and its distribution (Morison et al., 2008; Kang et al., 2009). In rainfed lowland, the drought occurs almost every year due to the low amount of rainfall and the short rainy season (Serraj et al., 2008; Miyan et al., 2015: Swain et al., 2017; Dewi and Whitebread, 2017). One of strategies that can be applied is planting drought stress tolerant genotypes of hybrid rice (Tuberosa, 2012; Todaka et al., 2015; Swain et al., 2017).

To obtain a new hybrid rice genotype, a selection needs to be undertaken. It is very difficult to perform a direct selection of the results obtained from the field in stress areas in accordance with the targeted environment, due to varying environmental conditions and drought stress often occurs at the most inopportune times (Fukai et al., 2008; Widyastuti et al., 2017). It is therefore necessary to find a method of selection which can identify drought tolerant genotypes of hybrid rice in advance. Good selection method is expected to be cheap, fast and reliably implemented in selecting genotypes simultaneously in large quantities and can separate the tolerant and susceptible genotypes (Lewis and Christiansen, 1981; Fukai et al., 2008; Sie et al., 2008; Rasheed et al., 2017).

The Polyethylene glycol (PEG) with molecular weight $\geq$ 6000 has been widely used in conducting research on the impact of water stress on the growth of crops including rice (Balch et al., 1996; Verslues et al., 2006; Wani et al., 2010; Afa, 2013; Afa et al., 2012, 2013; Widyastuti et al., 2017; Masoabi et al., 2018), but the results are still inconsistent with the those obtained from the field. Herawati (2010) concluded that the modification, which is the use of PEG 6000 as a selector agent still needs to be further done to obtain consistent results as in the field (Rao and FTZ, 2013; Mustikarini et al., 2013; Dantas et al., 2017; Kaceem et al., 2017). A testing modification can be conducted in the phase of seeds in nutrient culture. A direct selection obtained from the field through simulation of drought stress in rice fields was used as a comparison when anthesis simulated conditions of drought stress rainfed lowland. The aim of the present study was to evaluate the methods and variables in the early selection to obtain drought stress tolerant genotype of hybrid rice that could potentially be developed in the rainfed lowland.

MATERIALS AND METHODS

The experiment was conducted at the greenhouse of Research and Development Center for Biotechnology and Genetic Resources (BB-BIOGEN) Bogor. The plant materials used consisted of 8 hybrid genotypes produced by the system of 3 lines, 2 varieties of hybrid and two check varieties (sensitive and tolerant to the drought). Hybrid genotypes consisted of BI485A/BP3, BI485A/BP5, BI485A/BP10, BI485A/BP12, BI485A/BP15, BI599A/BP5, BI599A/BP15 and BI665A/BP6. Hybrid varieties consisted of Maro and HIPA 8, and the check varieties consisted of IR64 as drought-sensitive controls and Limboto as drought-tolerant control.

The design of the treatment used was 3 replications *split plot* design. The main plot was PEG 6000 consisting of 2 levels i.e. control (without PEG 6000) and PEG 6000 solution concentration of 25% in Yoshida solution (1976) and the subplot was rice genotypes consisting of 12 levels. The experimental unit was 5 seeds. One-week old normal sprouts (seedlings) of each genotype/variety with the equal root length were transferred into the growing media through the holes of Styrofoam that had been floated on the surface of the media/plastic tub with a capacity of 8 liters of nutrient solution for 7 days. Water that lost during the experiment due to transpiration was replaced by daily adding water/distilled water. During the growth experiment, the pH of medium was maintained at 4.5 by adding NaOH 1 N or HCl 1 N. Two plastic tubs were used as the main plot equipped with aerators, planted with 6 varieties/genotypes of hybrid rice per tube with 5 seedlings per experimental unit. These tubs were arranged according to the experimental design. The distance between the main plots was 25 cm, the distance for subplot was 10 cm and inter-group distance was 50 cm (Afa, 2013).

Variables observed were shoot height, root length, root dry weight and shoot, shoot root weight ratio (RSR), the drought level of leaves (SES IRRI score) and average reduction index of each variable was determined using the following formula recommended by Jiang and Lafitte (2007) and Afa (2013).

Reduction average (%) = [1 - (Vs/Vp)] x 100, where:

Vs = the value of the variable in the stress condition

Vp = the value of the variable in the condition without stress

Data were analyzed using analysis of variance and DMRT test was further performed using SAS 9.1 facilities test. Analysis of main component, correlation and block analysis were used to determine the contribution of variable and classification of drought tolerant genotypes.

RESULTS

Interaction of PEG 6000 and genotype significantly affected the height of shoot root length, root dry weight, shoot dry weight, root weight ratio and score of leaves drought level. These indicated that there were different responses between genotype from the treatment of 25% PEG 6000 concentration to the seedling phase, which gave an opportunity to get tolerant genotypes to drought stress (Afa, 2013) as seen in Table 1, 2 and 3.

In the treatment of PEG 6000, the highest average shoot height was obtained from genotype BI485A/BP10 of 10:11 cm with a decline only 40.1%, which did not differ significantly from genotype BI599A/BP15, BI485/BP15, BI485/BP12, BI485/BP3, BI599/BP5 and Maro varieties, and Limbo check varieties and IR64, sequentially of 9.59, 9.03, 8.82, 8.53, 7.97, 5.10, 8.95 and 8.44 cm. The lowest was in BI665A/BP6, reaching 6.74 cm with a decrease of 53.5%, but not significantly different from genotype BI485A/BP5, BI599A/BP5, BI485A/BP3 and BI485A/BP12 (Table 1).

On the variable of root length, PEG 6000 treatment generally contributed to the elongation of roots. PEG 6000 treatment resulted in the longest average root length in BI485A/BP5 genotype of 8.29 cm, increased to 23.4%, which was not significantly different from other genotypes but significantly different from IR64 and HIPA 8. Average root length of genotype BI485A/BP15, BI485A/BP12 and BI485A/BP10 was 8.11, 7.85, and 7.71 cm, respectively with an increase in root length attained 10.0, 26.6, 1.6%. These were significantly different from the check variety IR64 root length of 5.34 cm, which decreased by 38.4%. Limboto check varieties had longer roots than the control i.e. 6.39 cm, or increased to 11.7% (Table 1).

The increase in root length due to the treatment of PEG 6000 was not followed by an increase in root dry weight. The average reduction in root dry weight ranged between 31.0 - 63.1% (Table 2). The highest root dry weight was recorded in the Limboto check varieties that reached 4.00 mg with a reduction of 31.0%, which significantly different from the BI485A/BP5 genotype of 2:50 mg with 55.4% reduction of weight. BI599A/BP5 genotype had the lowest root dry weight of 2.40 mg with 63.1% weight reduction. The highest average of root dry weight was 485A/BP12 genotype and BI599A/BP15, reaching 3.50 mg for each with weight reduction of 45.3 and 41.7%, but not significantly different from the other genotypes (Table 2).

The decrease in root dry weight was associated with the efforts of the plant to spur the growth of root length, but impeding the accretion of root weight will result in small root density, which implies a reduction in root dry weight. The influence of PEG 6000 on the percentage of reduction of shoot dry weight ranged between 62.9-82.1% (Table 2). BI599A/BP15 genotype had a higher shoot dry weight (6.60 mg) than other genotypes, with low reduction in dry weight of 63.9%. Varieties of check Limboto had 6.20 mg crown dry weight with weight reduction of 62.9%. The highest percentage of reduction in shoot dry weight due to the treatment PEG 6000 was on Hipa-8 of 82.1%, while IR64 only 72.4% (Table 2).

The ratio of shoot root weight (RSR) increased in treatment PEG 6000. The lowest shoot root weight ratio was found in genotype BI599A/BP15 (0.54), which did not differ significantly from the other genotypes, but significantly different to BI665A/BP6 genotype and Hipa-8. BI665A/BP6 genotype and Hipa-8 had the highest RSR, reaching 0.74 and 0.80 respectively (Table 3).

Table 1. Influence of PEG 6000 solution on the height of shoot and the root length

Genotypes	The height of shoot (cm)			The length of root (cm)		
	Control	25% PEG 6000	Relative decrease (%)	Control	25% PEG 6000	Relative decrease (%)
BI485A/BP3	14.58 e	8.53 fghi	41.5	6.56 bcde	6.98 abcde	(6.4)
BI485A/BP5	15.92 de	7.42 ghi	53.4	6.72 bcde	8.29 ab	(23.4)
BI485A/BP10	16.89 bcd	10.11 f	40.1	7.59 abcd	7.71 abc	(1.6)
BI485A/BP12	16.36 cde	8.82 fghi	46.1	6.20 cde	7.85 abc	(26.6)
BI485A/BP15	18.78 ab	9.03 fgh	51.9	7.37 abcd	8.11 abc	(10.0)
BI599A/BP5	15.70 de	7.97 fghi	50.4	7.12 abcde	7.06 abcde	0.8
BI599A/BP15	15.88 bcd	9.59 fg	39.6	7.22 abcde	7.04 abcde	2.5
BI665A/BP6	14.50 e	6.74 i	53.5	7.25 abcde	6.98 abcde	3.7
Maro	18.26 abc	10.05 f	45.0	6.19 cde	6.56 bcde	(6.0)
Hipa 8	19.49 a	6.95 hi	64.3	5.42 e	5.75 de	(6.1)
IR64	15.93 de	8.44 fghi	47.0	8.67 a	5.34 e	38.4
Limboto	15.55 de	8.95 fgh	42.4	5.72 de	6.39 bcde	(11.7)

Notes: Numbers with the same letter are not significantly different from one another (DMRT test at α = 0.05; numbers in bracket indicated increasing).

Table 2. Influence of PEG 6000 solution to root and shoot dry weight

Genotypes	Root dry weight (mg)			shoot dry weight (mg)		
	Control	25% PEG 6000	Relative decrease (%)	Control	25% PEG 6000	Relative decrease (%)
BI485A/BP3	5.00 ef	3.00 hij	40.0	13.90 e	4.90 f	64.8
BI485A/BP5	5.60 def	2.50 ij	55.4	18.40 cd	4.00 f	78.3
BI485A/BP10	8.20 a	3.30 hij	59.8	24.30 a	6.20 f	74.5
BI485A/BP12	6.40 bcd	3.50 hi	45.3	21.00 abc	5.30 f	74.8
BI485A/BP15	6.30 bcd	3.40 hij	46.0	21.90 ab	4.90 f	77.6
BI599A/BP5	6.50 bcd	2.40 j	63.1	20.00 bcd	3.90 f	80.5
BI599A/BP15	6.00 cde	3.50 hi	41.7	18.30 cd	6.60 f	63.9
BI665A/BP6	4.60 fg	3.00 hij	34.8	17.30 d	4.00 f	76.9
Maro	7.20 b	3.30 hij	54.2	21.00 abc	5.50 f	73.8
Hipa-8	6.80 bc	3.20 hij	52.9	22.40 ab	4.00 f	82.1
IR64	5.50 def	3.00 hij	45.5	17.40 d	4.80 f	72.4
Limboto	5.80 de	4.00 gh	31.0	16.70 de	6.20 f	62.9

Notes: Numbers with the same letter are not significantly different from one another (DMRT at α= 0.05).

Table 3. Influence of PEG 6000 solution to the ratio of shoot root weight (NAT) and scores of leaves drought level (SES IRRI)

Genotypes	Ratio of shoot root weight (RSR)		Scores of leaves drought level	
	Control	25% PEG 6000	Control	25% PEG 6000
BI485A/BP3	0.37 d	0.63 bc	0.0 g	4.9 bc
BI485A/BP5	0.30 d	0.63 bc	0.0 g	8.3 a
BI485A/BP10	0.34 d	0.57 c	0.0 g	2.5 e
BI485A/BP12	0.30 d	0.66 bc	0.0 g	2.2 ef
BI485A/BP15	0.29 d	0.62 bc	0.0 g	3.1 de
BI599A/BP5	0.33 d	0.61 bc	0.0 g	7.0 ab
BI599A/BP15	0.33 d	0.54 c	0.0 g	2.1 e
BI665A/BP6	0.27 d	0.74 ab	0.0 g	7.4 ab
Maro	0.34 d	0.63 bc	0.0 g	1.5 ef
Hipa-8	0.31 d	0.80 a	0.0 g	4.2 cd
IR64	0.31 d	0.63 bc	0.0 g	5.3 abc
Limboto	0.34 d	0.65 bc	0.0 g	1.5 ef

Note: Numbers with the same are not significantly different from one another (DMRT at α = 0.05).

DISCUSSION

The root elongation in genotype BI485A/BP5 caused stunted growth, making the genotype lower than the others. in drought stress conditions, the growth of shoot more stunted than the growth of root (Wu and Cosgrove, 2000; Afa, 2013; Yokawa et al, 2014; Jin et al., 2015; Chen et al., 2017; Fang et al., 2017; Li et al., 2017). Extension or modification of root constitutes a form of adaptation plant to allow the root to gain more access to water. Hamim et al., (2008) and Ahadiyat et al., (2014) stated that the ability of plants to maintain the growth of the root is crucial as it absorbs water and nutrient in drought stress conditions. there is a positive relationship between root length and the ability of a genotype to adapt to the conditions of water deficit (Matsura et al., 1996; Tardieu, 2013; Basu et al., 2016; Gray and Brady, 2016; Polania et al., 2017).

Therefore, genotypes that have a relatively long root in 25% PEG 6000 was assumed to be tolerant to drought stress in the field. There was a balance between elongation of roots and shoot growth in some genotypes like BI599/BP15 which suggests that this genotype tolerant to drought. Thus, in assessing drought tolerant genotypes, the two variables can be taken into consideration.

The drought stress tends to increase the length of plant roots and shoot root weight ratio (Bañoc et al., 2000; Farooq et al., 2008; Nejad, 2011; Silva et al., 2012; Afa, 2013; Koevoets et al., 2016; Cai et al., 2017; Gu et al., 2017), because more carbohydrates translocated to the roots for its growth causing the shoot stunted to grow, and resulting in the increase of RSR. When water deficit increases, the growth of plants is prevented and the sizes of all part of the plant are varied (Praba et al., 2009).

Treatment of PEG 6000 caused the leaves become dry. Based on the scores of leaves drought level, genotypes BI485A/BP10, BI485A/BP12, BI485A/BP15, BI599A/BP15 and Maro varieties were not significantly different from Limboto check varieties (Table 3). Those genotypes were on the category of scores of leaves drought level between 0-3 that included in the category of drought tolerant based on IRRI SES criteria. Galle and Feller (2007) and Agbicodo et al., (2009) stated that limiting the development of leaves area is a defensive system of plants to survive during drought stresses, leading to the development of the roots to fetch water and closing of stomata to limit transpiration. The low level of scores of leaves drought on some hybrid genotypes tested was caused by the genotypes have the ability to furl the leaves, mainly in the tip of the leaves and leaves base remain opened, so that the transpiration can be reduced and photosynthesis remains continue (Figure 1).

The results of principal component analysis and correlation indicated that the variables that contribute to the diversity of seedling phase and correlated each other were root dry weight, shoot dry weight and the score of leaves drought level (Afa, 2013). These variables can be used as an indicator to classify the drought tolerant genotypes in hybrid seedling phase. The block analysis resulted in a dendrogram at similarity level of 64.0%, placing genotypes BI485A/BP12, BI485A/BP15, BI485A/BP10, BI599A/BP15 and varieties of Maro in the same group as Limboto (drought tolerant). Genotypes BI485A/BP3, BI665A/BP6, BI485A/BP5, BI599A/BP5 and HIPA-8 were in the same group as IR64 (drought sensitive) (Fig. 2).

Figure 1. Appearance of leaves of some hybrid genotypes and check varieties at a concentration of 25% solution of PEG 6000

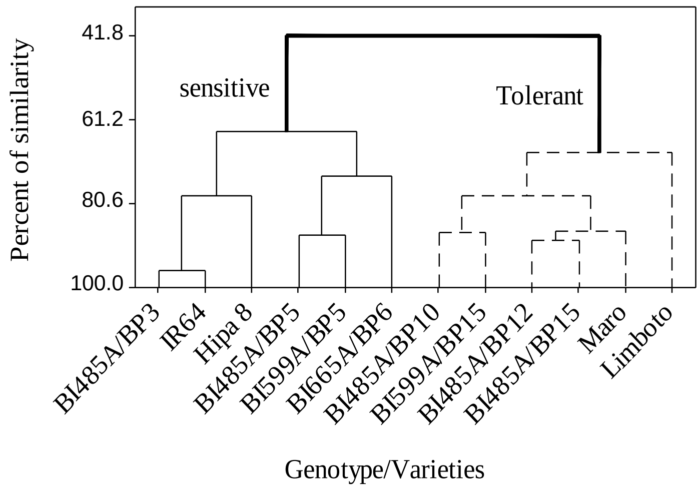

Figure 2. Dendrogram of drought tolerant hybrid rice genotypes based on variables of root dry weight, shoot dry weight and score of leaves drought level.

Therefore, the selection method using 25% solution of PEG 6000 in the seedling phase based on the criteria of root dry weight, shoot dry weight and score of leaves drought level may detect genotype BI485A/BP12, BI485A/BP15, BI485A/BP10, BI599A/BP15 and Maro varieties as drought tolerant varieties based on comparison with Limboto checks.

CONCLUSION

The use of PEG 6000 at a concentration of 25% (w/v) in the 7-day old seedling maintained at nutrient culture was able to early detect the genotypes of drought tolerant hybrid rice. Root dry weight, shoot dry weight and score of leave drought level were variables for the prediction of drought tolerant genotypes of hybrid rice. Genotype BI485A/BP12, BI485A/BP15, BI485A/BP10, and BI559A/BP15 were selected as drought tolerant genotypes and could potentially be developed in the rainfed lowland, but still needed field experiments.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

The authors extend their gratitude to the Agency for Agricultural Research and Development, Ministry of Agriculture of Indonesia for funding this research through KKP3T to Bambang S. Purwoko and teams.

AUTHOR CONTRIBUTIONS

LA designed and performleed the experiments and also wrote the manuscript. BSP, AJ, OH and ISD designed experiments and reviewed the manuscript. All authors read and approved the final version.

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