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The screening of soybean drought tolerant using polyethylene glycol 6000 at Entisol of Bengkulu Coastal Land

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Abstract The result showed that tested four soybean varieties (Anjasmoro, Dering 1, Devatra 2 and Grobogan) and the concentrations of PEG 6000 (0% control, 5% 10 %, 15% and 20%) did not interact in affecting the number of flowers and the number of pods. Soybean varieties varied in their tolerance to drought stress at Entisol. Based on all observed both vegetative and generative growth variables, the order of drought tolerance of soybean varieties (from the most to the least tolerant) were Anjasmoro, Dering 1, Devatra 2, and Grobogan. Increasing the concentration of PEG 6000 to the highest one of 20%, constantly reduced the vegetative and generative growths (plant height, number of leaves, number of flowers and number of soybeans pods). The predicted effective dose selection of PEG 6000 for soybean drought tolerant was 19.50%; it reduced yields up to 50%.

Keywords: Coast, PEG, Plant stress, Varieties

Introduction

The potential of coastal land in Bengkulu Province is about 5,250 hectares or an area of 27% of the total land area (Kementrian Kelautan dan Perikanan, 2018 and BAPEDA Bengkulu Province, 2017). The coastal lands are dominated by Entisol which pose many problems, especially related to its low ability to bind water and nutrients, low soil moisture, high temperature, and very low organic matter content (Bertham *et al*., 2019, and Bertham *et al.,* 2020). The utilization of coastal land for plant cultivation requires the right approaches, such as the addition of organic and biological fertilizers to the planting medium and using superior seeds that are suitable for Entisol land conditions in coastal areas.

The Ministry of Agriculture of Indonesia has released more than 80 superior varieties of soybeans to support national production. Dering 1 and 2 varieties are drought-resistant soybean varieties in such a way that they can be used as a drought tolerant base-line comparison (Balai Penelitian Tanaman

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Aneka Kacang dan Umbi, 2018). Furthermore, drought and its interaction with high temperatures are the main abiotic stress factors that affect the yield and stability of soybean production (Valliyodan *et al*., 2017). Drought tolerance does not only mean the ability of plants to survive under conditions of water scarcity, but also, more importantly, the ability of plants to produce optimally during periods of stress (Blum, 2009). Tolerant plants adapt more than susceptible plants (Mir *et al*., 2012). The techniques for identifying and evaluating of drought tolerance traits that are fast, accurate, and inexpensive can be carried out by observing morphological characters and secondary traits, which are highly correlated with yields under drought stress and are not seen in plants under non-stress conditions (Fleury *et al*., 2010; Fang and Xiong, 2015).

The morphological characteristics of soybean plants that are tolerant of drought stress are slow canopy wilt, high root:leaf ratio, wider root surface, high number of root nodules, high nodule dry weight (Tanaka *et al*., 2010; Ries *et al*., 2012). The soybean genotype PI 416937 has a good root system that allows it to maintain a constant rate of transpiration and maintain potential yield with a water pressure shortage of> 2.0 kPa compared to the genotype which has rapid canopy wilt (Fletcher *et al.,* 2007).

Drought stress during the germination of soybeans was simulated with PEG 6000. The water potential -0.41 MPa inhibited vigor index and the hypocotyl length in all tested varieties, but the inhibited germination percentage, lateral root number and dry weight of seedling in some varieties. However, the decrease in water potential -0.67 MPa inhibited almost germination variables in all varieties, except for the root length. Each tested soybean variety showed differences of germination response to drought stress (Herawati *et al*., 2020, Salazar-Henao *et al.,* 2016).

Soybean varieties of Dieng, Tidar and Sibayak showed less inhibition than others, but Anjasmoro, Burangrang, Galunggung, Kipas Putih and Tambora showed more inhibition of seedling growth. Based on index of reduction and drought sensitivity on germination variables, Dieng and Tidar were identified as tolerant varieties, whereas sixteen varieties as medium tolerant and seventeen varieties as sensitive ones. (Widoretno, 2011). The tolerant cultivar recorded lower percent reduction of shoot and root growth over control in soybean as reported by (Ange *et al*., 2016, Anaytullah *et al.,* 2008).

This research aimed to determine soybean varieties that are drought tolerant on Entisol in coastal land and to obtain a concentration of Polyethylene Glycol 6000 as a medium for selecting soybean tolerant to water stress.

Material and methods

Plant material

This experiment was carried out in the Greenhouse of the Faculty of Agriculture, University of Bengkulu (July-October, 2022). Furthermore, the soybean varieties used were Grobogan, Anjasmoro, and Dering 1 from Balitkabi, as well as the Devatra 2 variety, which was the superior variety of Bengkulu University. Dering 1 is used as a dry-resistant soybean variety produced by BALITKABI released by the Ministry of Agriculture of the Republic of Indonesia in 2016.

Healthy and pithy soybean seeds are soaked in water for 1 hour before planting. The moist seeds were smeared with the inoculum of the Rhizobium sp. to activate nodule formation. Furthermore, soybean seeds are planted with a depth of 2-4 cm as much as 2 seeds per polybag, after 2 weeks of maintaining a healthy plant and growing normally. Furadan 3G of about 1 g was applied per planting hole to treat fruit flies attacking soybean sprouts.

Growing media

The planting medium used was a mixture of Entisol soil taken from the coastal areas of Bengkulu City and chicken manure at a ratio of 1: 1. A polybags measuring 30 cm x 40 cm were used in this experiment. The dose of chicken manure used is 30 tons per hectare. The basic fertilizer used is 150 kg per hectare of urea, which is applied 3 times, namely 1/3 part of the preparation of the planting medium by mixing with the planting medium. 1/3 part is applied when the plants are 3 weeks old, and 1/3 part is applied when the plants start flowering. The dose of TSP fertilizer is applied as much as 250 kg per hectare and the dose of KCl fertilizer as much as 200 kg per hectare is mixed during the preparation of the planting medium.

Irrigation is carried out daily according to the calculation of the field capacity of the planting medium. Curacron 500EC and Decis 25ec, which are contact insecticides and gastric poison, are used for pest control. The disease controlled by Benomyl (Benlate) is effective in suppressing disease in soybeans (Agrios, 1988).

PEG 6000 application for drought tolerant

Polyethylene glycol 6000 (PEG 6000) with the chemical formula $H(OCH_2CH_2)nOH$ is used to hold water that is given during the irrigation process. In order to facilitate the application, a 100% concentration of PEG 6000 stock solution was made. Furthermore, dilution was carried out according to the concentration of the solution for treatment, namely 0% (without PEG 6000), 5%, 10%, 15% and 20%. The PEG 6000 treatment was carried out for seven weeks, namely when the plants were 2 weeks old after planting (2 WAP) until the plants were 8 WAP. The consideration of giving during this period is when the plant enters a stable vegetative stage until the plant has started to enter the pod filling stage. The PEG 6000 treatment was

carried out during the period when the plants needed water for their growth and production. Lack of water during this period can significantly reduce soybean production.

Experimental design

The water stress resistance test used two factors arranged in 2 factors factorial experiment in a Completely Randomized Design (CRD). The first factor was 4 varieties of national superior soybeans, namely: Grobogan, Anjasmoro, Devatra 2 and Dering 1. The second factor was the concentration of PEG 6000 consisting of 5 levels, namely: 0% (without PEG 6000 as a control), 5%, 10%, 15% and 20%. According to Mexal *et al.* (1975), PEG-6000 with a molecular weight of 6000 at a concentration of 5%, 10%, 15%, and 20% dissolved in distilled water, respectively, gave an osmotic potential of -0.3 bar, -1.9 bar, -4.1 bar, and -6.7 bar. From the two treatment factors, 20 treatment combinations, which were repeated 10 times, were obtained. The observed variables were plant height, number of leaves, number of flowers and number of pods and root performance.

Statistical analysis

The data were normalized using Z-Transformation before analyzing using X-CoStat Software version 6400 and Microsoft Excel 2010. The normal data were analyzed using the F test at 5% level to determine which treatment had a significant effect on the observed variables. If the processing was significantly different, the LSD test is performed at 5% to distinguish between water stress tolerant varieties and the most susceptible varieties using PEG 6000 as a medium for drought tolerant selection. The best Curve Analysis is used to determine drought tolerant selection doses that can reduce soybean growth and yield up to 50% from normal conditions.

Results

The raw data are roles to separate the independent and dependent variables. After determining the role of each variable, data normalization was carried out using the z-score. These normalized results are used for analyzing F test at 5% level. A summary analysis of variance was presented in Table 1. The effect of the interaction between the concentration of PEG 6000 and soybean varieties was only significantly different to the variable number of flowers and number of pods and had no significant effect on plant height and number of leaves. The unique factor of the soybean variety and the unique factor of the PEG 6000 concentration were significantly affected for all observed variables (plant height, number of leaves, number of flowers and number of pods).

Table 1. Anova Summary Effect of Varieties and PEG 6000 concentration on the growth and yield of soybeans

Growth Variable	F Count			CV (%)	R^2
	Varieties	PEG 6000 concentration	V x C Interaction		
Plant Height	9.3429*	15.6257*	1.5789 ns	24.92%	0.7819
Number of Leaves	4.1955*	17.1424*	1.5843 ns	26.60%	0.7529
Number of Flower	12.929*	13.7929*	3.8557*	17.55%	0.78995
Number of Pods	12.7075*	8.0089*	3.2506*	20.16%	0.75179

Note: The numbers followed by the same letter in the same column are not significantly different in the F test at 5% level.

Varieties

The results of the LSD test at a 5% level in the variables of plant height, number of leaves, number of flowers and number of pods can be seen in Table 2. Based on the LSD test at 5% level against 4 main variables as indicators of resistance observed quantitatively, namely plant height, number of leaves, number of flowers and number of pods against drought resistance, the most resistant to the most susceptible varieties can be sorted from the results, namely Anjasmoro, Dering1, Devatra 2, Grobogan.

Drought resistant was also carried out based on other morphological characteristics that were observed qualitatively, namely leaf area, wrinkles on the leaf surface, wilted leaves (Wilting) during the day and the color of the leaves that quickly changed to chlorosis followed by necrosis for susceptible plants.

Table 2. LSD Test Results at 5% level Effect of Varieties differences on plant height, number of leaves, number of flowers and number of soybeans fruits at 8 WAP

Varieties	Plant Height	number of leaves	Number of Flowers	Number of Fruits
Grobogan	75.62b	11.80b	17.96c	8.98 a
Anjasmoro	88.10a	15.18a	36.14a	9.70 a
Devatra 2	60.56c	14.44a	26.36b	4.38 b
Dering 1	78.78ab	14.38a	34.82a	3.82 b
LSD 0.05	10.4421	2.0147	6.5445	1.9677

Note: The numbers followed by the same letter in the same column are not significantly different in the F test at 5% level.

In addition, the determination of resistance to drought-resistant plants was able to maintain normal leaf numbers, little or no leaf wrinkles and no wilted leaves during the day (Figure 1).

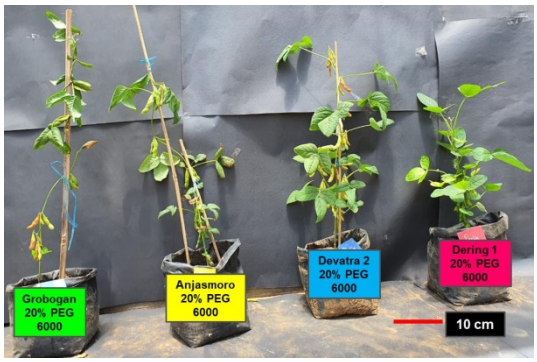

Figure 1. Growth and leaf condition of 4 soybean varieties in drought stress conditions using 20% PEG 6000

Drought resistance properties were also identified by the appearance of soybeans. Drought tolerant soybeans have longer root growth with a large number of lateral roots and root hairs, whereas susceptible plants are prone to stunted growth, having less the number of lateral roots and hair roots (Figure 2).

Figure 2. Root performance of four soybean varieties under drought stress conditions using 20% PEG 6000

PEG concentration

The results of the LSD test at 5% level of the effect of the single factor PEG 6000 concentration on the growth and yield variables of soybeans are presented in Table 3.

Table 3. LSD Test Results at 5% level Effect of PEG 6000 Concentration on plant height, number of leaves, number of flowers and number of soybeans fruits at 8 WAP

PEG 6000 Concentrations (%)	Plant Height (cm)	Number of leaves	Number of Flowers	Number of Pods
0	92.15 a	17.65 a	44.96 a	8.58 a
5	87.58 a	15.90 ab	29.55 b	8.15 ab
10	80.58 a	14.90 b	27.05 bc	6.10 bc
15	66.95 b	12.15 c	22.375bc	5.13 cd
20	52.58 c	9.15 d	20.175 c	3.15 d
LSD 0.05	11.6746	2.2525	7.3170	2.1999

Note: The numbers followed by the same letter in the same column are not significantly different in the F test at 5% level.

By increasing the concentration level of EG 6000 to 20% consistently reduced all observed variables (plant height, number of leaves, number of flowers and number of pods). The decrease in plant height was not significant prior to the concentration of 10% PEG 6000, but the increase in the concentration to 15% decreased the significant plant height up to 25.41%. The decrease in soybean plant height reached 42.94% by increasing the concentration of PEG 6000 to 20%.

The variable number of leaves also showed that there was not significant decrease with the provision of about 10%. A significant reduction in the number of leaves started to occur at a concentration of 15% PEG 6000 of 31.16%. By increasing the concentration to 20% PEG 6000 as a medium for selection decreased the number of leaves by 48.16%.

In contrast to the vegetative variable, the decrease in the number of flowers was significant at the 5% PEG 6000 distribution from the average number of flowers of 44.96 flowers per plant to 29.55 flowers per plant or a decrease of 34.28%. An increase in the concentration of about 15%, a decrease in the amount of interest, but not yet significant. Increasing the concentration to 20% of PEG 6000, there was a very significant decrease in the amount of interest that reached 55.12%. For the variable number of flowers, which is a very important variable on soybean production, it turns out that water stress is very influential.

The growth and yield characteristics of soybeans are significantly reduced under drought conditions during the period before and after flowering, while the maximum reduction was caused by PEG (16%) applied before flowering. The decrease in the number of fruit variables was also very significant. Furthermore, by applying 15% PEG 6000, the number of fruits reduced by 40.20%. The increase in the concentration of PEG 6000 to 20%, the number of pods formed significantly decreased, reaching 63.29%. Compared with the vegetative variable, the impact of drought stress is more dangerous when it occurs in the generative phase, namely at the time of flowering and pod formation. In addition, water stress during pod filling also

cause the empty pods to be higher. Although the pods are formed, the pod filling is less than perfect in such a way that it can reduce soybean yields by 80%, even for susceptible varieties of soybeans it can reach 100%.

Varieties x PEG concentration

The interaction between genotypes and PEG concentration was highly significant. A smaller percentage reduction in shoot and root growth from about 59.1 to 100% was observed for the genotypes. The reduction of shoot and root length was higher in susceptible genotypes, while reduction was less in the tolerant. The reduction of shoot length may be due to reduction of cell elongation by low water potential created by PEG. Cell elongation is mainly based on turgidity of the cell, which is reduced under PEG treatment causing reduction of shoot length.

There is an interaction between the soybean varieties tested and the PEG concentration on the variable number of flowers formed. All the resulting curves form a quadratic pattern with a different coefficient of determination. The curve regression equations resulting from orthogonal polynomial analysis are shown in Figure 1.

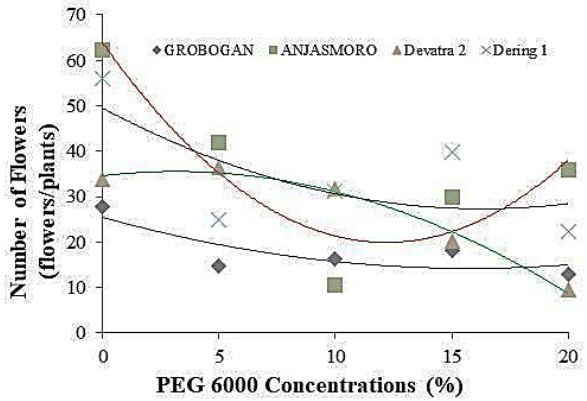

Figure 3. Interaction of Soybean varieties and PEG 6000 concentrations to numbers of flowers

The results of the polynomial and orthogonal tests were only explained the quadratic curve model on the interaction of PEG 6000 concentration with Dering 1 Variety of 45.70%, Grobogan of 64.72%, Anjasmoro of 83.86% and Devatra 2 were the highest of 98.62% ((Figure 3 and Table 1).

From the regression equation, it is known that the Anjasmoro soybean variety produces the lowest number of flowers, namely at a concentration of 10-15%. The Grobogan and Dering 1 varieties produced the same pattern, the lowest flower production was at a concentration of 15-20% PEG 6000. The Devatra variety shows a different pattern. The number of flowers increased

at the 5% PEG 6000 constellation, but the number of flowers decreased significantly and the least compared to the other 3 varieties at a concentration of 20% PEG 6000 (Figure 3).

The interaction of soybean varieties with a concentration of PEG 6000 had a significant effect on the number of pods that were formed (Figure 4). The response of Grobogan, Anjasmoro, and Dering 1 varieties produced the same quadratic pattern, namely an increase in the number of pods to a concentration of 5% PEG 6000, an increase in concentration up to 20% PEG 6000, a very significant decrease in the number of pods formed.

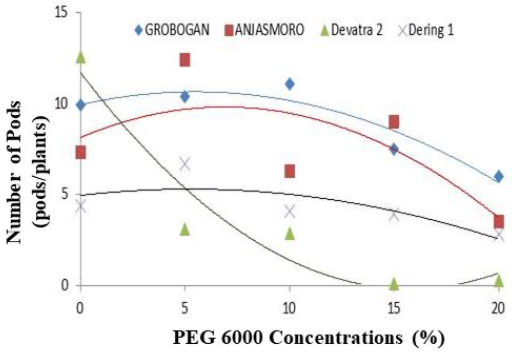

Figure 4. Interaction of Soybean varieties and PEG 6000 concentrations to numbers of pods

Although the number of pods formed was very large, the Grobogan variety was not completely filled. Therefore, the number of empty pods reached 90%, which was the lowest compared to the other three varieties. Meanwhile, the Devatra 2 variety produced the highest number of pods in entisol soil without PEG 6000. Furthermore, PEG 6000 in the range of 5% to 20% significantly reduced the number of pods. The number of pods not formed was at least 15% -20% PEG 6000 compared to the other 3 varieties. Out of the four varieties tested, the Grobogan variety was not very suitable for planting on entisol soil. Without PEG 6000 treatment (0%) the number of flowers and pods produced was the lowest compared to the other 3 varieties. Meanwhile, Dering 1 was the most tolerant variety because it was able to produce a high number of flowers and pods on entisol soil. The administration of PEG at a concentration of 20% reduced the number of flowers and pods produced for all species (Figure 3 and Figure 4). In order to determine the most appropriate PEG 6000 concentration for drought resistance selection media in soybeans, an accurate calculation of the cross-treatment variables is required using Best Curve Analysis.

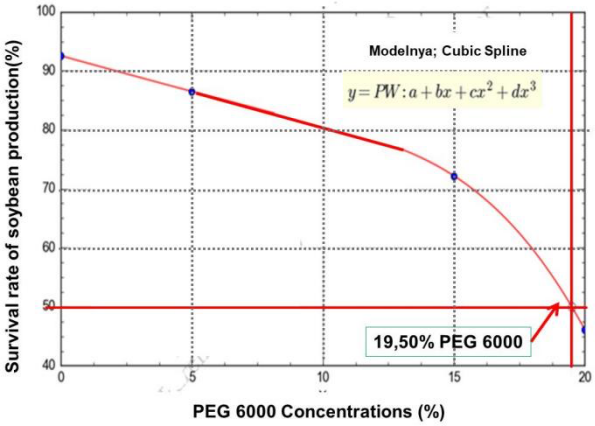

Figure 3. Best Curve Analysis for effective concentration of Soybean drought tolerant screening test

The analysis showed that the most appropriate model for describing the PEG 6000 concentration and soybean survival, and production was Cubic Spline. The concentration of PEG 6000 to select the effective drought resistance for soybeans (ED 50) is 19.50%. This dose reduces growth and yield by 50% compared to growth under normal conditions.

Discussion

Drought stress affects the root system of soybeans, which can cause small lateral roots which cannot develop properly. This leads to a plastic response to the root system by increasing the number of fibrous roots, reducing lateral root diameter, and reducing root biomass (Meister *et al.,* 2014; Salazar-Henao *et al.,* 2016). Changes in root anatomy, such as natural variations in aerenchyma formation in maize, reduce the metabolic potential per root length (Burton *et al.,* 2013).

The growth and yield characteristics of soybeans are significantly reduced under drought conditions during the period before and after flowering, while the maximum reduction was caused by PEG (16%) applied before flowering. The endogenous bioactive GA1 and GA4 content decreased under elevated drought stress (Hamayun *et al*., 2010). The decrease in the number of fruit variables was also very significant. Furthermore, by applying 15% PEG 6000, the number of fruits reduced by 40.20%. The increase in the concentration of PEG 6000 to 20%, the number of pods formed significantly decreased, reaching 63.29%. Compared with the vegetative variable, the impact of drought stress is more dangerous when it occurs in the generative phase, namely at the time of flowering and pod formation. In addition, water stress during pod filling also causes the empty pods to be higher. Although the pods are formed, the pod filling is less than perfect in such a way that it can

reduce soybean yields by 80%, even for susceptible varieties of soybeans it can reach 100%.

The interaction between genotypes and PEG concentration was highly significant. A smaller percentage reduction in shoot and root growth from about 59.1 to 100% was observed for the genotypes. The reduction of shoot and root length was higher in susceptible genotypes, while reduction was less in the tolerant. These results corroborate with earlier study of Dutta and Bera (2008) in mung bean. The reason for the reduction of shoot length may be due to reduction of cell elongation by low water potential created by PEG. Cell elongation is mainly based on turgidity of the cell, which is reduced under PEG treatment causing reduction of shoot length (Vijay, 2018).

In Summary, there was an interaction between soybean varieties and PEG 6000 concentrations with the number of flowers and pods. The order of drought tolerance of Soybean varieties according to all variable observation of vegetative and generative at Entisol were Anjasmoro-Dering 1-Devatra 2-Grobogan. Increasing of PEG 6000 concentration level to about 20% consistently reduced all vegetative and generative growth variables observation (plant height, number of leaves, number of flowers and number of soybeans pods). The effective dose range of PEG 6000 for soybean drought tolerant was 19.50%, the dose range can reduce yields to about 50%.

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