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DROUGHT RESISTANT SELECTION ON SOYBEAN SOMACLONAL VARIANTS

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ABSTRACT

This research was conducted to evaluate the yield potential of 19 somaclonal variants resulting from *in vitro* selection when planted under drought stress condition in the field. Field test was done by planting the variants, the parents, and checked varieties in the field during dry season, and was irrigated once a week for non-stress and once two weeks for drought stress treatment. Split-plot design arranged in a factorial (2 x 28) with three replications was used in this research. Observations were done on yield and yield components. Analysis of variance was used to see the difference between treatments and then it was continued with analysis using Honestly Significant Difference test to find out the best treatments. There was no interaction between genotype and drought stress on seed yield. Different genotypes showed a significant difference on this character. It indicated that the yield potential of selected variants was not affected by drought stress treatment. This research gave 10 variants having the potential to be developed as drought resistant genotypes. However, these ten potential genotypes need to be tested further in field trial to find out the yield adaptability and stability and their resistance to drought stress.

Keywords: somaclonal variants, *in vitro* selection, drought stress, soybean, selection

INTRODUCTION

National soybean production is currently experiencing a setback, in which only 45% of domestic demands can be covered by the Indonesian soybean farmer. There are some obstacles decreasing the soybean productivity in Indonesia, one of them is drought stress. Soybeans are often planted on dry land or after the main food crops at the end of the rainy season, so that water availability often becomes a limiting factor. Directorate General of Food Crops, Ministry of Agriculture since 2003, has sought increased productivity and expansion of land cultivated through Bangkit Kedelai Program to reduce soybean imports. The expansion of planting areas directed towards rained (non-irrigation) and dry land has not been used optimally, while the development of high yielding varieties tolerant of environmental stress is expected to make a real contribution to the improvement of soybean production.

Varieties play an important role to improve the productivity of soybean. Therefore, productivity improvement programs need to be supported by the development of superior varieties of high yielding and tolerant to environmental stress. This will assist the development of soybean in the area that has problems of water availability on a specific planting period. Although drought stress is one factor limiting soybean production, drought tolerant soybean varieties are not many which have been released. From 1000 soybean germplasm selection number in Indonesia, only around 62 varieties have been cultivated by farmers (Suhartina, 2005). However, from this amount, there is no information about the level of tolerance to drought stress. The result of screening on 30 varieties of soybeans at germination and vegetative stages in the greenhouse and *in vitro* in the laboratory, which has been done previously, showed that most soybean varieties tested were classified in the group of drought sensitive. Varieties that showed good tolerance and drought stress were Tidar and Dieng (Widoretno *et al*., 2007).

The availability of superior varieties has an important role in increasing the productivity of soybean. Therefore, productivity improvement program needs to be supported by the

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development of superior soybean varieties which have a high yield and tolerance to environmental stress. This will help the development of soybeans on land that had a problem with water availability at a specific period of planting.

In vitro selection technique has been used to improve tolerance to drought stress for maize (Matheka *et al*., 2008), wheat (Almansouri *et al*., 2001, and sugarcane (Yadav *et al*., 2006). *In vitro* selection for tolerance to drought is usually done by culturing cells/callus/tissues on selective media containing polyethylene glycol (PEG, HO (CH2-CH2) n-CH2-CH2OH, n ≥ 4). Then the cells/tissues/callus resistant to PEG selective medium are regenerated into the plant (Farshadfar *et al*., 2012; Wani *et al*., 2010; Matheka *et al*., 2008; Widoretno *et al*., 2002). Polyethylene glycol of high molecular weights has been used to simulate drought stress in plants as no penetrating osmotic agents lowers the water potential in a way similar to soil drying (Hassanein, 2010).

Previous research showed that the somaclonal variants resulting from *in vitro* selection of strain MLG 2999 were 2B17, 2S3, 2S7, 2S13, 2S14; somaclonal variants of 3B6, 3B7, 3B14, 3B17, 3B18, 3B20, 3S7, 3SE2, 3SE4 resulted from *in vitro* selection of strain B 3731; and somaclonal variants of 8B3, 8B4, 8B5, 8B6 and 8B12 resulted from *in vitro* selection of strain MSC 8606 (Widoretno *et al*., 2002). However, the yield and the level of tolerance to drought stress in the field of these variants were unknown. Therefore, it is necessary to carry out field screening of these somaclonal variant results of *in vitro* selection to find out the yield and the resistance to drought stress in the field.

MATERIALS AND METHODS

The materials used in this research were 19 variants of *in vitro* selection on PEG media, i.e. 2B17, 2S3, 2S7, 2S13, 2S14, 3B6, 3B7, 3B14, 3B17, 3B18, 3B20, 3S7, 3SE2, 3SE4, 8B3, 8B4, 8B5, 8B6 and 8B12 derived from the *in vitro* selection of strains MLG 2999, MLG 3731 and MSC 8606 which were obtained from the results of previous research (Widoretno *et al*., 2002). For comparison, we used the clones and varieties tolerance of MLG 2805, Dieng and Tidar and sensitive clones and varieties of MSC 9019, Burangrang and Tambora.

The research was conducted from June to September 2009 in the experimental field of the Faculty of Agriculture, Brawijaya University, Jatikerto village, Kromengan district, Malang regency, with elevation of 303 asl. and alfisol soil. The design used in these experiments was split plot design which was arranged in a factorial (2 x 28) with three replicates. The main plot was irrigation treatment, and the sub plot was soybean variant consisting of 20 variants. The parameters observed included yield and yield components. To see the differences in variables observed among the treatments, the analysis of variance (ANOVA) was performed followed by Tukey Significancy test or Honestly Significant Difference Test.

Drought-resistant selection was done through field testing with the planting of selected variants obtained from *in vitro*, their parents and checked varieties in the field during the dry season. Testing is done by giving irrigation once a week for non-stress environment and every other week for drought treatment. Every variant in this experiment was planted in separate plot with the size of 3 x 2 m of each plot and planting distance of 30 x 20 cm. Each variant was planted with 50 seeds per plot and was repeated three for each variant.

RESULTS AND DISCUSSION

Observation on seed yield showed that variant treatment gave a highly significant effect on seed yield but water stress treatment had no effect on seed yield. It showed that 19 variants produced as the result of in vitro selection were proven unaffected by drought treatment, which was indicated by statistical result obtained between the normal irrigation and drought stress. Based on the analysis of variance, it can also be ascertained that difference in seed yield per hectare among the variants tested was caused by the differences in genotypes of each variant. The average value of seed yield per hectare in 19 variants that were planted in drought conditions ranged from 8.5 to17.8 quintal per ha, while the average yield on national varieties used for comparison ranged from 13.8 to 22.4 quintal per ha (Table 1).

Irrigation given once every other week in drought treatment had no effect on seed yield of variants. It could be due to the alfisol soil, clay type, which was used in the experiment. This type allows the soil to have a high ability to hold water and to remain moist. In addition, the rainfall during experiment was 75 mm which caused the level of water stress stay unreached.

Table 1. Average of seed yield on 19 soybean drought resistant variants derived from *in vitro* selection

No	Somaclonal variants	Seed yield (ku per ha)
1	B3731 (parent)	16.1 efghi
2	3B6	11.4 abcde
3	3B7	16.4 fghi
4	3B14	13.5 bcdefgh
5	3B17	13.0 abcdefg
6	3B18	12.7 abcdefg
7	3B20	16.4 fghi
8	3S7	11.8 abcdef
9	3SE2	17.8 hij
10	3SE4	13.0 abcdefg
11	MSC8606 (parent)	9.3 ab
12	8B3	11.2 abcd
13	8B4	13.9 bcdefgh
14	8B5	8.5 a
15	8B6	17.4 ghi
16	8B12	10.3 abc
17	MLG2999 (parent)	17.3 ghi
18	2S3	14.1 cdefghi
19	2S7	11.4 abcde
20	2S13	11.5 abcde
21	2S14	13.9 bcdefgh
22	2B17	13.7 bcdefgh
	Check genotypes	
23	MLG2805	18.8 ij
24	MSC9019	15.9 defghi
25	Burangrang	13.8 bcdefgh
26	Dieng	22.4 j
27	Tambora	16.3 fghi
28	Tidar	14.9 cdefghi
	HSD 5 %	**4.72**

Remarks: Values followed by the same notation indicate that there is no significant difference based on HSD 5%

Based on the field test of the 19 variants selected *in vitro*, 10 variants which were recommended for further drought resistance test, ie., 3SE2, 8B6, 3B20, 3B7, 2S3, 2S14, 8B4, 2B17, 3B14 and 3B17 were obtained (Table 1). This was based on the test results comparing the mean value presented in Table 1 showing that 10 variants provided an average seed yield per hectare which was not statistically different from the yield of check genotypes of Dieng, MLG 2805 and Tambora with the highest average seed yield per hectare.

Results of correlation analysis performed on several yield components (seed weight.plant^{-1}, number of pod per plant and 100 seed weight) of soybean seed yield per hectare showed a linear regression relationship for seed weight per plant (r= 0.99) (Figure 1a) and number of pod per plant (r= 0.83) (Figure 1b) for seed yield per hectare, whereas the correlation between 100 seed weight and seed yield per hectare showed a quadratic regression relationship (r= 0.12) (Figure 1c).This indicated that the seed yield per hectare was more significantly influenced by seed weight per plant and number of pod per plant in the form of linear regression relationships. In other words, an increase in soybean seed weight per plant and pod number per plant would be followed by the increasing soybean seed yield per hectare linearly. Meanwhile, the weight of 100 seeds of soybean would only increase seed yield per hectare up to a certain point then it would reduce seed yield in case of further improvements in the weight of 100 seeds.

Soybean yield is determined by number of pod per plant, number of seed per plant and seed weight and these traits are the most important yield component of soybean (Ohashi and Nakayama, 2009; Kokubun *et al*., 2001; Kobraee and Shamsi, 2011a). Since the numbers of seeds per pod are genetically controlled, the number of seeds is determined predominately by the number of pods per plant, which is largely dependent on the number of floral buds that initiates pods and attains maturity (Desclaux *et al*., 2000). Soybean plants produce an abundance of floral buds, but 40–80% of the flowers and pods initiated eventually abort under conventional cultivation. The individual seed weight is a product of the rate and the duration of seed filling; it is generally determined during seed filling after the pod number had been fixed (Desclaux *et al*., 2000; Brevedan and Egli, 2003). Tischner *et al*., (2003) mentioned that seed set is determined by the number of ovules per.pod^{-1}, the frequency of embryo abortions, and the number of pods per plant.

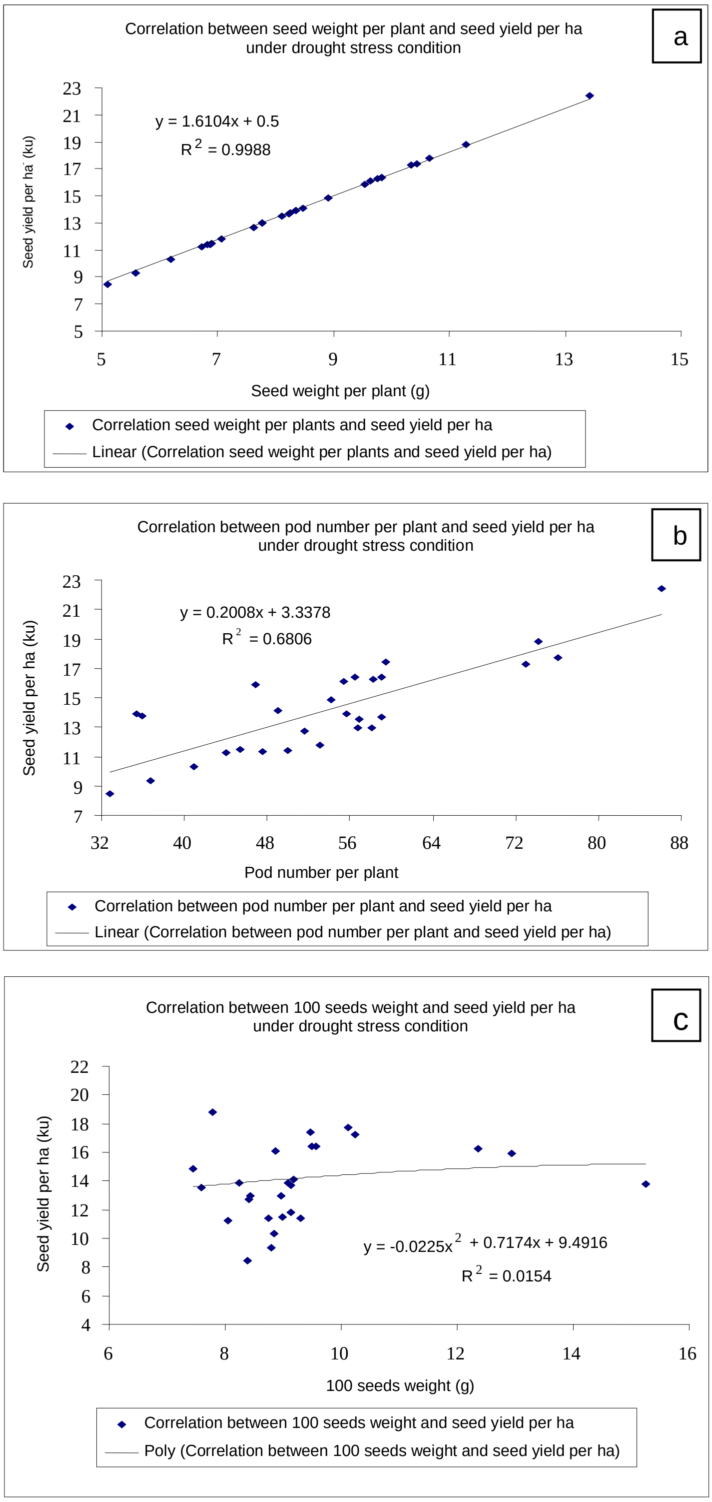

Figure 1. Correlation between yield per ha and yield components: seed weight per plant (a), pod number per plant (b), and 100 seeds weight (c)

Number of pod, seed per plant and 100-seed weight per plant of soybean were affected by irrigation regimes and cultivar (Kobraee *et al*., 2001). Kuswantoro *et al*. (2011) reported that pod number per plant was polygenetically controlled and significantly correlated to seed yield. Pod number is the most important yield component that is injured when stress conditions occurred at early flowering stage (Kobraee and Shamsi, 2011b). Water deficit reduced pod number per plant via decreases in flowering and increases in pod abscission (Desclaux *et al*., 2000). Drought stress decreased photosynthetic rate, leading to carbohydrate deprivation and ultimately inducing pod abortion (Tischner *et al*., 2003). Abortion of flowers and pods was due to water stress in flowering and pod set stages as the main reason for reducing number of pod and seed.plant^{-1}. In addition, drought stress increases leaf senescence and indeed reduces source size in plant (Kobraee and Shamsi, 2011c). Kobraee *et al*., (2011) reported that water stress in seed–filling stage produced grains in smaller number (32%) due to the reduction of grain sizes and weights by shortening duration of grain growth and filling period. Under drought stress conditions, cultivars that had more seed yields than the other ones being more capable of producing sub stem can be more successful in moderating stress effects or in compensating damages caused by stress by producing more sub branches.

Yield components such as seed weight and pod number per plant on soybean variants selected had also been observed. In addition, seed yield per hectare was also observed for resistance to drought on further tests.

CONCLUSIONS AND SUGGESTIONS

There was variability observed in the yield of soybean variants tested, which were derived from *in vitro* selection under drought stress condition. Variant yield was lower than yield of checked variety of Dieng. There was indication that some variants had higher yield than their parents. The results of this study indicated that ten variants were potentially to be developed as drought resistant genotypes. However, the genotypes need to be further tested in field to find out the yield adaptability, stability and its resistance to drought stress.

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