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## RESEARCH ARTICLE

# A GREENHOUSE TEST FOR VIABILITY AND GROWTH YIELD OF VARIOUS SOYBEAN VARIETIES UNDER WATER SHORTAGE CONDITION

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### ABSTRACT

Polyethylene glycol (PEG), a non-ionic, non-toxic, and non-metabolic polymer, and is hard to be broken down by microorganisms. This compound is therefore can be used to induce drought stress to the cultivated plants. The current data were the first published data from three consecutive years of study with the aims to test the adaptability level of seven soybean varieties and find out the two most adaptive varieties before the next step of this three year-based-research. The study applied an experimental study with different concentrations of PEG i.e.: 0%, 10%, 20% and 30% (w.v<sup>-1</sup>) PEG. There were two different phases of study namely test of viability and test of growing ability on the field. The viability test showed that Deja 1 and Depak Kuning were the two most viable varieties in comparison with those three others Dega, Dena 1, Detap 1, and Anjasmoro. Of the physiological characters, those two most viable varieties showed also the best sensitivity index between medium to tolerant. The Tukey test revealed that the treatment of PEG gave a significant effect on the plant's growth as well as yield, although the PEG did not cause significant effect on the chlorophyll content, total chlorophyll and carotene. In terms of proline content, it showed that the PEG reveal that the Anjasmoro variety increased the proline content sharply.

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## INTRODUCTION

In agriculture, drought is defined as a period when the rain falls are lesser than its normal average and will cause the total amount of water in the rhizosphere to be lesser than its normal level for plant's growth leading to a lower yield than supposed to be (Osmolovskaya et al., 2018). Moreover, Kumar et al. (2018) suggested the use of tolerance plants to overcome the drought. To have tolerant soybean plants, Budisantoso and Kamsinah, (2009) for example, reported the use of mutagenic agent colchicine, and Zuyasna et al., (2016) used radioactive irradiation toward the soybean. However, according to Purba et al., (2013), the last two methods were not effective in producing tolerant plants since the use of mutagenic agent colchicine will cause multinucleate plants (Budisantoso and Kamsinah, 2009) and still requested fieldwork before getting a tolerant individual plant. Plants that are forced to be adapted to drought might cause growth reduction as well as their yield; the later the drought comes the more severe the plant's growth. The drought which comes during the vegetative growth phase, for example, will cause dwarfism and reduction in leaves width

In another hand, the drought which comes, during the plant's generative phase may cause a more severe effect like reduction in the total number of blooming flowers, reduce the total number of pods and 100 seeds weight (Budisantoso dan Kamsinah, 2009; (Suryantiet al., 2015), because of plant's physiological disorders like blocking of nutrient translocations and photosynthesis. Hamayun et al., (2010) reported an approach to have such individual plants tolerant to drought by using the polyethylene glycol (PEG) especially because of the PEG is known as a non-ionic long polymer, stable and soluble in water so reduce the water potential (Zuyasna et al., 2016). Some research reports have been published for wheat and rice (Govindarajet al., 2010), and soybean (Hamayun et al., 2010). Purwoko et al. (2013) showed that the use the PEG at different concentrations of 5%, 10%, 15%, and 20% might reveal an osmotic potential of -0.03, -0.19, -0.41, and -0.67 MPa. Moreover, Widyastutiet al., (2016) stated that the use of the PEG at 20-25% caused a reduction in water potential as much as -6.7-9.9 bar. Meanwhile, in a normal situation, the water potential of field capacity is 0.33 bar, reduction of water potential by -9.9 bar will become the critical point to the plant, the plants, therefore, are unable to absorb both water and

Purwoko *et al.*, (2013) also stated that plant's enforcement to drought might be given either during seeds' germination, vegetative growth, and generative growth phases; the drought stress which is given along the seed's germination phases leads to affect the viability of the seeds. Osmolovskaya *et al.* (2018) said that reduction in water absorption will be followed by a reduction in the seeds viability because of the reduction in activity of hydrolytic enzymes. Meanwhile, Budisantoso and Hartiko (2001); Suryanti *et al.* (2015) reported that drought stress which happens during the vegetative and reproductive phases might cause lowering the leaf's size, lowering the photosynthesis rate, lowering the total number of flowers and pods, and finally reduce the total plant's yield but the proline content increased. Applying the PEG at the hydroponic system to groundnuts with a combined growing media of coco peat and rice-husks charcoal (4:1 v.v<sup>-1</sup>), Adisyahputra (2005), reported that the higher concentrations of PEG caused the more severe effect to the plants. However, Song *et al.*, (2010), disagree with the previous data that in their report on paddy-rice varieties might tolerance to drought stress due to the application of the PEG at 20%. Musa (2008) tested the use of the PEG of 100 g.L<sup>-1</sup> to the sugar cane callus and found that of the tolerance varieties 55% callus grew further to form the plantlets at the osmotic potential of -0.5 MPa. The varieties with a mediocre tolerance and sensitivity, however, produced a lower percentage (7% and 4%) of callus grew further and concluded that tolerant capacity of each species or variety was strongly dependent on individual sensitivity to drought stress. Widoretno (2003) stated that the PEG reduces the proliferation, plant's tissues, and even the shoots-regeneration; because of drought stress leads to the increase of proline level in the endogenic and the explant tissues as an important plant physiological response like keeping the turgor pressure at a normal level because of the level is lowering down it might affect further to the physiological and biochemical processes. According to Husni (2006), a factor to keep the turgor pressure is a reduction in osmotic and ability in accumulating soluble nutrients like sugar and amino acids especially in form of proline. Under drought stress, the proline level in the leaves increases because it is function as an osmoregulator to the plants. The excessive production of proline, however, increases the plant's tolerance to drought stress. Such research is testing the tolerance level of soy bean varieties because the PEG level on their growing media becomes a prerequisite. The current study was aimed to force the adaptability level of soybean varieties then selected 2 varieties among them as the most adaptive ones and finally, it is expected to have the tolerant soybeans to drought stress physiologically.

## MATERIAL AND METHODS

7 soybean varieties namely Deja 1, Dega, Dena, Detap, Depak Kuning, Argomulyo, and Anjasmoro obtained from the Balitkabi were used along with this study. The PEG was initially diluted with aquadest to make concentrations of 0%, 10%, 20% and 30% (b.v<sup>-1</sup>). The current study was split into two stages namely: 1. the seeds viability level to germinate under the drought stress because of PEG treatment and 2. test of the plant's growing ability on the field as well as the plant's yield and the proline level contained in the leaf. In the first stage, the soybean seeds were soaked in the PEG solution for 12 hours (Yuanasari *et al.*, 2015). The reduction in plant's viability is calculated with the following formula:

$$\text{Reduction in average viability (\%)} = \left\{1 - \left(\frac{Vs}{Vp}\right)\right\} \times 100$$

Remarks:  $Vs$  and  $Vp$  is average score of reduction in viability at drought stress ( $Vs$ ) and an optimum condition ( $Vp$ ). The soybean seeds were grown in the poly bags and after 10 days after plant (DAP) the germs were then treated with the PEG with an interval of 2 days alternating with water until the plants reached 60 days old. Such parameters like leaf size, total biomass, clean assimilation rate and plant's yield were observed by pulling out the plants from their growing media followed by statistical analysis applying the t-Test at a significant rate of 95 and 99% and followed by the Tukey test at 95% for understanding the differences among the treatments. The obtained data of each parameter was then calculated for its sensitivity index (S) toward drought stress using the formula of Adisyahputra (2005)

$$S = \frac{(1 - Y / Yp)}{(1 - X / Xp)}$$

Remarks: Y and Yp, each of them is an average score for observed data of particular soybean variety at particular drought stress (Yp) and an optimal condition (Y). X and Xp, each of them is an average score for observed data of all soybean varieties at particular level of drought stress (Xp) and an optimal condition (X).

The sensitivity index is grouped as follows:

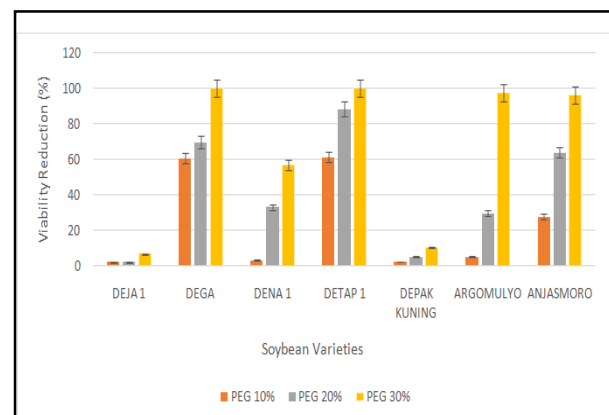
$S \leq 0,5$  tolerance to drought stress.

$0,5 < S < 1$  a mediocre level of tolerance to drought stress.

$S \geq 1$  sensitive to drought stress.

## RESULTS

The current data revealed that the Deja 1 and Depak Kuning varieties showed as the least severe varieties when water shortage is given during their germination time (Figure 1),



**Figure 1. The histogram of viability level among the soybean seeds test for their adaptability in viability treated with the PEG**

where the PEG concentration of 30% (b.v<sup>-1</sup>) could only reduce their viability of less than 10%. On the other hand, a much higher level of viability reduction was noted from the rest of tested varieties even leads to death on Dega variety. Interesting phenomenon showed by the Argomulyo variety, which was quite promising when the PEG concentration was

**Table1. The Tukey test of the effect of variety to growth rate and yield parameters**

Variety	Fresh weight 1	Fresh weight 2	Dry weight 1	Dry weight 2	Total No of pods	Chlorophyll A	Total Chlorophyll	Carotene
Deja 1	3.750bc	10.669a	0.693ab	2.603a	13.250ab	11.928a	0.791bc	2.413a
Dega	3.969abc	10.137a	0.918a	2.773a	7.750b	11.557a	0.949ab	2.333a
Dena	5.447a	10.880a	0.909a	3.183a	14.083ab	12.422a	0.985a	2.453a
Detap	2.853c	6.263a	0.569b	2.008a	10.333b	10.066a	0.6290c	1.965a
Depak Kuning	2.852c	9.157a	0.598b	2.718a	17.083a	9.981a	0.7979b	1.930a
Argomulyo	5.034ab	7.167a	0.792ab	2.385a	11.583ab	11.303a	0.929ab	2.274a
Anjasmoro	3.128c	6.835a	0.601b	2.152a	13.333ab	10.547a	0.160d	2.382a

Remarks: Numbers followed by the same letters in the same column showed not significant at the Tukey at 95% different.

**Table 2. The Tukey test of the PEG application to the plant's yield**

PEG concentrations	Fresh weight 1	Fresh weight 2	Dry weight 1	Dry weight 2	Total number of pods	Chlorophyll A content	Total chlorophyll	carotene
Control	4.554 a	11.489 a	0.840 a	3.226 a	17.238 a	10.834 a	0.759 a	2.147 a
PEG 10%	3.509 b	9.0981 ab	0.763 ab	2.651 ab	11.761 b	10.767 a	0.746 a	2.159 a
PEG 20%	3.598 ab	7.7889 b	0.664 ab	2.049 b	11.333 b	10.8617 a	0.726 a	2.215 a
PEG 30%	3.827 ab	6.544 b	0.636 b	2.258 b	9.619 b	11.997 a	0.764 a	2.478 a

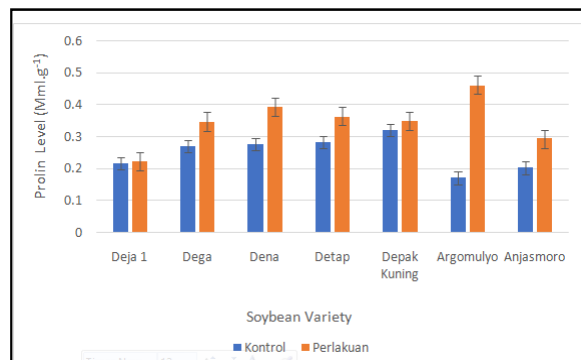
Remarks: Numbers followed by the same letters in the same column showed a non significant result at 95%.

**Table 3. The growth sensitivity index of soybean following the PEG applications**

No	Varieties	The PEG concentrations (%)	Fresh Weight 20 days after planting (DAP)	Dry weight 20 days after planting (DAP)	Fresh weight 40 days after planting (DAP)	Dry weight 40 days after planting (DAP)	Clean assimilation rate
1	Deja 1	0	0	0	0	0	0
		10	0.506	1.833	0.907	1.323	0.071
		20	1.32	1.154	3.362	4.925	0.196
		30	0.575	2.166	2.191	2.967	0.049
			Mediocre-sensitive	Sensitive	Sensitive	Sensitive	Tolerance
2	Dega	0	0	0	0	0	0
		10	0.648	3.549	0.470	0.372	0.226
		20	0.268	1.578	1.367	1.071	0.842
		30	0.157	0.160	1.021	0.926	0.681
			Mediocre-sensitive	Sensitive	Sensitive	Sensitive	Mediocre
3	Dena	0	0	0	0	0	0
		10	0.821	5.381	0.964	1.167	0.618
		20	0.334	1.958	0.348	0.348	0.200
		30	0.36	1.659	0.498	0.414	0.204
			Mediocre-sensitive	Sensitive	Mediocre	Mediocre-sensitive	Mediocre-sensitive
4	Detap	0	0	0	0	0	0
		10	0.345	1.249	0.379	0.746	15.348
		20	1.315	6.569	4.525	0.153	6.989
		30	0.556	1.229	0.210	0.088	1.870
			Mediocre-sensitive	Sensitive	Mediocre-sensitive	Mediocre	Sensitive
5	Depak Kuning	0	0	0	0	0	0
		10	0.231	1.498	0.537	0.681	0.029
		20	0.087	0.371	0.218	0.167	0.336
		30	0.378	2.173	0.730	0.313	0.181
			Mediocre	Mediocre	Mediocre	Mediocre	Tolerance
6	Argo Mulyo	0	0	0	0	0	0
		10	0.189	0.891	0.301	0.058	0.500
		20	0.236	1.329	6.252	5.693	0.505
		30	0.111	0.441	2.396	2.935	0.238
			Mediocre-sensitive	Sensitive	Sensitive	Sensitive	Sensitive
7	Anjas Moro	0	0	0	0	0	0
		10	0.498	2.545	1.245	1.189	0.150
		20	0.128	1.386	2.166	2.659	0.058
		30	0.056	0.039	1.459	2.055	0.413
			Mediocre-sensitive	Sensitive	Sensitive	Sensitive	Mediocre

this variety does no longer show its strong viability. Unfortunately, this study did not analyze further its physiological character which made this variety has totally performance on facing the give water shortage. The Tukey test (Table 1) applied for all growth rate parameters of various soybean varieties showed that from the fresh weight 2, the dry weight 2, total number of pod per plant, and total chlorophyll, which were analyzed at the end of the study showed a consistency in physiological effect of those two varieties with the strongest viability (Deja 1 and Depak Kuning).

In this table these two varieties revealed a consistent results of highest or at least higher than the rest varieties. Means tht the better viability during germination time the better growth rate would be resulted. However, these two most viable varieties, showed similar carotene content with the rest varieties. Of the plant's yield parameters which are related with the effect of PEG concentrations from absent to the highest of 30% (Table 2). The control block (the PEG was absent) always showed the highest number in all parameters analyzed. Means that at field capacity of water availability in the soil, all varieties of soybean grew well.



**Figure 2. The level of Proline contains in the individual soybean plant**

However, when the concentrations were increased the results seemed a slight inconsistency, where the numbers at the concentration of 30% PEG not always the least number one. Analysis of soybean-growth sensitivity toward the PEG concentrations given (Table 2) showed that Depok Kuning was the only consistent variety starting from the germination viability test up to the plant's yield. In this table the Depok Kuning variety has either mediocre level of sensitivity or tolerant, the Deja 1 variety, however, showed an inconsistency in fresh weight and dry weight observed at 20 and 40 days after planting and the rest varieties showed more sensitive to the PEG concentration given. Figure 2 supported all data of the current study especially when observations were focused on the seeds' germination viability as well as growth and yield production (Table 1 and Table 2). At these two varieties, the level of proline, which was released by the plant to overcome water shortage showed almost similar level between control and treated plants. But, at the rest varieties, the proline level showed much higher than the control plants.

## DISCUSSION

Anonymous (2016), described that the Deja 1 variety is known as a tolerant variety to excessive water, the current data, therefore, partially completing the earlier report, since the Deja 1 variety was also tolerant to water shortage as shown by the reduction of its germination viability by less than 10% following the application of the PEG, which caused water shortage in the soil (Table 1). This fact-finding showed that the Deja 1 variety has a superior characteristic as it could be tolerant in both situation of excessive water and also in the drought stress. However, the last finding of tolerant in the drought stress condition, needs a further study before declaring it. Genetically, the Deja 1 variety is a crossbred between Tanggamus and Anjasmoro varieties, while the Depok Kuning came from a selection results (Anonymous, 2016), and so, it might be concluded that these two varieties, of Deja 1 and Depok Kuning, have already a tolerance character to drought stress. On the other hand, the Dega and Detap 1 varieties, at the PEG concentration of 30% (b.v<sup>-1</sup>) reduce their viability rate of up to 100%, no seed was viable to germinate. The PEG which is given to the soil, blocks the water absorption process by the seeds, while additional water becomes important factor for the seed to germinate (Suryanti *et al.*, 2015); to increase the activity of hydrolytic enzymes, to allow the embryonic cells to grow and develop germs (Ai, 2010).

On the Tukey test (Table 1), the plant's growth rate and yield of the soybean varieties, somehow, showed that the Dega, Dena, and Argomulyo had a relatively similar vegetative growth rate, but they were different from Deja 1, Detap, Depok Kuning, and Anjasmoro. Of the yield parameter for example, the Depok Kuning variety produced the highest number of pods per plant, although these numbers, statistically, were not significantly different from those pods numbers produced by the Dena and Deja 1 varieties. These data, indicate that of the viability, growth rate and plant's yield parameters, the Depok Kuning, Dena and Deja 1 were concluded as suitable varieties for being treated as parental seeds in the next planting season. The Tukey test of PEG effect to the plant's growth and yield (Table 2) showed that the concentrations of PEG given showed a significant different to fresh, and dry weight and the total number of pods per plant, but showed a different result to total chlorophyll a and carotene parameters. The current data, therefore, parallel to those reported previously by Hamayun *et al.*, (2010); where the effects of the drought stress on soybeans were strongly dependent on the concentrations of the PEG. That study also stated, that both fresh and dry weight, will reduce when the PEG is administered to soil during the early vegetative growth phase up to the early generative phase of the plants; the current study, however, revealed that the PEG concentrations applied did not significantly affect to the chlorophyll content.

Moreover, this study also showed that the PEG concentrations reduced the plant's yield not significantly, meant that the PEG induced drought stress and block water and nutrient absorption only since at the PEG concentration of 30% (b.v<sup>-1</sup>) did not reach its lethal dose, that might be because of the use of water in irrigating the plants in between the application of PEG lead to a small recovery in the plants. Of the sensitivity index, calculated from the clean assimilation rate, the current data showed that the Deja 1 and Depok Kuning had a tolerance sensitivity. Meanwhile, the rest varieties had a mediocre level of tolerance to sensitivity. The clean assimilation rate is calculated from the total amount of water in the soil, to represent assimilation rate of the plant dry matters. The higher the clean assimilation rate the better environment for the plant's growth. Of the proline level contains in the leaf, the study noted that the proline level increased in all varieties (Figure 2), indicating the PEG applied to the growing media caused the drought stress to the treated plants (Husni, 2006). The level of drought stress depends on the plant's variety as shown by the Deja 1 and Depok Kuning where the accumulation levels were relatively smaller than other varieties.

## CONCLUSION

The current study might conclude that the Deja 1 and Depok Kuning showed a better viability and sensitivity index than the rest varieties and so will be used in the next year's planting season to reach an adaptable variety to drought stress.

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