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In Vitro Study of Potato (*Solanum tuberosum* L.) Tolerant to the Drought Stress

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Abstract

In vitro preliminary studies is candidate tolerant of potato (*Solanum tuberosum* L.) to the drought stress. This study aimed to determine the characters of potato after being exposed to *in vitro* drought stress conditions using Polyethylene Glycol PEG. This research was conducted at the Tissue Culture Laboratory, UPT Balai Benih Induk Hortikultura Dinas Pertanian Provinsi Sumatera Utara, in Medan and other places in January 2015 until May 2015. This study used Completely Randomized non Factorial Design namely PEG (P) comprising of two levels, namely: P1 = 5 000 mg · L^{-1}, P2 = 6 000 mg · L^{-1}. The results showed that increasing the concentration of PEG resulted in reduction of the percentage of plantlets survival, reduced plantlets height and plantlets dry weight but the increase in the total protein and leaf chlorophyll. Although the banding pattern is relatively the same, there is a brighter visible banding pattern on potato plantlets with OPAA 09 in the range of 65 bp to 75 bp with sequences of GTGGGTGCCA.

Keywords: tolerant; drought stress; *in vitro*; RAPD.

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1. Introduction

Potato is one of the alternative commodities that supports government programs for food security. In recent years, the need for potatoes is likely to increase and this plant is one of the priority commodities to be developed. Potato in Indonesia is a source of carbohydrate in the food diversification program. Polyethylene Glycol (PEG) is reported to be able to hold water so that it is not available to plants. The amount of PEG solution to hold water depends on its molecular weight and concentration [1], it is soluble in water, not toxic to plants, and is not easily absorbed, making PEG as an effective compound to stimulate drought conditions [2]. Selection methods were developed using a solution of PEG that could be expected to identify potato plants respond to drought stress *in vitro* thus it might also consistent *ex vitro*. Van Sint January et al. [3] also argued the consensus that drought tolerant plants in field are also mediated at the cellular level. The results of this study were expected later to produce potato plants that did not require too much water in the metabolism and could be grown in the lowlands. The research focused on PEG and RAPD although reproducibilty of the RAPD profile is still the centre of debate [4].

2. Materials and methods

The experiment was conducted at the Tissue Culture Laboratory Unit Laboratorium Kultur Jaringan UPT/Balai Benih Induk Hortikultura Dinas Pertanian Propinsi Sumatera Utara, Medan and other places in January 2015 to May 2015. This study used a half strength basal media Murashige and Skoog ((½ MS) and Completely Randomized non Factorial Design namely PEG (P) comprising two levels, namely: P1 = 5 000 mg · L^{-1}, P2 = 6 000 mg · L^{-1}. Explants were then planted on 1/2 MS medium with the addition of PEG. PEG effect was separately extracted from a factorial treatment. The parameters observed at the end of the study was percentage of plantlets survival (%), plant height, dry weight, total protein and leaf chlorophyll (Faculty of Agriculture Laboratory, UISU), RAPD analysis was done in Laboratorium of Institute of Research and Development of Agriculture—Center for Research and Development of Biotechnology and Genetic Resources of Agriculture, Bogor.

3. Results and discussion

3.1. Percentage of planlet survival

Concentration of PEG was significantly reducing the percentage of plantlets survival. The percentage of plantlets survival at the age of 6 wk was only 47 % after 6 000 mg · L^{-1} PEG treatment. The higher the concentration of PEG treatment, the lower the plantlets survival percentage. It meant that potato explants were sensitive to *in vitro* PEG treatment.

Results of research were arranged in the following Table:

Treatment of PEG	Effect of PEG on plantlets height (mm) (weeks after planting)					
	1	2	3	4	5	6
P1	1.80 $^{(a)}$	2.05 $^{(a)}$	3.79 $^{(a)}$	5.37 $^{(a)}$	6.38 $^{(a)}$	6.79 $^{(a)}$
P2	2.01 $^{(a)}$	2.21 $^{(a)}$	3.39 $^{(a)}$	4.32 $^{(b)}$	4.77 $^{(b)}$	5.11 $^{(b)}$
Note: numbers followed by the same letters on the same column is not significant with test level at α 0.05						

Table 1: Plantlet height (mm).

Table 1 showed that in this case, the decline in water potential by the addition of PEG decreased the proliferation of tissue, the growth and the shoot regeneration. This is reasonable because it caused the decrease in cell growth, cell wall synthesis and

Treatment of PEG	Effect of PEG on plantlets dry weight (g) (6 wk after planting)
	Dry weight (g)
P1	0.77 [(a)]
P2	0.74 [(a)]
Note: numbers followed by the same letters on the same column are not significantly different with test level at α 0.05	

Table 2: Plantlets dry weight (g).

Treatment of PEG	Effect of PEG on total protein (%) (6 wk after planting)
	Total protein (g)
P1	0.73 [(a)]
P2	0.83 [(a)]
Note: numbers followed by the same letters on the same column are not significantly different with test level at α 0.05	

Table 3: Effect of PEG on total protein (%).

protein synthesis. The results of our previous studies revealed that the content of proline increased at higher PEG treatment [5].

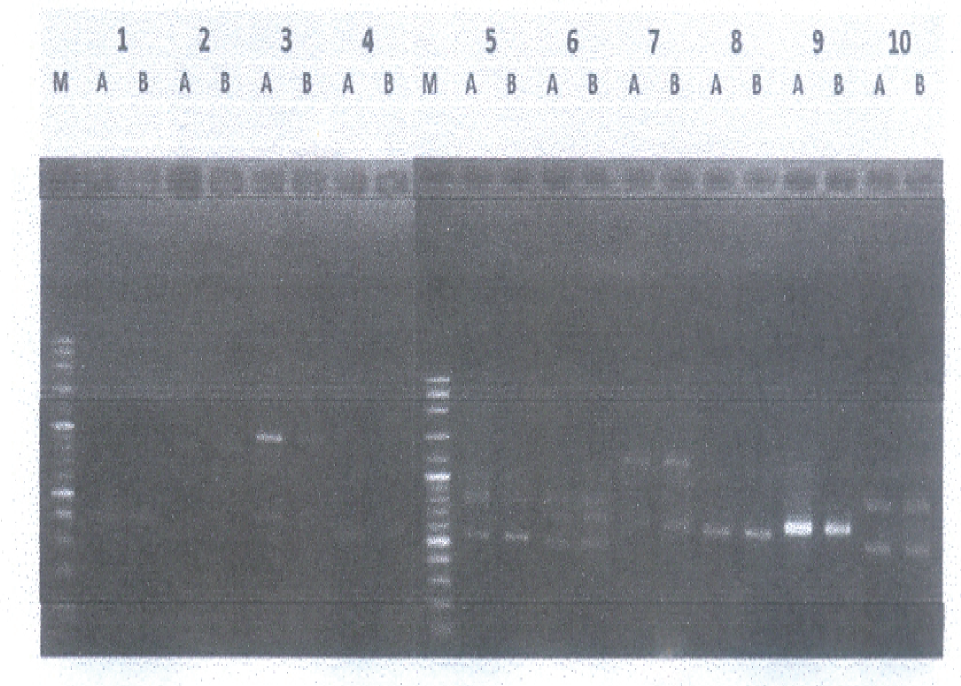
3.2. Plantlets dry weight

The use of PEG as osmoticum stress was as suspected, could reduce the cell elongation and expansion, which in turn lowered the plantlets dry weight. Charloq et al. [6] and Sirait et al. [5] also reported that PEG treatment had significantly lowered the number of roots, nodes and leaves, as well as the plantlets height and dry weight.

The higher the concentration of PEG treatment the lower the dry weight (g). PEG on 6 000 mg $\cdot$ L^{-1} lowered caused the water potential of the medium lower so that the water of the medium was inhibited into the plantlets. The absence of water was one of the abiotic stresses which inhibited the growth and the development of plants. Water is also an important reagent in photosynthesis and hydrolysis reactions. The higher levels of total protein and chlorophyll contained in plantlets were the markers of drought tolerance or an adaptation of the plantlets (Table 3 and Table 4).

Treatment of PEG	Effect of PEG on leaf chlorophyll (unit $\cdot$ mm^{-3}) (6 wk after planting)
	Leaf chlorophyll (unit $\cdot$ mm^{-3})
P1	30.6 [(a)]
P2	34.2 [(b)]
Note: numbers followed by the same letters on the same column are not significantly different with test level at α 0.05	

Table 4: Effect of PEG on leaf chlorophyll (unit $\cdot$ mm^{-3}).



Notes:
1. Primer OPE 05
2. Primer OPAA 01
3. Primer OPAA 09
4. Primer OPAA 19
5. Primer OPK 12
6. Primer OPX 17
7. Primer OPC 20
8. Primer OPAA 03
9. Primer OPAA 06
10. Primer OPAA 10

M= Ladder 100 bp
A= Potato plantlets
B= Potato tubers

Figure 1: Electrophoresis results of DNA amplification with some primers (selected) of *in vitro* potato plantlets after PEG treatment.

Although the banding pattern is relatively the same, there is a brighter visible banding pattern on potato plantlets with OPAA 09 in the range of 65 bp to75 bp with sequences GTGGGTGCCA (Figure 1).

4. Conclusions

Increasing the concentration of PEG resulted in reduced percentage of plantlets survival, the plantlets height and dry weight. Conversely, the higher the PEG concentration treatment, the higher the total protein and leaf chlorophyll in the plantlets. There was a brighter banding pattern on potato plantlets with OPAA primer sequences of GTGGGTGCCA 09 with the size of 65 bp to 75 bp. This marker was expected to be a factor of differentiation in candidate of Potato tolerant (*Solanum tuberosum* L.) to the *in vitro* drought stress.

5. Suggestion

In vitro drought tolerant potato needs to be evaluated in physiological, biochemical, molecular character and in the field experiment.

References

[1] Mexal J, Fisher JT, Osteryoung J, Patrick RCP. Oxygen availability in polyethylene glycol solutions and its implications in plant-water relation. Plant Physiology 1975;55:20–24.

[2] Mullahey JJ, West SH, Cornell, JA. Effects of simulated drought bypolyethylene glycol on Bahiagrass germination [Online] from http://cat.inist.fr/?aModele=afficheN&cpsidt=3216841. Seed Science and Technology 1996;24:219–224. [[Accessed on March 15th 2016].

[3] Van Sint Jan V, de Macedo CC, Kinet JM, Bouharmont J. Selection of Al-resistant plants from a sensitive rice cultivar, using somaclonal variation, *in vitro* and hydroponic cultures. Euphytica 1997;97:303–310.

[4] Bardakci, F. Random amplified polymorphic DNA (RAPD) markers. Turkish Journal of Biology 2001; 25: 185–196.

[5] Sirait BA, Roselyna IR, Charloq. Growth and proline content of potato (*Solanum tuberosum* L.) *in vitro* candidate tolerant to drought of origin callus. In: Proceedings of the 3rd Annual International Conference Syiah Kuala University (AIC Unsyiah) 2013, in conjunction with the 2nd International Conference on Multidisciplinary Research (ICMR), 2–4 Oct. 2013. Muchlisin ZA, Darmadi, Supradan MD (Eds.). Banda Aceh, Indonesia. p. 366–370.

[6] Charloq, Panjaitan E, Sirait BA. Study of early screening of potato (*Solanum tuberosum* L.) as a result of drought stress (*in vitro*). Paper prented in the 2nd Annual International Conference Syiah Kuala University 2012 & The 8th IMT-GT Uninet Biosciences Conference, 22nd to 24th November 2012, Banda Aceh, Indonesia, p. 403–405.