

The effects of different concentration of sucrose and various auxin on in vitro shoot and microtuber formation of red potato (*Solanum tuberosum*, L. var Desiree)

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Abstract. The experiment was aims to study the effect of different sucrose concentrations (30 , 90, 120 and 150 g.L⁻¹) and various types of auxin (IAA, IBA, NAA) on in vitro shoot and microtuber formation of Red Potato (*Solanum tuberosum*, L. var Desiree). Young stem cutting from planlet cultured on half strength MS medium. All types of auxin use the concentration of 0.25 mg.L⁻¹. The research conducted a completely randomized design (CRD) factorial. The data obtained were analyzed by analysis of variance (ANOVA) and DMRT test at 0.05. Parameters observed included growth of microtuber and shoot growth, such as number of shoots and shoot length. The observations result for 3 months, show that the higher the concentration of sucrose, can stimulate tuber formation but suppress shoot growth. Early tuber initiation (36,7 DAP) and optimum number of tuber was observed in 150 g.L⁻¹ sucrose, whereas at 30g.L⁻¹ had no effect to tuber induction. In addition, high number of microtuber was observed in NAA compare to other auxin. Observational data on shoot growth showed that the higher concentration of sucrose will inhibit bud formation. The 90 g.L⁻¹ sucrose concentration showed the best number of shoots, which averaged 1.5 shoots/plantlet. But at a concentration of 30g/L sucrose, explants are only able to form callus. In the bud length parameter, IAA gave the best response that is an average of 3cm/shoot.

1. Introduction

The high productivity of potato is still constrained by availability of quality potato seeds, conventional cultivation techniques, as well as limited places with high temperatures and temperatures suitable for potato cultivation. In general, potato cultivars cultivated by farmers are highland potatoes (700 masl) such as Granola and Atlantic. But there are also potatoes that can grow well in medium plains such as Desiree cultivars [1][2]. That potatoes are also called “Red potatoes” because they have reddish bulbs [3]. These cultivars can potentially be developed to meet national potato needs.

The process of developing these cultivars must be supported by the provision of superior seeds through in vitro culture techniques. Through this technique, tubers (G0) and micro shoots can be produced in large quantities in a relatively short time. The composition of the media and growth regulating substances play an important role in spurring the growth of tubers and potato shoots in vitro. In addition, sucrose concentration is also one of the factors that need to be considered. Some researchers claim that sucrose is able to induce potato micro tubers [4]–[6]. Therefore, this study aims



to determine the effect of some sucrose concentrations on in vitro shoots of Desiree potatoes in inducing micro bulbs.

2. Methods

This research was held at the Plant Tissue Culture Laboratory, Department of Agricultural Production, Jember State Polytechnic. Material used in this study were sucrose (30, 90, 120 and 150 g.L⁻¹), various types of auxin (0.25 mg.L⁻¹ IAA, IBA, NAA), young stem cutting from planlet, half strength MS medium. The research conducted a completely randomized design (CRD) factorial. The data obtained were analyzed by analysis of variance (ANOVA) and DMRT test at (p<0.05). Parameters observed included percentage of callus, microtuber and shoot formation, and also growth of microtuber and shoot, such as number of shoots and shoot length.

3. Results and discussion

3.1 Emergence, number and fresh weight of microtubers

Table 1. Comparison of means and interaction of Auxin type and sucrose levels in the media for microtubers emergence (DAP).

Auxin (0,25 mg.L ⁻¹)	Sucrose Level				Mean of Auxin
	30 g.L ⁻¹	90 g.L ⁻¹	120 g.L ⁻¹	150 g.L ⁻¹	
IAA	0.0	45.6	46.8	40.6	55.8 ^a
IBA	0.0	45.0	45.4	40.2	55.2 ^a
NAA	0.0	35.6	34.6	29.2	47.4 ^b
Mean of Sucrose	0.0 ^a	42.1 ^c	42.3 ^c	36.7 ^b	

^a at 30g.L⁻¹ no tuber appears

^b The number followed by the same letter are not significantly different at (p<0.05) level of Duncan's test.

Table 2. Comparison of means and interaction of Auxin type and sucrose levels in the media for microtubers number per planlet.

Auxin (0,25 mg.L ⁻¹)	Sucrose Level				Mean of Auxin
	30 g.L ⁻¹	90 g.L ⁻¹	120 g.L ⁻¹	150 g.L ⁻¹	
IAA	0.0	0.2	0.1	0.7	0.3 ^a
IBA	0.0	0.4	0.4	0.9	0.4 ^a
NAA	0.0	1.1	1.5	1.5	1.0 ^b
Mean of Sucrose	0.0 ^a	0.6 ^{ab}	0.7 ^b	1.0 ^c	

^a The number followed by the same letter are not significantly different at (p<0.05) level of Duncan's test.

Table 3. Comparison of means and interaction of *Auxin type* and sucrose levels in the media for fresh wight of microtubers (g).

Auxin (0,25 mg.L ⁻¹)	Sucrose Level				Mean of Auxin
	30 g.L ⁻¹	90 g.L ⁻¹	120 g.L ⁻¹	150 g.L ⁻¹	
IAA	0.0	1.2	0.4	0.6	0.6
IBA	0.0	1.2	1.4	1.2	1.0
NAA	0.0	1.6	1.8	1.8	1.3
Mean of Sucrose	0.0 ^a	1.3 ^b	1.2 ^b	1.2 ^b	

^a The number followed by the same letter are not significantly different at (p<0.05) level of Duncan's test.



Figure 1. Emergence of microtuber



Figure 2. 90 days old microtubers of red potato

Based on the ANOVA test, giving some sucrose concentrations combined with several types of auxin did not show a real effect. The emergence of microtubers (**Figure 1**) was first seen in the media with the addition of NAA and 150 g.L^{-1} sucrose at 29.2 DAP (**Table 1**). In the treatment media also obtained the highest number of tubers, which is an average of 1.5 tubers per plantlet (**Table 2**). Both of these factors show a very real influence on a single basis. The formation of micro tubers is strongly influenced by sucrose as an energy source. Tubers on potato plants are also a place of energy storage for plants. In the process of micro tuber formation, sucrose as an external source of carbon is able to stimulate cell development to form tubers [7], [8]. The higher the concentration of sucrose affects the process of cell differentiation set the Sink tissue [9][10]. This is seen in the media with the addition of 30 g.L^{-1} of sucrose is not able to stimulate tuber formation, only grows callus.

Type of auxin also affects tuber formation. NAA is able to accelerate tuber formation faster compare to other types of auxin tested. NAA act to increase the formation of nodes in potato stems which then become a place for tubers to grow[11]. The role of NAA is also seen in the process of cell division and enlargement at the stolon, then swelling occurs at the stolon tip[12].

In the parameters of fresh weight of micro tubers, the effect of interactions between auxin types and some sucrose concentrations also did not show any significant differences, nor did auxin factors singly. But the sucrose factor alone affects the fresh weight of tubers (**Table 3**), although between 90- 150 g.L^{-1} sucrose concentration was not significantly different based on the results of Duncan's test. The difference is seen only at a concentration of 30 g.L^{-1} which is no tuber that grows. Of the several concentrations tested, the fresh weight of tubers that were induced in a medium with a concentration of 90 g.L^{-1} showed a value greater than the level of other concentrations. The greater the concentration of sucrose is thought to be the cause of reduced tuber cell volume. Sucrose as a carbon and energy source for plants, also plays a role in the process of regulating osmotic pressure, therefore there is a change in osmotic potential so the absorption of water becomes reduced to increase the volume of tuber cells [10].

3.2 Number and length of shoots

Table 4. Comparison of means and interaction of *Auxin type* and sucrose levels in the media for number of shoots.

Auxin ($0,25 \text{ mg.L}^{-1}$)	Sucrose Level				Mean of Auxin
	30 g.L^{-1}	90 g.L^{-1}	120 g.L^{-1}	150 g.L^{-1}	
IAA	0.0	2.0	2.2	1.4	1.4
IBA	0.0	2.2	1.6	1.0	1.2
NAA	0.0	3.0	1.6	2.0	1.7
Mean of Sucrose	0.0^a	2.4^c	1.8^{bc}	1.5^b	

^a The number followed by the same letter are not significantly different at ($p < 0.05$) level of Duncan's test.

Table 5. Comparison of means and interaction of *Auxin type* and sucrose levels in the media for shoot length (cm).

Auxin (0,25 mg.L ⁻¹)	Sucrose Level				Mean of Auxin
	30 g.L ⁻¹	90 g.L ⁻¹	120 g.L ⁻¹	150 g.L ⁻¹	
IAA	0.0 a	6.6 f	5.1 e	0.3 ab	3.0 ^a
IBA	0.0 a	0.5 b	0.5 b	0.2 ab	0.2 ^b
NAA	0.0 a	2.9 d	1.3 c	0.3ab	1.1 ^b
Mean of Sucrose	0.0 ^a	3.2 ^b	2.3 ^b	0.3 ^a	

^a The number followed by the same letter are not significantly different at (p<0.05) level of Duncan's test.

**Figure 3.** Shoots formation in MS medium containing 90 g.L⁻¹ sucrose

ANOVA analysis results showed that the interaction factor between sucrose concentration and some auxin types did not significantly affect the number of shoots (**Table 4**), but had a significant effect on shoot elongation. The interaction between IAA and 90 g.L⁻¹ of sucrose produces the longest shoot, which is an average of 6.6 cm (**Table 5**). The results of the analysis also show that the level of sucrose concentration alone affects the number and length of shoots. Sucrose at a concentration of 90 g.L⁻¹ is able to produce better shoots (**Figure 3**), which is an average of 2.4 shoots per explant as well as the length of shoots from that concentration on average 3.2 cm. The higher the concentration of sucrose added is thought to suppress shoot growth. Seen on sucrose with a concentration of 150 g.L⁻¹ produces the least number of shoots and is shorter than other concentrations. High sucrose concentration can quickly accumulate on the shoots of explants so that it stimulates stolon formation, not bud formation[13].

The role of auxin shows significant differences in shoot elongation. Whereas in the formation of shoots, some types of auxin show unreal differences. The role of IAA in bud elongation looks better because the resulting shoots are longer than other types of auxin, which is an average of 3 cm. IAA is a hormone that is also produced endogenously by plants [14]. It is suspected that the role of IAA added to the media is able to interact with IAA produced by the plant itself, so that it is more optimal to stimulate stem lengthening [15].

4. Conclusions

From this study it can be concluded that the combination of several types of auxin and sucrose concentration did not show any significant differences in the formation of micro tubers and shoots, but the interaction between the two was seen in the stem length parameters. The single auxin and sucrose factors greatly influence the tuber formation process. The use of NAA and 150 g.L⁻¹ sucrose was able to bring up the first tuber, which was 36.7 DAP and produced more tubers than the other treatments, namely an average of 1 tuber per plantlet. But at 90 g.L⁻¹ sucrose, it produces more bulbs than higher

concentrations of sucrose. Visually, the media with the addition of 30 g.L⁻¹ sucrose only occurs in the formation of callus which is unable to form tubers or shoots. The most shoots (2.4 shoots per explant) and the longest (3.2 cm per shoot) were seen in the media with an addition of 90 g.L⁻¹. From the auxin factor, IAA is better able to stimulate stem elongation than other types of auxin.

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6. References

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