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4 The effect of murashige and skoog (MS) modified medium and several types of auxins on the growth of stevia (*Stevia rebaudiana bertonii*) in vitro

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Abstract. The aims of this research is to testing the use of several types of auxins with the same concentration (0.2 ppm IAA; 0.2 ppm NAA; 0.2 ppm IBA) and modification of MS media (1/4 MS; 1 / 2MS; MS full). Parameters observed included roots number, roots length, plantlets length and velocity of root emergence. The result shown that roots appeared influenced by the modification of MS media, the fastest root appearance was in the treatment using full MS and 1/2 MS on 14 and 15 DAS (day after showing). The several types of auxin and the modification of MS media were very significant. The number of roots parameter also resulted from the interaction between the auxin type and the modification of MS media which had a very significant effect. At the end of the observation (30 DAS) highest number of roots with an average of 4.23 was found in the using NAA treatment, with 1/2 MS. The modification of MS media is more optimal increasing roots length is at 1/2 MS. The modification of MS media which is more optimal in increasing length of plantlets at full media and was not significantly different with 1/2 MS.

1. Introduction

Stevia is currently a plant that has the potential to be developed on a large scale in Indonesia [1]. This plant is a natural sweetener that has the potential to be an alternative to natural sweetener plants beside sugar. Stevia's sweetness level is up to 200-300 times higher than sugar [2]. The sweet taste produced from stevia comes from the content of steviolosides and rebaudiosides [3]. Sweeteners made from stevia are high in antioxidants and low calories, making them good for diabetics too [4]. The process of developing stevia to obtain quality, uniform, multiplied seedling in large quantities in a relatively short time can use in intro culture techniques [5]. Technically, the stevia plantlet must be sturdy and well rooted before it is ready to be acclimated. Some of the obstacles in acclimatizing seedlings are slow growth due to poor root systems.

From several previous studies, the use of MS media modification had an effect on the growth of stevia roots [6]. In addition, the type of auxin also affects the growth of the roots of the stevia plantlet [7]. In previous studies, researchers had also conducted research to stimulate the multiplication of the stevia shoots [8], [9] but the micro shoots were not immediately acclimatized. Further research is needed to determine the right type of auxin and optimal modification of MS media to improve plantlet performance and in the particular to stimulate roots to be ready for acclimatization. Based on the previous explain, the purpose of this research is to testing the use of several types of auxins with



same concentration (0.2 ppm IAA; 0.2 ppm NAA; 0.2 ppm IBA) and modification of MS media (1/4 MS; 1 / 2MS; MS full).

2. Material and methods

2.1. Collection of plant material

The plant were collected from green house of Plant Tissue Culture Laboratory, Department of Agricultural Production, Politeknik Negeri Jember-Indonesia.

2.2. Surface sterilization

Explants were washed thoroughly under running tap water for 10 min, followed by treatment with 2g.L⁻¹ fungicide and bactericide for 15 min. Then, the explants were sterilized in 70% ethanol for a minute, and finally with 5% Clorox for 2 min and washed 5 times with sterile distilled water, and then internode explants were cut to a size of 1 cm and leaves explants were cut to a size 1 cm²

2.3. Culture medium

MS medium containing 3% sukrose, gelled with 0,7 % agar and 0,25 mg.L⁻¹ IAA supplementation is used basic media in the study. In addition, different concentration of MS medium (1/4 MS, 1/2 MS and full MS) and Some of auxin types (without auxin, IAA, NAA and IBA) with same concentration (0,2 ppm) is added as treatment.

2.4. Sub culturing

Explant were cultured on MS 0 media first for 2 weeks. Then new stem and leaves subcultured on each treatment medium. Incubation in the growth chamber at 22oC with 18 hours of light and 6 hours of darkness.

2.5. Parameters and data analysis

This research tested the response of growing internode and leaf explants at different MS concentration and different auxin type using a factorial complete randomized design (CRD) with 5 replication. The data obtained were analyzed by Analysis of Variance (ANOVA) and DMRT test at ($p < 0,05$). The parameters observed related to roots growth include: roots initiation time, length of plantlet, number and length of roots.

3. Result and discussion

3.1. Number and length of roots and length of plantlets

Table 1. Average roots number, roots length and plantlets length at different modification of MS media and several types of auxin treatment at the age of 30 DAS

Treatment	Level	Parameters		
		Roots number	Roots length (cm)	Plantlets length (cm)
Types of auxin	Without Auxin	3.24 b	1.06 ab	6.86 a
	IAA	1.79 c	1.10 a	6.50 a
	NAA	4.23 a	0.96 b	6.81 a
	IBA	1.68 c	0.75 c	5.29 b
Modification of MS media	Full	2.81 b	1.22 b	8.30 a
	1/2 MS	3.46 a	1.37 c	8.23 a
	1/4 MS	1.93 c	0.30 a	2.58 b

¹The number followed by the same letter are not significantly different at ($p < 0,05$) level of Duncan's test.

The Result show that the single factor types of auxin and modification of MS media Table 1 and their interaction factors had a very significant effect on the roots number parameter formed in Figure 1. The highest of roots number with an average of 4.23 was found in the treatment using NAA, and average of 3.46 was found in the treatment using modification of 1/2 MS media. However in general, the result of interaction between two factors be able to reach an average of 6.04 roots per plantlets. Meanwhile on roots length parameter show that the factors types of auxin and modification of MS media affected of roots length. The Longest roots mean was 1.1 cm more stimulated by IAA and was not significantly different with control (without auxin). The base medium which is more optimal increasing of roots length is at 1/2 MS media modification.

The length of plantlets parameter in Table 1 showed that length of plantlets was influenced by the auxin types and the MS media concentration. The Highest length of root mean was 6.86 cm more stimulated by control and was not significantly different from NAA and IAA. The base medium which is more optimal in increasing length of plantlets at full media and was not significantly different from 1/2 MS media concentration.

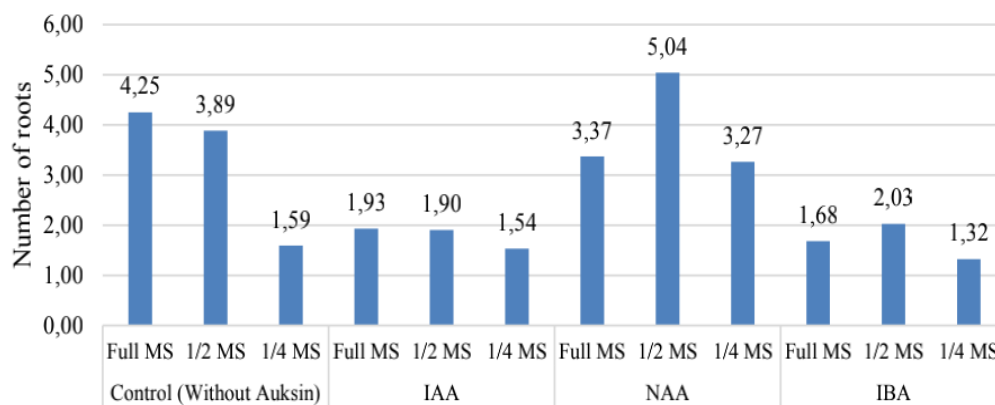


Figure 1. Average of roots number in the types of auxin and modification of MS media treatments at age 30 DAS

The results of data analysis above indicated that the addition of auxin which functions as a regulator of enlargement and elongation of cells in the meristem area. Beside that, auxin is used in vitro culture to stimulate callus induction, callus morphogenesis to form roots and shoots and encouraging somatic embryogenesis. The use of auxin (IAA, NAA and IBA) at certain concentrations gives different responses, if the right of auxin concentration can stimulate roots well, but it is too high it can be toxic to plant itself [7], [10].

3.2. Roots emergence

The results of data analysis using ANOVA showed that modification of MS media had a very significant effect on the appearance of the roots for the first time, while some of auxin types did not show significant differences. The observation data also showed that the interaction between modification of MS media and types of auxin was not significantly different in stimulate the roots emergence. The results of Duncan continued test analysis of the effect modification of MS media on roots emergence are shown in the following Table 2.

Table. 2 Average of roots emergence

Modification of MS media	Roots emergence (DAS)
MS Full	14.95 a
1/2 MS	15.25 a
1/4 MS	20.40 b

The number followed by the same letter are not significantly different at ($p < 0,05$) level of Duncan's test.



Figure 2. Growth of stevia plantlets at the age of 30 DAS on basic media; a) Full MS; b) 1/2 MS; c) 1/4 MS

The fastest of roots emergence mean was 15.25 DAS more stimulated by 1/2 MS media modification and was not significantly different with control. This shows that the media components usually consist of macro and micro nutrients, amino acids, vitamins, sugar as carbon sources, supplements are able to provide nutrients for the growth of roots and shoots of stevia. Where is MS media there are 40 mM of N elements in the form of NO_3^- and 29 mM in the form of NH_4^+ . MS media is currently the basic medium whose composition is able to support the tissue culture of other plants [11]. The excess of nitrate, potassium, and ammonium in MS media is one of the advantages of this medium.

4. Conclusions

From this study it can be concluded that roots appeared influenced by the modification of MS media, the fastest root appearance was in the treatment using full MS and 1/2 MS basic media on 14 and 15 DAS. In addition, from the parameters number and length of roots, the several types of auxin and the modification of MS media were very significant. Apart from that, the number of roots parameter also resulted from the interaction between types of auxin and modification of MS media which had a very significant effect. At the end of the observation (30 DAS) highest number of roots with an average of 4.23 was found in the using NAA treatment, with 1/2 MS media modification. However in general, the result of interaction between two factor were able to reach an average 6.04 roots per plantlet. The longest of roots on average were 1.1 cm more stimulated by IAA and not significant with control. The best medium which is more optimal in increasing roots length is at 1/2 MS. The Highest of roots length mean was 6.86 cm more stimulated by control and was not significantly different with NAA and IAA. The base medium which is more optimal in increasing of plantlets length at full media and was not significantly different from 1/2 MS media.

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