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Plantlet Formation and Acclimatization of Sugarcane cv. PS 881 with Different Types and Concentration of Auxin

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Abstract. This research has been carried out with a view to induce rooting and plantlet formation, followed by acclimatization. Micro shoots of sugarcane cv. PS 881 were cultured on Murashige and Skoog medium supplemented with different types and concentration of auxins for root induction. This research conducted experimentally using a split-plot design. The main plots were three types of auxins, which consisted of IAA, IBA, and NAA. The subplots were auxin concentrations with four levels, i.e. 0 µM, 5 µM, 10 µM, and 15 µM. Significantly faster root emergence time and higher number of roots observed in the Murashige and Skoog basal medium supplemented with 10 µM NAA. The best root length obtained in the Murashige and Skoog basal medium supplemented with NAA 0 µM. Plantlets derived from NAA 10 µM treatment showed the best performance during acclimation with a 100% survival rate. NAA at a concentration of 10 µM considered to be the best treatment in plantlet formation and acclimatization of sugarcane cv. PS 881. This study showed that the use of MS medium with 10 uM NAA is able to increase the growth of PS 881 sugarcane plantlets. The results of this study can increase the availability of high quality seedlings and increase national sugar production.

Key words: sugarcane; PS 881; auxin; rooting; acclimatization

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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of the most important agricultural commodities and is the primary raw material for sugar. Sugarcane cv. PS 881 is one of the superior sugarcane cultivars and is widely cultivated. Sugarcane cv. PS 881 has a high yield of 10.22 ± 1.62%, resistant to several pests and diseases including stem borer and shoot borer, and toleran to diplopia (Indonesian Sugar Research Institute, 2014). However, this sugarcane cultivar is susceptible to various parasites, including parasitoids and stem and shoots borer (Meidalima, 2014), and diseases such as red leaf spot caused by the fungus *Eriosphaeria sacchari* and *pokkahbung* disease caused by the fungus *Fusarium moniliforme* Sheld. Var. subglutinans Wr. Et Rkg (Kristini et al., 2012; Putra et al., 2013). Those diseases resulted in a decrease in sugarcane production. Fungal disease is complicated because it can form a chlamydospore that can survive for a long time in the soil.

Sugarcane propagation through *in vitro* techniques is the right step to obtain superior sugarcane seeds free from viruses and diseases. Sugarcane propagation, which included callus induction, somatic embryogenesis, and shoot multiplication to produce micro shoots, has been carried out in the previous studies. This research focused on plantlet formation and acclimatization, which therefore needs a good rooting system. Root induction requires the correct use of the type and concentration of auxin. Root induction is an essential part of the formation of sugarcane plantlets, ultimately determining the success of acclimatization (Ajadi et al., 2018; Shankar et al., 2018). An excellent rooting system of sugarcane cv. PS 881 will facilitate the next stage of culture, the acclimatization stage. Acclimatization is the process of adapting plantlets into new environments *ex vivo* from *in vitro* conditions. This study's objectives were to observe the effect of various types and concentrations of auxins on the plantlet formation of sugarcane cv. PS 881. This research was expected to provide information about the formation of sugarcane cv. PS 881 plantlets, so that it can increase the supply of sugarcane cv. PS 881 seeds and increase national sugarcane production.

METHODS

The experiment has been conducted from March to October 2019 at the Laboratory of Plant *In Vitro* Culture, Faculty of Biology, Jenderal Soedirman

University. The research material used was PS 881 cultivar sugarcane shoots, which were regenerated from callus using MS medium supplemented with 5 µM BAP. The shoots used were 2 cm long, with two leaves in each shoot and no roots.

The root formation of sugarcane plantlets was carried out on Murashige and Skoog (MS-1962) medium with IAA, IBA, and NAA at various concentrations. The study was an experimental study with a split-plot design with three replications. The main plots were the types of auxin consisting of IAA, IBA, and NAA whereas the subplots were auxin concentrations at 0 µM, 5 µM, 10 µM, and 15 µM. Murashige and Skoog basal medium was supplemented with 20 g/l sucrose, and the pH of the media was set at 5.83. The explants were incubated at 24°C under 24 h light period (from the fluorescent light tube). The variables observed were the growth of sugarcane plantlets, with parameters measured consisted of the number of roots, root length, number of shoots, number of leaves, and plantlet height.

Acclimatization

The plantlets obtained were then taken away from the culture media and washed cleanly to remove all media residue. The plantlets were then put on a pot tray containing acclimatization media consisting of soil, compost, and rice-husk-charcoal, and covered with plastic. The acclimatization was carried out for the whole three weeks, with a regular cover opening. The plastic hood was opened after three weeks, and plants were further maintained in the greenhouse.

Data collection and statistical analysis

All treatments were carried out in three replications. Plantlet formation data were recorded every day after planting for the next eight weeks. The data were expressed as an average of three repetitions. The data obtained were analyzed using analysis of variance (ANOVA), followed Duncan Multiple Range Tests (DMRT) with a confidence level of 95% if there were significant differences between treatments.

RESULTS AND DISCUSSION

The results showed that the root formation of sugarcane cv. PS 881 was observed in all treatments. It was observed that the explants showed good developmental responses as characterized by the increasing number of roots, increasing root length, new leaf formation, increasing plant height, and new shoot formation in all treatments. Application of auxin group in plant *in vitro* culture has an essential role in the formation of plantlets, whereas the selection of auxin types and concentrations determine the growth and differentiation of explants (Pop et al., 2011; Zazımalova et al., 2010). The growth of all explants in this study showed that the treatment of IAA, IBA, and NAA resulted in good responses of the cultured explants (Zhao, 2014).

The results (Table 1) also showed that the 10 µM NAA treatment resulted in the fastest root emergence time, which was 2.15 days after planting (dap), although this treatment did not show any significant difference with the 5 µM NAA treatment and 5 µM IAA treatment. According to Tesfa & Admassu (2016), the use of NAA to stimulate rooting is more effective than other kinds of auxin. Nurhayani et al. (2018) also reported that NAA and IBA is the most effective auxin compared to IAA. Goel et al., (2010) reported that NAA is the most stable auxin for initiating the roots of sugarcane variety CoS 99259. According to Kaur & Kapoor (2016), auxin plays an important role in cell lengthening and enlargement, as well as cell differentiation, and root induction. NAA is a type of auxin, which is effective for stimulating root formation but has a narrow concentration range. Tolera (2016) stated that NAA and IBA are types of auxin which are often used in vegetative propagation of sugarcane because they have a high rhizogenic ability and are stable in plant tissue.

The longest time needed for root to emerge was 3.99 dap, as shown by plantlets grown on control MS medium (0 concentration) for IBA and NAA. It was also seen that shoots grown on 0 µM IAA produced root as fast as that of 5 µM IBA. It is ssuggested that the shoots planted on 0 µM IAA contained sufficient concentration of endgenous IAA capable of stimulating root induction. Media without any addition of exogenous auxin is still capable of inducing root formation and growth. According to Elazab & Shaaban (2015), sucrose in *in vitro* rooting media provides an energy supply that is used in explant morphogenesis. States that growth, such as the formation of bud meristems, requires high energy. Therefore, different sugar concentrations also influence the growth of shoots in cultured plants. Sucrose, which is converted into essential materials such as cell wall materials, proteins, and other materials, has a role in stimulating root formation and growth. Plants can hydrolyze sucrose into glucose and fructose (Samudera et al., 2019). The glucose produced will then be processed through glycolysis and Krebs cycle to produce the much-needed energy in the form of ATP and NADH. Tesfa & Admassu (2016) also suggested that explant root growth can still occur when it is cultured on media containing sucrose even when the media do not contain any auxin.

Table 1. Effect of different types and concentration of auxins on the time of roots initiation in sugarcane cv. PS 881.

Treatment	Average day after planting
IBA 0 µM	3.99[a]
NAA 0 µM	3.99[a]
IBA 10 µM	3.58[ab]
NAA 15 µM	3.44[ab]
IBA 15 µM	3.30[ab]
IAA 10 µM	3.15[ab]
IAA 15 µM	3.12[ab]
IAA 0 µM	3.10[ab]
IBA 5 µM	3.10[ab]
IAA 5 µM	2.97[bc]
NAA 5 µM	2.21[c]
NAA 10 µM	2.15[c]

Note: Numbers followed by the different letter show significantly different value in DMRT (≤0.05)

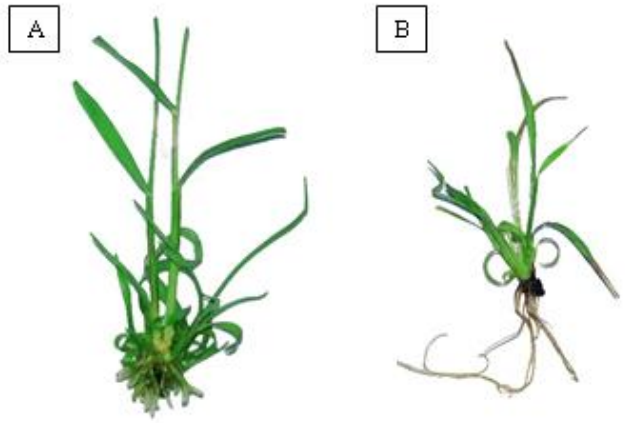

Figure 1. Number of roots formed. a) plantlet on MS medium with 10uM NAA, b) plantlet on MS medium with 0uM IBA

The average number of roots formed (Table 2) shows that the 10 µM NAA treatment also produced the highest average number of roots (6.19 roots/explants), although it was not significantly different from the 5 µM NAA treatment. Both treatments resulted in the formation of a large number of roots with blunt root tip, large in size, and short. This result is consistent with the root emergence data and also in line with the result obtained by Dibax et al. (2011), who studied the root induction in sugarcane cv. RB931003 and cv. RB98710 using 10.74 µM NAA. Moreover, Redae & Ambaye (2018) reported that 2 mg/l NAA (10.74 µM) and 0.5 mg/l BAP (2.22 µM) were the best treatments to induce rooting of C86-165 cultivar of sugarcane. According to Ali et al. (2012), auxin will be more effective at low concentrations and can directly conjugate with endogenous auxin. Solangi et al. (2016) stated that the use of auxin at low concentrations is very effective in accelerating root growth. Auxin has a role in cell enlargement, division, and differentiation (Nurhayani et al., 2018; Sari et al., 2014). Root formation starts from the metabolism and differentiation of cells that produce new cells in the tissue (Sofian et al., 2018). Auxin is known to be needed in the physiological stage of adventitious rooting (Pop et al., 2011).

Table 2. Effect of different types and concentration of auxins on the number of roots of sugarcane cv. PS 881.

Treatment	Average Number of root
NAA 10 µM	6.19 [a]
NAA 5 µM	5.42 [a]
IBA 5 µM	4.27 [b]
NAA 15 µM	4.27 [b]
IAA 10 µM	3.82 [bc]
IAA 15 µM	3.63 [bc]
IAA 0 µM	3.58 [bc]
IAA 5 µM	3.51 [bcd]
IBA 10 µM	3.30 [bcd]
IBA 15 µM	3.25 [cd]
NAA 0 µM	2.57 [de]
IBA 0 µM	2.12 [e]

Note: Numbers followed by the different letter show significantly different value in DMRT (≤0.05)

The least number of roots formed was observed on the explants grown on control MS media (0 concentration) for IBA and NAA. Although under those treatments explants produced the least number of roots, the roots formed were longer than those of explants' grown on 5 and 10 µM NAA media. In addition, the roots formed have sharp tips and small size. Auxin at high concentrations can inhibit the induction of root and explant growth. This inhibition could be caused by the increased ethylene level stimulated by a high auxin level in the media. Ethylene is an inhibitor of root formation (Tolera, 2016).

The root length in response to the interaction between type and concentration of auxin showed that the 0 µM NAA treatment (without the provision of NAA) has the longest root length average (3.69 cm/root), although it was not significantly different with IAA concentration of 0 and 5 µM and IBA concentration of 0, 5, 10, and 15 µM (Table 3). It can be

said that the most effective treatment for root length was shown by 5 µM IAA and 5 µM IBA treatments. Although 10 µM IBA produced slightly longer root, but it was not significantly different with that of 5 µM IBA. Sugarcane cv. PS 881 explants grown under these treatments produced similar root length as of the 0 µM NAA treatment. According to Mustafa & Khan, (2012), IBA is believed to play an essential role in the regulation of root development, IBA at small concentration will affect the root length. IBA plays a significant role in the rhizogenesis of sugarcane. IBA can act as a precursor for the release of indole-3-acetic acid (IAA), which can affect several other processes, including cell division, differentiation, and elongation.

The shortest roots length produced by a 15 µM NAA treatment, which had no significant difference with the that of 10 and 5 µM NAA treatments. NAA is an auxin that is not so effective in promoting root lengthening, and tends to initiate the formation and addition of lateral roots.

The observation of the number of shoots formed showed that the formation of sugarcane shoots was controlled by the type of auxin used. The effect of both auxin concentrations and the interaction between type and concentration of auxins had no effect of shoot number. IAA application resulted in the most significant average number of shoots, 1.81 shoots/explants Tabel (4). It is suggested that this shoot formation was caused by high concentrations of endogenous cytokinin originating from the previous culture period under BAP treatment. BAP is known as a cytokinin which is easily conjugated and stored for a longer time. Sugarcane bud formation originates from the formation of meristematic centers activity (meristems) which lead to the formation of organs that are controlled by exogenous and endogenous growth regulators (Schaller et al., 2014). Cytokins play a role in increasing the activity of meristem tissue by stimulating specific proteins (Feng et al., 2017). Cytokinin directly plays a role in the interphase stage in which DNA transcription and RNA translation occur during protein synthesis. Proteins will make up enzymes such as polymerases that function to extend DNA chains, ligase enzymes used to combine DNA fragments, and kinase enzymes that regulate cell cycle enzymes. These three enzymes make cell division far more effective (Hayati & Nurchayati, 2010).

The results of analyses on the number of leaves and plant height showed that all treatments tested did not significantly affect the number of sugarcane cv. PS 881 leaves and plant height. The addition of 10 µM NAA in the culture media resulted in the highest number of leaves with 23 leaves. On the other hand, 0 µM IBA produced the least number of leaves with nine leaves. There was a correlation between the number of shoots and leaves formed at 10 µM NAA. The increased number of shoots always accompanied by the addition of leaves.

Table 3. Effect of different types and concentration of auxins on the root length of sugarcane cv. PS 881.

Treatment	Root Length (cm)
NAA 0 µM	3.69 [a]
IAA 5 µM	3.35 [ab]
IBA 10 µM	3.05 [ab]
IBA 5 µM	3.00 [ab]
IAA 0 µM	2.64 [ab]
IBA 15 µM	2.58 [ab]
IBA 0 µM	2.57 [ab]
IAA 10 µM	2.30 [bc]
IAA 15 µM	2.26 [bc]
NAA 5 µM	1.11 [cd]
NAA 10 µM	0.62 [d]
NAA 15 µM	0.49 [d]

Note: Numbers followed by the different letter show significantly different value in DMRT (≤0.05)

Figure 2. Number of shoots formed a) plantlet on MS medium with 5 µM IBA, b) plantlet on MS medium with 5 µM IAA, c) plantlet on MS medium with 5 µM NAA

Table 4. Effect of different types of auxins on shoot number of sugarcane cv. PS 881.

Treatment	Average number of shoot	
IAA	1.81	a
IBA	1.59	ab
NAA	1.39	b

Note: Numbers followed by the different letter show significantly different value in DMRT (≤0.05)

Plantlets were acclimatized in media consisted of soil, compost and charcoal husk. Acclimatization results showed that plantlets originating from 10 µM

NAA and 5 µM IBA treatments showed good adaptation in the *ex vivo* environment with a percentage of survival rate reaching 100%. Plantlets obtained from the 10 µM NAA treatment were characterized by the fastest root emergence and the highest number of roots. Simultaneously, the plantlets from the 5 µM IBA treatment had functional morphology with a good root system and a high number of shoots. Plantlets originating from other treatments showed unsatisfactory results, and some of them died. It suggests that a weak rooting system might be the cause of plantlet death. Root has a vital role in the success of plant acclimatization. Nurhayani et al. (2018) stated that acclimatization process is influenced by the number and size of the roots of the plantlets formed. Acclimatization is carried out when the root formed or when the rood primordia initiated. Those root primordia will generally grow to true root on the planting medium.

Sukmadjaja & Mulyana (2011) reported that plantlet size could also be a determining factor in the success of acclimatization. Larger plantlets have better adaptability than the smaller ones. Plantlets size has an important role in the success of acclimatization, and large size plantlets have higher percentage of survival compared to small-sized plantlets (Raynalta & Sukma, 2013). According to Sukmadjaja & Syakir (2014), the selection of a container or place for acclimatization was also one of the factors which have to be considered. This study shows that the use of MS media with 10 uM NAA was able to increase the growth of PS 881 sugarcane plantlets. This fast growth of plantlets, originated from MS media containing 10 uM NAA, might have been caused by the fast root induction, large number of roots and leaves. Good rooting system will facilitate the ability of plantlets to absorb water and minerals from soil, which in turn will facilitate photosynthesis to take place in the leaves, eventually manivested into plant growth. Good leave will facilitate better photosynthetic process in the newly acclimatized plants. The results of this study can increase the availability of high quality seedlings and increase national sugar production.

CONCLUSION

The root formation of sugarcane cv. PS 881 shoots is influenced by the type and concentration of the auxin used. Treatment of NAA at a concentration of 10 µM resulted in the fastest rood emergence time, largest number of root and shoot, as well as acclimatization success with a percentage of survival reaching 100%.

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