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EXPLANT STERILIZATION AND IN VITRO CULTURE OF PURPLE SWEET POTATO TO OPTIMIZE NORMAL SHOOT FORMATION

Sterilisasi Eksplan dan Kultur In Vitro Ubi Jalar Ungu untuk Meningkatkan Pembentukan Tunas Normal

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ABSTRACT

Sweet potato (*Ipomoea batatas* L.), a tuber-producing plant, is a functional food that produces carbohydrates while meeting nutritional needs. Propagation of sweet potato through tissue culture is often hampered in the initial culture due to contamination. The study aimed to optimize sterilization of explants and growth of *in vitro* culture of purple sweet potato. Tubers of purple sweet potato cv. Antin 2 were *ex vitro* cultured through a semi-immersion system to produce shoots. The shoots as explants were sterilized with alcohol, fungicide, and sodium hypochlorite (P1); alcohol and sodium hypochlorite (P2); sodium hypochlorite (P3), and washed on sterile distilled water as control (C). The explants were then cultured on MS solid medium in tubes and jars. Growth and multiplication of shoots were carried out on MS solid medium added with cytokinins (BA and kinetin) at different concentrations. The results showed that the best sterilization method was obtained in the sodium hypochlorite (P3) and alcohol-sodium hypochlorite (P2) treatments, with sterile shoots reaching 100%. Planting the explants in jars gave higher normal shoot formation (85–100%) than that in tubes (12.5–48%). The use of kinetin at 0.5–1 mg L^{-1} gave good shoot vigor. The best axillary shoot multiplication was found on media with 0.5 mg L^{-1} BA. Growing explants on the semi-immersion system and sodium hypochlorite sterilization produced the highest sterile ones, whereas culturing three explants promotes normal growth straight after sterilization.

[***Keywords:*** *fungicide, sterilization, purple sweet potato, tissue culture*]

ABSTRAK

*Ubi jalar (*Ipomoea batatas *L.), tanaman penghasil umbi, memiliki peran sebagai pangan fungsional yang menghasilkan karbohidrat sekaligus mampu memenuhi kebutuhan gizi. Perbanyakan ubi jalar melalui teknik kultur jaringan sering kali terkendala pada saat penyediaan eksplan awal karena kontaminasi. Penelitian ini bertujuan untuk mengoptimasi sterilisasi eksplan dan pertumbuhan* in vitro *ubi jalar ungu. Umbi dari ubi jalar ungu cv. Antin 2 ditumbuhkan secara* ex vitro *melalui sistem semi-perendaman untuk menghasilkan tunas. Tunas selanjutnya disterilisasi dengan alkohol, fungisida, dan natrium hipoklorit (P1); alkohol, natrium hipoklorit (P2); natrium hipoklorit (P3), dan pembilasan dengan air destilasi steril sebagai kontrol (C). Tunas tersebut kemudian dikultur pada media padat MS dalam tabung dan botol. Pembesaran dan multiplikasi tunas dilakukan pada media padat MS dengan penambahan sitokinin (BA dan kinetin) pada berbagai konsentrasi. Hasil penelitian menunjukkan bahwa sterilisasi terbaik diperoleh pada perlakuan perendaman dalam sodium hipoklorit (P3) serta akohol dan sodium hipokloit (P2) dengan persentase tunas steril mencapai 100%. Penanaman eksplan dalam botol memberikan persentase tunas normal lebih tinggi (85–100%) daripada dalam tabung (12,5–48%). Penggunaan kinetin 0,5–1 mg L^{-1} menghasilkan vigor tunas yang baik. Multiplikasi tunas aksiler terbaik diperoleh dalam media dengan penambahan BA 0,5 mg L^{-1}. Penyediaan sumber eksplan dengan sistem semirendam dan sterilisasi dengan sodium hipoklorit menghasilkan eksplan steril terbaik, sedangkan penananam tiga eksplan meningkatkan pertumbuhan normal eksplan segera setelah sterilisasi.*

[Kata kunci: fungisida, sterilisasi, ubi jalar ungu, kultur jaringan]

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.), a tuber-producing plant from the Convolvulaceae family, grows in tropical and subtropical areas. Sweet potato tubers contain not only high carbohydrates but also vitamin A, C, and minerals. Sweet potato belongs to the functional food, where its tubers contain 0.53–0.73 mg Fe, 0.23–0.27 mg Zn, 23.04–29.97 mg Ca, 21.30–25.40 mg Mg, 42.00–46.33 mg P, and 308.67–328.67 mg K per 100 g dry tubers (Sanoussi et al. 2016). The demand for sweet potatoes is very high in Europe, used as chips, ice cream, noodles, and other foods. The highest sweet potato producer at present is Asia (75% of world production), with the largest producer is China. Indonesia is one of the important sweet potato producers in Asia, but its productivity is lower than China's (Suryani 2016). Indonesia is the fourth largest sweet potato producer globally, contributing 4.35% of the world's sweet potato plantations.

Research and development of sweet potatoes in Indonesia are carried out by the Indonesian Legumes and Tuber Crops Research Institute (ILETRI) in Malang. To date, at least 23 superior sweet potato varieties based on productivity, harvest time, nutritional content, and resistance to pests and diseases were released in Indonesia. The population of sweet potatoes in the field is 33,000–50,000 plants ha^{-1}. The potential yield of superior sweet potatoes is 25 t ha^{-1}, but the average yield at farmer level is only 12-16 t ha^{-1} (Abadi et al. 2013). Sweet potato Antin 2 variety released by ILETRI has 24.5 t ha^{-1} productivity, the tubers contain 0.9% fiber, 0.6% protein, 22.2% starch, 22.1 mg 100^{-1} g vitamin C, and 130.2 mg 100^{-1} g anthocyanin with reddish-purple skin and purple flesh (Balitkabi 2016).

One of the constraints in sweet potato cultivation in Indonesia is the lack availability of superior planting materials for the farmers. Farmers generally use cuttings for planting material, but the use of cuttings more than four planting times will reduce the yield of sweet potato due to the lower juvenility of the cuttings (Wahyuni 2016).

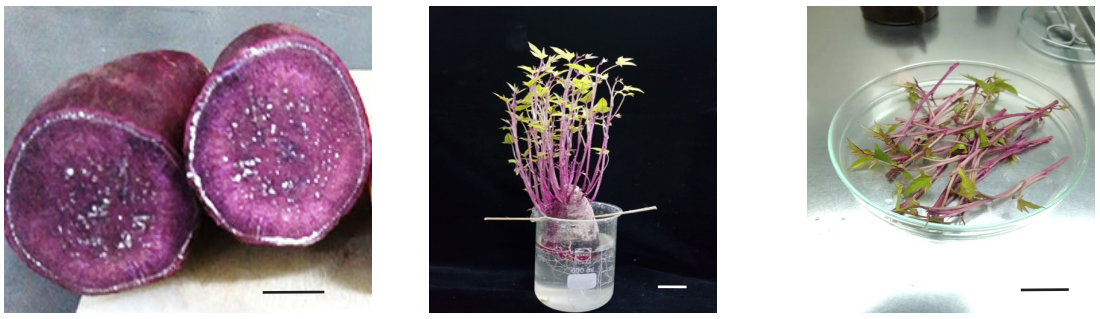
Tissue culture is one of the sweet potato propagation techniques to produce clonal, mass, and pest-free seeds. Plant tissue culture produces identical and uniform plants from the parent. Plant tissue culture is carried out by planting parts of the plant such as organs, tissues, and cells on nutrient media under sterile conditions. Plant tissue culture can also be used to conserve superior plant germplasm (Chauhan et al. 2019; Pacheco et al., 2016). Conservation of sweet potato germplasm in the field has several obstacles, such as biotic and abiotic stresses. Whereas germplasm storage with seeds is difficult to obtain uniform offspring because sweet potato is a heterozygous cross-pollinated plant (Wahyuni 2012).

Propagation of sweet potato by tissue culture has been carried out mainly in Africa and America, but it is rarely applied in Indonesia. The explants used included leaves, axillary shoots (Hang et al. 2016; Kim et al. 2015), and meristem tips (Alula et al. 2018). Contamination is one of the major problems in *in vitro* plant culture, especially in tuber and rhizome crops, including sweet potatoes. Ziralu (2021) reported high fungal contamination in *in vitro* sweet potato culture. Bakhsh et al. (2017) also reported a low rate of plantlet regeneration after sterilization. Explant contamination rate is high in sweet potato *in vitro* cultures despite using hydrogen peroxide (H_2O_2) (Bakhsh et al. 2017; Jena and Samal 2011) and mercury chloride ($HgCl_2$) (Hammond et al. 2014). The use of hydrogen peroxide and mercury chloride as a sterilant is considered dangerous and should be avoided. Hydrogen peroxide is a mutagen, corrosive and reactive, oxidizing and producing toxic gases in fires (Ellis 2001). Mercury is a chemical that is persistent and bio-accumulative in ecosystems. Hence, it harms human health and the environment that its use is limited. Its waste management is regulated through the Indonesian Ministry of Health No. 5 of 2016. The study aimed to optimize the sterilization of explants and the growth of purple sweet potato in *in vitro* culture using safe sterilants.

MATERIALS AND METHODS

Preparation of Explant Sources

The research was conducted at Laboratory of Plant Cell Culture and Micropropagation, Indonesian Research Institute for Biotechnology and Bioindustry, Bogor, Indonesia. The planting material used was purple sweet potato cv. Antin 2. *Ex vitro* explant induction was carried out by a semi-immersion system, where tubers were cut horizontally into two parts (Figure 1a) and placed in a 600 mL Beaker glass filled with 400 mL of water. The tuber was supported by sticks on the edges to keep only partially of the tuber immersed in water (Figure 1b). Shoots that emerged from the tubers were cut and used as explants (Figure 1c).



a b c

Figure 1. Explant preparation of purple sweet potato; (a) tubers, (b) shoots appeared from tubers after two weeks on a semi-immersion system, (c), shoots used as explants. Bar scale = 5 cm.

Explant Sterilization

The shoots were cut and washed with detergent and running water and then sterilized using alcohol 75% (for 15 seconds), benomyl 0.25% (15 minutes), and sodium hypochlorite 0.65% (10 minutes) as shown in Table 1.

In P1 treatment, nodes were sprayed with alcohol, soaked in benomyl fungicide, soaked in sodium hypochlorite (NaOCl), and rinsed with sterile distilled water. In P2 treatment, nodes were sprayed with alcohol, soaked in sodium hypochlorite, and rinsed with sterile distilled water, while in P3 treatment nodes were only soaked in sodium hypochlorite and rinsed with sterile distilled water. Rinsing nodes with sterile distilled water was used as control. Sterilization was carried out in a Laminar Air Flow (LAF) cabinet

The sterilized explants (1 node with 1.5 cm height) were planted in tubes (2.5 cm diameter and 15 cm height) and jars (5.5 cm diameter and 9 cm height). One explant was cultured per tube and three explants per jar. The media volume was 12.5 mL in the tube and 30 mL in the jar. The shoot induction medium was MS (Murashige & Skoog 1962) with the addition of 30 g L sucrose, 3.5 g L^{-1} gelzan, without growth regulators. The pH of the medium was adjusted to 5.7 before being autoclaved at 121 ^{o}C and 1 kg cm^{-2} for 20 minutes. The cultures were incubated in a culture room at 26 ± 2 ^{o}C under 20 µmol photons m^{-2} sec^{-1} light intensity at 12-hour a day photoperiod. The percentage of contamination, shoot conditions (normal or abnormal growth of planlet after sterilization), and shoot vigor (node number, leaf number, and planlet height) were observed six weeks after culture.

The study was arranged in a completely randomized design with nine replications. The data were subjected to analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT at α = 5%) was used for further analysis when the data were significantly different.

Plantlet Multiplication and Growth

Plantlet multiplication and growth were carried out by adding cytokinins to the media. Sweet potato shoots (one node with 1.5 cm height) from the previous stage (axenic culture/sterile node) were planted on solid MS medium with the addition of kinetin and 6-benzyladenine (BA) at 0.5, 1, and 2 mg L^{-1}. Medium without hormones was used as control. Plantlet growth in each treatment was observed after six weeks of culture.

The experiments were arranged in a completely randomized design with ten replications for each treatment. The data were subjected to ANOVA and followed by DMRT (α = 5%) when the data were significantly different.

Table 1. Sterilization treatments for sweet potatoes nodes

Treatment	Alcohol	Benomyl	Sodium hypochlorite	Sterile distilled water
Control (C)	-	-	-	+
P1	+	+	+	+
P2	+	-	+	+
P3	-	-	+	+

Notes: + = present, - = absent

RESULTS AND DISCUSSION

Sterilization and Post-Sterilization Shoot Conditions

Shoots appeared on the tuber after two weeks of incubation with a semi-immersion system. One sweet potato tuber produced 26 shoots of 21 cm height and each shoot had on average 4.5 nodes and leaves (Figure 1b). Explant contamination appeared one week after culture. The highest numbers of sterile explants were obtained in P2 treatment. Both explants planted in tubes and jars were 100% sterile (0% contamination), followed by P3 where the sterile explants reached 100% in jars and 96.15% in tubes (Figure 2). The results showed higher rates of sterile explants than those of the previous studies (Amissah et al. 2016; Hammond et al. 2014; Onwubiko et al. 2015). Amissah et al. (2016) sterilized shoots with higher concentrations of alcohol and NaOCl, but the contamination percentage remained high (94.6–100%). *Ex vitro* shoot induction as explant material increased the rate of sterile explants. P1 treatment produced the lowest sterile explants, 59.5% in tubes and 12.5% in jars (Figure 2), and no sterile explant produced from control either planted in tube or jar.

NaOCl is a common disinfectant used for surface sterilization. Its use is generally with a concentration of 0.1–1% NaOCl or 10–20% Clorox or commercial bleach for 10–20 minutes (Ahmadpoor et al. 2022; Felek et al. 2015; Kim et al. 2015; Lazo-Javalera et al. 2016; Oo et al. 2018). Soaking explants in high concentrations of NaOCl for a long time causes the explants to necrosis and die. Felek et al. (2015) reported that using 0.5% NaOCl for 10 minutes produces a high clean survival explant with low contamination. Kim et al. (2015) used 3% NaOCl to sterilize internode and leaves of sweet potato for 8 and 9 minutes. Hence, we use 0.65% NaOCl soaking treatment for 10 minutes.

The use of benomyl (P1 treatment) aimed to avoid fungal contamination because generally the main contaminant in sweet potato explants was fungi

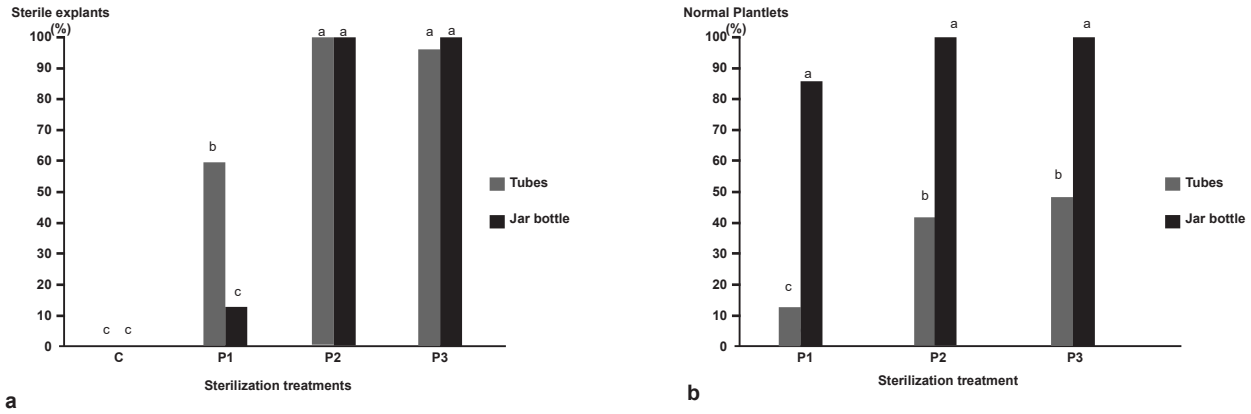


Figure 2. The percentage of sterile shoots of purple sweet potato after sterilization treatments (a) and the percentage of explants growing normally due to sterilization treatment (b) at six weeks after culture in tubes and jars; P1 = alcohol, fungicide, NaOCl; P2 = alcohol, NaOCl; P3 = NaOCl, C = control. Bars with the same letters are not significantly different.

(Hammond et al. 2014; Ziraluo 2021). However, in this study, *ex vitro* shoot induction using a semi-immersion system was conducted in a controlled room, not in the field or greenhouse. So, fungicide immersion seemed not necessary. In this study, all the contaminants were bacteria. Contamination was still high in the sodium hypochlorite immersion treatment after soaking in fungicides. Soaking with fungicides is assumed to give dangerous signal conditions to bacteria. Therefore, the bacteria developed a self-defense mechanism after fungicide immersion treatment, which caused sterilization at the next stage would be less effective (sodium hypochlorite immersion).

Zhang et al. (2020) reported an increase of antibiotic resistance genes in greenhouse soil after fungicide treatment. Fungicide treatment enhances bacterial community in the soil and soil animal gut (Zhang et al. 2019). Sinta et al. (2019) stated an increase in bacterial contamination in a long and less effective sterilization treatment because the bacteria built their defense systems by forming endospores. Benomyl is a broad-spectrum systemic fungicide that inhibits tubulin polymerization (Kara et al. 2020; Zhou et al. 2016). The increase of bacterial resistance after fungicide treatment needs to be studied further.

The shoots with P1 treatment planted in jars gave the highest contamination rate (87.5%) because three explants were grown in each jar, so contamination easily spread to other explants in the same jar. Jena & Samal (2011) also found endogenous bacteria and fungi in sweet potato *in vitro* culture. Endogenous bacteria were difficult to be sterilized by surface sterilization (Amissah et al. 2016), but controlled *ex vitro* explants induction could reduce contamination levels even with lower sodium hypochlorite concentrations compared to previous research.

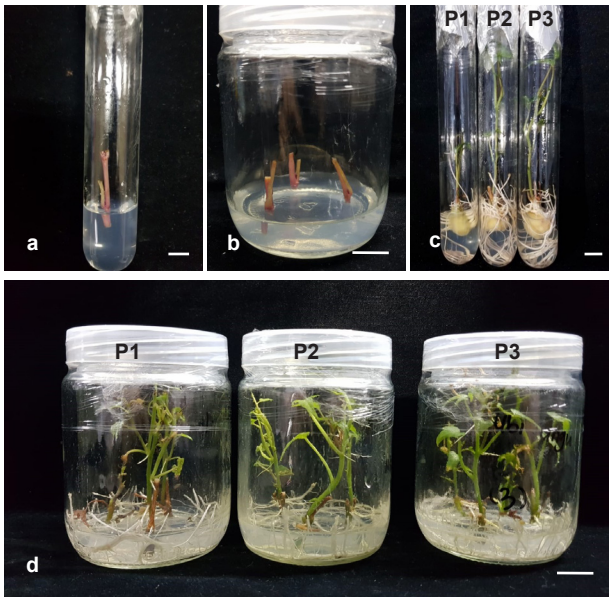


Figure 3. Sterilized explants of purple sweet potato cultured in tubes (a) and jars (b) and growth of the explants in three sterilization treatments (P1 = alcohol, fungicide, NaOCl; P2 = alcohol, NaOCl; and P3 = NaOCl) after six weeks on tubes and jars (d) . Bar scale = 1 cm.

Explant conditions were observed after six weeks of culture to determine the effect of sterilization on the growth of sweet potato shoots. The results revealed that the sterilization caused abnormal growth in explants where shoots did not grow (Table 2, Figure 2b & 3). While shoots in the P1 treatment were planted in jar bottles, the percentage of shoots growing normally reached 85.7% (Table 2, Figures 2a & 2b). Interestingly, explants from all sterilization treatments (P1, P2, and P3) planted in tubes formed calli, whereas explants grown in jars did not produce any callus with all sterilization treatments (Figure 3c-d). Only one explant was planted in the

tube, while three explants were in the jar (Figure 3a-b). Abnormal plantlets in jars only occurred in P1 treatment (14.3%) because of browning (Table 2). Amissah et al. (2016) reported malformation of sweet potato shoots and explant death occurred after sterilization treatment, where 100% of nodes cultured in tubes did not grow after benomyl and chloramphenicol treatments.

Sterilization treatment in this study produced more vigorous plantlets with faster growth than that resulted in previous studies. Plantlet height reached 6.8 cm after six weeks of culture in tubes with P3 sterilization treatment (Table 2). The shoots from P1 explants in the tubes grew shorter and less vigor than that of other treatments. Planting explants in jar gave more vigorous shoots for each sterilization treatment. Roots of sweet potato had already developed in all treatments after four weeks (Figure 3c-d), faster than that reported by Amissah et al. (2016), where new roots began to appear after 12 weeks of culture. Amissah et al. (2016) used 10%, 20%, and 30% NaOCl, but 10% NaOCl produced high contamination, while 20% and 30% NaOCl produced more sterile explant, but plantlet growth was significantly impaired.

In this study we used lower concentration of NaOCl. The effect of high concentrations of sterilant on plant growth was also reported by Huh et al. (2015), Felek et al. (2015), and Oo et al. (2018). To reduce contamination at the beginning of *in vitro* culture and prevent the use of high concentrations of sterilant, it is common to use explants from sterile parts of the plant, such as inner layers of oil palm ortet (Pratiwi et al. 2021), inner integuments of rubber trees (Tisarum et al. 2020), or isolated plants in a greenhouse as a source of explant (Saptari et al. 2017).

Table 2. Sterilant effect on shoot growth of purple sweet potato in tubes and jar.

Compartment	Sterilization treatment	Abnormal plantlets (%)	Node number	Leaf number	Plantlet height (cm)
Tubes	P1	87.5	2.5 d	2.0 d	3.5 b
	P2	59.3	3.5 cd	2.0 d	5.0 ab
	P3	52.0	4.3 bc	3.3 c	6.8 a
Jars	P1	14.3	5.3 b	4.7 b	4.6 ab
	P2	0.0	6.6 a	5.6 a	5.0 ab
	P3	0.0	5.1 b	5.1 ab	5.8 ab

P1 = alcohol, fungicide, NaOCl; P2 = alcohol, NaOCl; P3 = NaOCl
Numbers in the same column followed by the same letters are not significantly different according to Duncan's multiple range test at $\alpha \leq 0.05$.

Plantlet Growth and Multiplication

Plantlet growth and multiplication were carried out by adding cytokinins (BA and kinetin) to the media; media without hormones were used as control. The tallest plantlets and the highest number of leaves and of nodes were found in the media with kinetin treatments and without hormones (Table 3, Figure 4). The highest axillary multiplication rate was found in the addition of 2 mg L^{-1} BA. Plantlets in the media added with cytokinin formed calli; however, they were able to form roots as well. The highest number of roots was found in 0.5–1 mg L^{-1} kinetin treatment. Based on plantlet height, leaf number, node number, root number in this study, the use of kinetin 0.5–1 mg L^{-1} gave the best vigor. The result is different from that reported by Hang et al. (2016), where the highest shoot growth was found in the treatment of 1 mg L^{-1} kinetin and 1 mg L^{-1} BA. In this study, BA did not increase shoot height, leaf number, and root number. Sweet potato plantlets in this study produced roots even on media without hormones. On the contrary, Hang et al. (2016) reported that adding NAA in the media was necessary to produce 100% rooted plantlets. This different response is presumably because of the different varieties used.

This research shows that *in vitro* culture of sweet potatoes can be conducted on media without hormones to minimize media costs. The use of free-hormone media produced on average 7.7 nodes every six weeks. High node multiplication of sweet potato on free-hormone

Table 3. Effect of BA and kinetin on the growth of plantlet of purple sweet potato after six weeks of culture.

Treatment	Shoot height (cm)	Axillary shoot number	Leaf number	Node number	Root number	Callus formation
Control	5.85 a	1.0 b	8.4 a	7.7 a	2.3 bc	No
BA 0.5 mg L^{-1}	4.33 ab	1.0 b	3.5 b	3.7 b	2.0 cd	Yes
BA 1 mg L^{-1}	3.22 ab	1.0 b	3.2 b	4.1 b	1.3 de	Yes
BA 2 mg L^{-1}	1.66 b	1.2 a	1.4 b	2.2 b	0.9 e	Yes
Kin 0.5 mg L^{-1}	6.60 a	1.0 b	7.9 a	7.5 a	3.0 ab	Yes
Kin 1 mg L^{-1}	6.22 a	1.0 b	6.7 a	6.4 a	3.3 a	Yes
Kin 2 mg L^{-1}	6.17 a	1.0 b	7.4 a	6.3 a	2.3 bc	Yes

Numbers in the same column followed by the same letters are not significantly different according to Duncan's multiple range test at $\alpha \leq 0.05$.

medium makes sweet potato seeds affordable even though it comes from tissue culture (Ogero et al. 2011). In another study, low-cost sweet potato production carried out by replacing MS medium with Easy Grow vegetative fertilizer successfully reduced the production cost up to 46% by producing 3.5–4 nodes (Ogero et al. 2011). In this study, 7.7 nodes were produced every six weeks so that the multiplication rate and media prices were almost the same. The availability of sweet potato *in vitro* culture can be used to conserve its germplasm or to produce virus-free plants through meristem culture.

CONCLUSION

In vitro culture of purple sweet potato cv. Antin 2 was successfully carried out by optimizing the sterilization process supported by *ex vitro* induction of explants using a semi-immersion system. Sterile explants were obtained in sodium hypochlorite sterilization treatment, which shoots grew vigorously. Growing explants on the semi-immersion system and sodium hypochlorite sterilization produced the highest sterile ones, whereas culturing three explants in jar promoted normal growth straight after sterilization. Plantlets grew abnormally in the tube after sterilization. The use of kinetin 0.5–1 mg L^{-1} gave good shoot vigor. The best multiplication of axillary shoots at 1.2 folds was found on media with BA 0.5 mg L^{-1}, while the highest node multiplication at 7.7 folds was obtained on media without hormones.

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