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Shoots Multiplication Media Formulation of Purple Sweet Potato (Ipomoea batatas L.) Using Photoautotrofic Techniques

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Abstract: *Purple sweet potato (Ipomoea batatas L.) has the potential to be used as functional food due to its anthocyanin content, a pigment that causes tuber meat purple. The unavailability of purple sweet potato seeds which are free of pests and diseases caused the need for in vitro multiplication methods of sweet potato seeds using photoautotrophic culture techniques. Photoautotrophic is a method of micropropagation using sugar-free medium, thus the need for carbohydrates for growth depends on photosynthesis. Photoautotrophic techniques need to be developed to increase the resistance of plantlets when transferred to ex vitro conditions. The study aimed to obtain a planting medium formulation on the rate of shoots multiplication in the photoautotrophic technique. The research conducted was a formulation with the simplification of MS medium by reducing macronutrients to ½; and 1/3 of its normal concentration in the photoautotrophic technique with radiation levels of 8, 16 and 24 hours using a completely randomized design (CRD). The results obtained were in the form of media ½ MS + BAP 3 mg / L + NAA 1 mg / L gave the best media formulation in the photoautotrophic technique for multiplication of shoots with number of leaves, number of shoots, and more plant nodes than other medium formulations at the irradiation level of 8, 16 and 24 hours.*

Keywords: *Ipomoea batatas, photoautotrophic culture, simple medium*

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I. Introduction

Increased consumption of sweet potato as a functional food is quite important in manifesting food diversification. Functional food according toBPOM in[(1)] is food that naturally or has been processed, contains one or more compounds based on certain physiological functions that are beneficial to health. As an example for antioxidants, lowers blood pressure, lowers cholesterol levels, lowers blood sugar levels, increases calcium absorption, and others (Furuta *et al.* 1998 in[(2)]). Because purple sweet potato are dominated by purple, their antioxidant activity is certainly related to their anthocyanins[(3)].

Propagation of sweet potato plants is generally done using cuttings, namely stem cuttings or shoot cuttings. Propagation of plants with stem cuttings or shoot cuttings continuously has a tendency to decrease yield in the next generation[(4)]. Therefore, to spur the development and production of purple sweet potato plants, technology is required to support the availability of seeds that are free from large numbers of pests and diseases. One alternative method that can overcome this problem is plant tissue culture techniques.

The application of tissue culture techniques can produce sweet potato seeds massively on a large scale, uniformly in a relatively short time and available continuously, the plants produced are healthier and the quality of seeds is more guaranteed because they are free of pathogens compared to conventional methods[(5)(6)(7)].

One step that must be taken in *in vitro*propagation of seedlings is the multiplication of shoots which is the key to the success of *in vitro* propagation techniques. However, tissue culture techniques are also characterized by high air humidity, low light intensity, low CO_2 concentration, high ethylene concentration, limited air movement, low air porosity, and the presence of sugar content in the culture medium. These conditions often cause plant production of tissue culture to experience stomatal damage, thinning of the epicuticular wax layer, excessiveelongation of shoots, decrease in chlorophyll concentration and hyperhydration, thus the intensity of growth and chances of living in *ex vitro* environments is low, also often at risk of contamination and producing plantlets with low survival percentage at the acclimatization stage ((Lucchesini*et al*., 2001; Hazarika, 2003; Hazarika, 2006; Chaum*et al*., 2011 in[(8)]). In addition, the success of tissue culture techniques is also influenced by the type and composition of the medium and culture environment, hence it determines the production cost of *in vitro* plant propagation, it can be said that the utilization of tissue culture techniques requiresrelatively expensive production costs. Thus, it is necessary to manipulate the medium and culture environment by simplifying the medium on photoautotrophic culturetechnique.

Several studies on increasing plants by simplifying the *in vitro*planting medium have been carried out, but are still limited to the simplification of the composition and type of planting medium.

The research results ofDyah [(9)] show that the reduction ofmacronutrients in MS mediumto 1/2; 1/3 even 1/4 can be used to grow red ginger and the decrease in sugar concentration from 20 g/1 to 10 g/1 in NPK medium with a certain concentration can maintain the growth of red ginger. It is similar to the research results ofSupriati [(10)]that show that the optimal medium for *in vitro* shoots multiplication of kapok banana Amorang is ¼ MS medium with the addition of 1 ppm BA. Under treatment of 16 hours photoperiod incubation condition.

Photoautotrophic culture is a type of *in vitro* culture providing the condition that chlorophyll explants maintained in an *in vitro* medium without sugar with increased lighting will grow and develop in a photoautotrophic manner, consequently the need for organic material, in whole or in part, can be met endogenously[(11)(8)]. In this study, photoautotrophic culture techniques simplified MS medium, by reducing macro nutrients to ½ and 1/3 of its normal concentration with various combinations of BAP and NAAconcentrations. According to Wattimena*et al*., 1992 ***in***Pratiwi, Siregar [(12)], one of the factors that determines the success of tissue culture is the growth regulator. BAP (Benzyl Amino Purine) is a growth regulating substance belongs tocytokinin class which if combined with NAA (Naphthalene Acetic Acid) will encourage cell division and the formation of plant morphogenesis. NAA is a synthetic growth regulator that can regulate various processes of cell growth and elongation. Auxin generally inhibits shoot growth, while the combination of high cytokinin concentrations with low auxin is important in the formation of buds and leaves. Fereol*et al*., (2002). In tissue culture both groups of growth regulators proved to play a role in supporting tissue growth when used at the right concentration.

Based on the description above, the multiplication of shoots becomes a very important stage in the propagation of seeds through tissue culture. The aim of this study was to obtain a medium formulation to stimulate the multiplication of purple sweet potato shoots in the photoautotrophic technique.

II. Material And Methods

Location and Time of Research: The research was conducted at the In Vitro Unit of Agrotechnology Laboratory, Faculty of Agriculture, Halu Oleo University. The research was carried out from July to October 2017.

Materials and tools : The plant material used was derived from the tuber of purple sweet potato plants (Ipomoea batatas L.) obtained from the farmers, and the ingredients used were Murashige and Skoog (MS), agar, sucrose, BAP, NAA, NaHCO3, HCL, NaOH, 96% and 70% alcohol, liquid ingredients, bayclean, tissue, acrylic box, heat resistant plastic, rubber band and aquadest. The plant material used was in vitro shoots produced by shoot inductions. The media tested for shoots multiplication were MS (1/2 and 1/3) of its normal concentration of macro nutrients formula combined with BAP and NAA.

Sugarless MS medium which will be formulated according to the treatment. The making of the medium was produced with the standard procedure for making MS medium. Macro nutrient concentrations of ½ and 1/3 were prepared by diluting the concentration of macro stock based on the treatment needed. All macro nutrient stock solutions, ZPT and agar was added into a 1000 ml measuring glass. Followed by the measurement of the pH of the medium using pH meter. The medium was heated until the gelatin was dissolved, then removed and added 250 µl/L bayclean, the medium did not been autoclaved. Furthermore, medium that had been given 25 ml of bayclean per bottle was stored in the culture room.

The study used Completely Randomized Design (CRD) on MS medium treatment which had macro nutrients of ½ and 1/3 of its normal concentration with the addition of various combinations of BAP concentrations (0,1,2 and 3) mg/L + NAA (0.5, 1 and 2) mg/L and replicated 3 times.

Optimization of Multiplication Medium Formula of purple sweet potato shoots :The leaves of in vitro shoots that were formed were reduced until the remaining two leaves with one plant nodes one bud were then transferred to the shoots multiplication medium according to the treatment.

All treatments were put into acrylic box with chemical substance of NaHCO3 inside, then added HCL 1 N as a source of CO2 which was being replaced with new one every 8 hours. An hour before NaHCO3 with HCL 1 N being replaced, the acylic box was fully opened so that the plantlets inside the box received CO2 supply freely then after an hour the box was closed again, thus the supply of CO2 was obtained from the chemical. And the acrylic boxes were incubated at temperature of 26-27°C in culture rooms in an in vitro environmental growing treatments of 8, 12, and 16 hours of irradiation. The subculture was conducted routinely until the culture was 8 weeks after the planting.

III. Result and Discussion

The entire series of shoots multiplication activities, culture was maintained in an acrylic box. Multiplication of shoots and subcultures was a multiplication of shoots at the age of 8 weeks after planting which was carried out autotrophically by simplifying medium to ½ and 1/3 MS without using sugar as a carbon source enriched with a combination of BAP and NAA concentrations on various irradiation treatments that can be seen in the following tables:

Table 1, The results of variance were classified as high, which ranged from 5-6 variants indicating that in MS medium formulations with the addition of the combination of BAP + NAA concentrations significantly affected the number of leaves with irradiation rates of 8,16 and 24 hours at culture age of 8 MST. In general, the highest number of leaves was obtained in the media formulation of ½ MS + BAP 3 mg/L + NAA 1 mg/L at all levels of irradiation given. The highest number of leaves had 6,50; 7,48 and 8,00more strands, compared to other MS medium formulations.

Table 1. Effect of various MS medium formulations enriched by combination of BAP and NAA concentrations on the number of leaves with irradiation of 8, 16 and 24 hours at the age of 8 MS

TREATMENT	8 HOURS	16 HOURS	24 HOURS
M1= ½ MS + BAP 0,5 mg/L+ NAA 0,5 mg/L	5.53 e	5.80 ef	6.55 e
M2= ½ MS + BAP 0,5 mg/L + NAA 1 mg/L	5.55 e	6.00 e	7.00 d
M3 = ½ MS + BAP 0,5 mg/L + NAA 2 mg/L	5.57 e	6.00 e	7.00 d
M4 = ½ MS + BAP 1 mg/L + NAA 0,5 mg/L	5.58 e	6.50 d	7.00 d
M5 = ½ MS + BAP 1 mg/L + NAA 1 mg/L	5.60 e	6.50 d	7.00 d
M6 = ½ MS + BAP 1 mg/L + NAA 2 mg/L	5 80 de	6.00 e	7.20 cd
M7 = ½ MS + BAP 2 mg/L + NAA 0,5 mg	6.0 cd	6.50 d	7.30 bc
M8 = ½ MS + BAP 2 mg/L + NAA 1 mg/L	6.10bcd	6.50 d	7.50 bc
M9 = ½ MS + BAP 2 mg/L + NAA 2 mg/L	6.13 bc	6.70 c	7.50 bc
M10 = ½ MS + BAP 3 mg/L + NAA 0,5 mg/L	6.60 a	7.00 b	7.60 b
M11 = ½ MS + BAP 3 mg/L + NAA 1 mg/L	6.50 a	7.40 a	8.00 a
M12 = ½ MS + BAP 3 mg/L + NAA 2 mg/L	6.40 ab	7.00 b	7.50 bc
M13 = 1/3MS +BAP 0,5 mg/L+ NAA 0,5mg/L	5.00 f	5.70 f	6.00 fg
M14 = 1/3MS + BAP 0,5 mg/L + NAA 1 mg/L	4.85 fg	5.70 f	6.00 fg
M15 = 1/3MS + BAP 0,5 mg/L + NAA 2 mg/L	5.10 f	5.46 g	6.10 f
M16 = 1/3MS +BAP 1 mg/L + NAA 0,5 mg/L	5.00 f	5.40 g	6.20 f
M17 = 1/3MS + BAP 1 mg/L + NAA 1 mg/L	5.00 f	5.00 h	6.20 f
M18 = 1/3MS + BAP 1 mg/L + NAA 2 mg/L	5.00 f	5.00 h	5.80 gh
M19 = 1/3MS + BAP 2 mg/L + NAA 0,5 mg/L	4.80 fg	5.00 h	5.77 gh
M20 = 1/3MS + BAP 2 mg/L + NAA 1 mg/L	4.60 g	5.00 h	5.70 gh
M21 = 1/3MS + BAP 2 mg/L + NAA 2 mg/L	4.55 g	4.70 i	5.60 h
M22 = 1/3MS + BAP 3 mg/L + NAA 0,5 mg/L	4.00 h	4.50 i	5.50 h
M23 = 1/3MS + BAP 3 mg/L + NAA 1 mg/L	4.00 h	4.50 i	5.00 i
M24 = 1/3MS + BAP 3 mg/L + NAA 2 mg/L	4.00 h	4.00 kj	5.00 i

The number followed by the same letter indicates that their is no significant difference in Duncan's multiple range test α=0,05

This shows that the reduction of nutrients more than ½ of its normal concentration shows slow growth, besides reducing nutrient, growth in leaves also depends on the combination of concentrations of growth regulators used. The combination of BAP and NAA concentrations will synergize in stimulating leaf growth because BAP plays a role in stimulating synthesis RNA and protein in various tissues which can further encourage cell division. In addition, BAP can also stimulate tissue to absorb water from the surroundings, thus the process of protein synthesis and cell division can run well. Furthermore, BAP is a synthetic growth regulator that is not easily overhauled by an enzyme system of plants, hence it can spur induction and multiplication of shoots. (Utami, 1998; Chaerudin*et al.* 1996 ***in***Munarti [(13)]). In addition to the nutrient content of medium and growth regulators, light irradiation also determines the ability of explant to spur growth, particularly leaf formation. It is very dependent on light irradiation. It is proofed that the number of leaves increases along the increasing irradiation time. The 24-hour irradiation results in the highest number of leaves compared to the irradiation rate of 8 and 16 hours in all planting medium formulations used.

Table 2 shows that the MS medium formulation with the addition of a combination of BAP + NAA concentrations significantly affected the number of shoots at all levels of irradiation. At the age of 8 MST, the growth of shoots in all MS medium formulations showed increased growth of shoots along with increasing levels of irradiation. Shoot growth in the medium formulation of ½ MS + BAP 3 mg/L + NAA 1 mg/L and media ½ MS + BAP 3 mg/L + NAA 2 mg/L produced the highest number of shoots at 24 hours irradiation, namely 2.00 shoots each. Whereas for irradiation of 8 and 16 hours produced the highest number of shoots in MS ½ MS + BAP 1 mg/L + NAA 2 mg/L (M6) and ½ MS + BAP 2 mg/L + NAA 0.5 mg (M7) medium

formulations of 1.40, respectively the shoots were higher formed at 24-hours irradiation. This finding indicates that in addition to reducing macro nutrients, the duration of irradiation will cause slow growth of shoots.

Table 2. Effect of various MS medium formulations enriched by combination of BAP and NAA concentrations on the number of shoots with irradiation of 8, 16 and 24 hours at the age of 8 MST

TREATMENT	8 HOURS	16 HOURS	24 HOURS
M1= ½ MS + BAP 0,5 mg/L+ NAA 0,5 mg/L	1.26 ab	1.23 ab	1.46 def
M2= ½ MS + BAP 0,5 mg/L + NAA 1 mg/L	1.23 ab	1.23 ab	1.45 ef
M3 = ½ MS + BAP 0,5 mg/L + NAA 2 mg/L	1.23 ab	1.30 ab	1.51 cde
M4 = ½ MS + BAP 1 mg/L + NAA 0,5 mg/L	1.26 ab	1.23 ab	1.55 cd
M5 = ½ MS + BAP 1 mg/L + NAA 1 mg/L	1.26 ab	1.30 ab	1.53 cde
M6 = ½ MS + BAP 1 mg/L + NAA 2 mg/L	1.40 a	1.40 a	1.55 cd
M7 = ½ MS + BAP 2 mg/L + NAA 0,5 mg	1.40 a	1.40 a	1.45 ef
M8 = ½ MS + BAP 2 mg/L + NAA 1 mg/L	1.20 ab	1.20 ab	1.65 b
M9 = ½ MS + BAP 2 mg/L + NAA 2 mg/L	1.20 ab	1.20 ab	1.60 bc
M10 = ½ MS + BAP 3 mg/L + NAA 0,5 mg/L	1.26 ab	1.30 ab	1.66 b
M11 = ½ MS + BAP 3 mg/L + NAA 1 mg/L	1.26 ab	1.30 ab	2.00 a
M12 = ½ MS + BAP 3 mg/L + NAA 2 mg/L	1.20 ab	1.20 ab	2.00 a
M13 = 1/3MS +BAP 0,5 mg/L+ NAA 0,5mg/L	1.20 ab	1.30 ab	1.51 cde
M14 = 1/3MS + BAP 0,5 mg/L + NAA 1 mg/L	1.30 ab	1.30 ab	1.53 cde
M15 = 1/3MS + BAP 0,5 mg/L + NAA 2 mg/L	1.10 b	1.10 b	1.60 bc
M16 = 1/3MS +BAP 1 mg/L + NAA 0,5 mg/L	1.30 ab	1.30 ab	1.45 ef
M17 = 1/3MS + BAP 1 mg/L + NAA 1 mg/L	1.20 ab	1.20 ab	1.40 fg
M18 = 1/3MS + BAP 1 mg/L + NAA 2 mg/L	1.30 ab	1.30 ab	1.30 h
M19 = 1/3MS + BAP 2 mg/L + NAA 0,5 mg/L	1.20 ab	1.20 ab	1.35 gh
M20 = 1/3MS + BAP 2 mg/L + NAA 1 mg/L	1.20 ab	1.20 ab	1.40 fg
M21 = 1/3MS + BAP 2 mg/L + NAA 2 mg/L	1.30 ab	1.30 ab	1.40 fg
M22 = 1/3MS + BAP 3 mg/L + NAA 0,5 mg/L	1.23 ab	1.23 ab	1.30 h
M23 = 1/3MS + BAP 3 mg/L + NAA 1 mg/L	1,17 b	1.16 b	1.30 h
M24 = 1/3MS + BAP 3 mg/L + NAA 2 mg/L	1.20 ab	1.20 ab	1.30 h

The number followed by the same letter indicates that their is no significant difference in Duncan's multiple range test α=0,05

This finding indicates that the reduction of the macro nutrients of MS medium to ½ of its normal concentration with the addition of a combination of BAP and NAA concentrations for a long time can provide a good response to the number of shoots. These results also show that the longer the irradiation the more shoots are formed, it can be assumed that the shoots capable of utilizing light to support cell growth aided by the presence of growth regulators added to the medium. According toWindi, Wattimena [(14)], the results of observations from several researchers reported that cultures incubated in the dark morphogenesis were inhibited, even though they had been given carbohydrates from the medium. This is similar to the findings of Wattimena, (1991)*in*Pratiwi, Siregar [(12)]that the duration of irradiation in tissue culture impacts the content of endogenous hormones. The role of irradiation in tissue culture against morphogenesis may also be replaced by the addition of regulatinggrowth substances into the medium. In morphogenesis, the duration of irradiation is related to the energy received by the tissue. Plants that grow on long irradiation have a higher amount of endogenous auxin compared to short irradiation ones.

According toKieber [(15)], the presence of auxin and cytokinin in the medium can stimulate parenchymal tissue cells to divide. Cytokinins have been known to play an important role in almost all aspects of plant growth and development including cell division, bud initiation and growth, and the development of photomorphogenesis.

Table 3. Effect of various MS media formulations enriched bycombination of BAP and NAA concentrations on the number of plant nodes with irradiation of 8, 16 and 24 hours at age 8 MST

TREATMENT	8 HOURS	16 HOURS	24 HOURS
M1= ½ MS + BAP 0,5 mg/L+ NAA 0,5 mg/L	5.16 efg	5.93 d	6.11 de
M2= ½ MS + BAP 0,5 mg/L + NAA 1 mg/L	5.16 efg	5.96 d	6.93 c
M3 = ½ MS + BAP 0,5 mg/L + NAA 2 mg/L	5.31 def	5.96 d	6.96 c

M4 = ½ MS + BAP 1 mg/L + NAA 0,5 mg/L	5.33 de	6.33 cd	7.00 c
M5 = ½ MS + BAP 1 mg/L + NAA 1 mg/L	5.36 cde	6.40 c	7.00 c
M6 = ½ MS + BAP 1 mg/L + NAA 2 mg/L	5.53 cde	5.93 d	7.06 bc
M7 = ½ MS + BAP 2 mg/L + NAA 0,5 mg	5.83 bcd	6.33 cd	7.03 c
M8 = ½ MS + BAP 2 mg/L + NAA 1 mg/L	5.90 bc	6.33 cd	7.10 bc
M9 = ½ MS + BAP 2 mg/L + NAA 2 mg/L	5.90 bc	6.63 bc	7.23 bc
M10 = ½ MS + BAP 3 mg/L + NAA 0,5 mg/L	6.33 ab	6.93 ab	7.23 bc
M11 = ½ MS + BAP 3 mg/L + NAA 1 mg/L	6.50 a	7.26 a	8.223 a
M12 = ½ MS + BAP 3 mg/L + NAA 2 mg/L	6.26 ab	6.86 b	7.36 b
M13 = 1/3MS +BAP 0,5 mg/L+ NAA 0,5mg/L	5.00 fgh	5.40 e	6.00def
M14 = 1/3MS + BAP 0,5 mg/L + NAA 1 mg/L	5.00 fgh	5.46 e	6.00def
M15 = 1/3MS + BAP 0,5 mg/L + NAA 2 mg/L	5.00 fgh	5.33 e	6.06 ef
M16 = 1/3MS +BAP 1 mg/L + NAA 0,5 mg/L	4.76 hi	5.33 e	6.13 d
M17 = 1/3MS + BAP 1 mg/L + NAA 1 mg/L	4.73 hi	4.93 f	6.13 d
M18 = 1/3MS + BAP 1 mg/L + NAA 2 mg/L	4.53 hi	4.93 f	5.80efg
M19 = 1/3MS + BAP 2 mg/L + NAA 0,5 mg/L	4.53 hi	4.93 f	5.76fgh
M20 = 1/3MS + BAP 2 mg/L + NAA 1 mg/L	4.40 ij	4.86 f	5.56 gh
M21 = 1/3MS + BAP 2 mg/L + NAA 2 mg/L	4.36 ij	4.60 fg	5.56 gh
M22 = 1/3MS + BAP 3 mg/L + NAA 0,5 mg/L	3.90 j	4.40 g	5.46 h
M23 = 1/3MS + BAP 3 mg/L + NAA 1 mg/L	3.88 j	4.33 gh	4.90 i
M24 = 1/3MS + BAP 3 mg/L + NAA 2 mg/L	3.90 j	4.00 h	4.86 i

The number followed by the same letter indicates that their is no significant difference in Duncan's multiple range test α=0,05

Table 3. The results of variance showed that the MS medium formulation with the addition of a combination of BAP + NAA concentrations significantly affected the number of plant nodes at all irradiation levels at the age of culture of 8 MST. Irradiation observations of 8, 16 and 24 hours showed the highest number of plant nodeswas obtained in MS medium formulation of ½ MS + BAP 3 mg/L + NAA 1 mg/L (M11) medium formulations at 6.50, 7.26 and 8.22 at 8 MST, respectively.

Reduction of nutrient concentrations to ½and 1/3 of its normal concentration on MS medium can still stimulate the increase in the number of plant nodes at all levels of light irradiation. The number of plant nodes indicates a growth response as well as measuring the environmental influences that are applied. This is based on the large number of plant nodes are the easiest parameter to measure growth that can be seen [(16)].

In general, the results of this research show that the reduction of macro nutrients of MS medium up to ½ and 1/3 of its normal concentrationwas more efficient by using LED lighting types and the utilization of NaHCO3 chemicals with the addition of HCL to produce CO2, thereby spurring multiplication of shoots on the treatment of macro nutrient reductionof MS medium. The results of this study provide evidence thatphotoautotrophic technique culture of sweet potato with the quality of explant growth results that are close to the quality of growth on MS medium can be obtained using medium formulation of½ MS + BAP 3 mg/L + NAA 1 mg/L at all levels of irradiation showing the best research results as an alternative substitute medium in an*in vitro* sweet potato culture.

IV. Conclusion

Medium formulations of ½ MS + BAP 3 mg/L + NAA 1 mg/L in photoautotrophic cultures at irradiation levels of 8, 16 and 24 hours were the best formulations for shoot multiplication with more leaves, shoots and plant nodes compare to other MS medium formulations. Further research is needed by using various types of LED light and NaHCO3 concentration to stimulate photosynthesis rates in autotrophic culture techniques.

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