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In vitro Propagation of Malaysian Cassava (*Manihot esculenta* Crantz) Variety through Low Cost Tissue Culture Media

Huzaimah Mahdi, Rebicca Edward

Department of Plant Science, Faculty of Resource Science and Technology, University Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

Abstract*—Cassava (*Manihot esculenta *Crantz) is a perennial woody plant belongs to Euphorbiaceae family and listed as one of the most important source of carbohydrates around the world. In Malaysia, Cassava is an important industrial crop for starch processing and food industries. Hence, an* in vitro *propagation technique is needed to produce these highly demand industrial crop. In this study, the Malaysian cassava variety which was Putih variety was cultured onto low cost tissue culture media by using nodal explants. The low cost media were tested using locally available ingredients which were 2 ml/L Maxigreen50 liquid fertilizer as the substitute to MS salt, 3%(w/v) table sugar as the substitute to sucrose, agar-agar strip (14 g/L), corn flour (20 g/L), and tapioca flour (20 g/L) as the substitute to Phytagel powder. The low cost media were supplemented with young coconut water of Matag variety at the concentration of 0, 25, 50, 75, and 100 mL/L. The results showed that the best low cost media for the induction of shoot multiplication, height and number of leaves was the low cost media supplemented with 2 mL/L Maxigreen50 liquid fertilizer, 14 g/L agar-agar strip + 20 g/L corn flour, and 100 mL coconut water for cassava Putih variety.*

Keywords— In vitro, Manihot esculenta, low cost, shoot multiplication.

I. INTRODUCTION

Manihot esculenta or also known as Cassava, Tapioca, Manioc, Yuca (Spanish), and Ubi Kayu (Malay) is a perennial woody shrub from Euphorbiaceae family which is native to Central and South America (FAO, 2000). Cassava is categorized as an important source of carbohydrate after rice and corn which provide important component of diet to more than 800 million of people around the world (Richardson, 2013). In Malaysia, Cassava is mainly cultivated for the large scale industrial purposes for starch processing industries (Lian and Idris, 2000). According to Department of Agriculture (DOA) Sarawak (2015), the Cassava industry for production of chip and snack production has been increasingly in demand and been a source of income generation for the small scale farmer (DOA Sarawak, 2015). Hence, tissue culture propagation technique is needed for the rapid production of plantlets which have uniform genetic characteristics and free from diseases. However, the high cost of the chemical ingredients for the preparation of tissue culture media becomes one of the problems for the application of tissue culture technology (IAEA, 2004). Thus this research will investigate the suitable low cost tissue culture media which use available and cheap materials as the substitute to the high cost of chemical used in conventional tissue culture media in order to reduce the cost of cassava plant production.

II. MATERIALS AND METHODS

The vegetative germplasms of local Cassava (*Manihot esculenta* Crantz) variety in Sarawak which was Putih variety was obtained from Agriculture Research Centre (ARC) Semongok. The nodal cuttings of three months old's cassava Putih variety which consists of node number 2 to node number 4 from the shoot tip were excised into 1.0-1.5 cm length before surface sterilized by immersing in 70% ethanol for 1 minute followed by agitation in 25% Clorox (active ingredients: 5.25% Sodium Hypochlorite (NaOCl)) for 10 minutes with two drops of Tween-20 before rinsed with sterile distilled water for five minutes. The sterile nodal explants were cultured onto the control media which was full strength MS media with 7 g/L Phytagel, 30 g/L sucrose, and Benzylaminopurine (BAP) at 1.0 mg/L and 1-Naphthaleneacetic acid (NAA) at 0.01 mg/L, whereas in the low cost tissue culture media, the materials used for the low cost media were 2 mL/L Maxigreen50 liquid fertilizer (22-16-12+2MgO+TE) as the substitutes to MS salt, 30 g/L table sugar as the substitutes to sucrose, 14 g/L agar-agar strip with or without combination with 20 g/L corn flour and 20 g/L tapioca flour as the substitute to Phytagel powder, and young coconut water of Matag variety at the concentrations of 0 mL, 25 mL, 50 mL, 75 mL, and 100 mL as the substitutes to BAP and NAA. There were six replicates on each

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treatment and five explants in each replicate. The observation on growth parameters such as number of shoots, plant height, and number of leaves were observed on each four weeks interval until week 12. One way analysis of variance (ANOVA) was used for analyzed the data and comparison of mean by using Tukey test ($p<0.05$).

III. RESULTS AND DISCUSSION

The Effect of Different Concentrations of Coconut Water and Different Types of Gelling Agents on the Mean Number of Shoots of Cassava in Low Cost Media

For the number of shoots, there was a significant difference ($p<0.05$) on the mean number of shoots produced from the nodal explants where the highest mean number of shoots was obtained from control treatment, T1 (1.0 mg/L BAP + 0.01 mg/L NAA) which was 3.77±0.43 in cassava Putih variety (Table 1). For the low cost media, there were no shoot multiplication recorded in which only one shoot per nodal explant was grown from T4 (agar-agar strip with 50 mL coconut water), T5 (agar-agar strip with 75 mL coconut water), T6 (agar-agar strip + corn flour with 100 mL coconut water), T8 (agar-agar strip + corn flour with 25 mL coconut water), T9 (agar-agar strip with 50 mL coconut water), T10 (agar-agar strip + corn flour with 75 mL coconut water), and T11 (agar-agar strip + corn flour with 100 mL coconut water) which were 1.00±0.00 (Table 1).

Table.1: Mean number of shoots, heights and leaves of cassava Putih variety on low cost media of different concentrations of coconut water and different types of low cost gelling agents after 12 weeks cultures

Treatment	Gelling agent	Plant growth regulator	Mean no. of shoots	Mean shoot heights	Mean no. of leaves
T1	Phytagel	1 mg/L BAP + 0.01 mg/L NAA	3.77 ± 0.43^{c}	6.97 ± 0.13^{g}	26.37 ± 0.61^{f}
T2	Agar-agar strip	0 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T3	Agar-agar strip	25 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T4	Agar-agar strip	50 mL/L coconut water	1.00 ± 0.00^{b}	3.65 ± 0.08^{b}	2.90 ± 0.41^{b}
T5	Agar-agar strip	75 mL/L coconut water	1.00 ± 0.00^{b}	4.45 ± 0.10^{c}	0.00 ± 0.00^{a}
T6	Agar-agar strip	100 mL/L coconut water	1.00 ± 0.00^{b}	5.59 ± 0.09^{d}	3.20 ± 0.41^{b}
T7	Agar-agar strip + corn flour	0 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.67 ± 0.48^{c}
T8	Agar-agar strip + corn flour	25 mL/L coconut water	1.00 ± 0.00^{b}	3.69 ± 0.13^{b}	4.70 ± 0.47^{e}
T9	Agar-agar strip + corn flour	50 mL/L coconut water	1.00 ± 0.00^{b}	5.56 ± 0.13^{dz}	0.00 ± 0.00^{ax}
T10	Agar-agar strip + corn flour	75 mL/L coconut water	1.00 ± 0.00^{b}	6.23 ± 0.10^{e}	3.53 ± 0.51^{c}
T11	Agar-agar strip + corn flour	100 mL/L coconut water	1.00 ± 0.00^{b}	6.87 ± 0.10^{fz}	5.77 ± 0.43^{gy}
T12	Agar-agar strip + tapioca flour	0 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T13	Agar-agar strip + tapioca flour	25 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T14	Agar-agar strip + tapioca flour	50 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T15	Agar-agar strip + tapioca flour	75 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T16	Agar-agar strip + tapioca flour	100 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

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These findings were in agreement with the findings obtained by Daud *et al.* (2011) on *in vitro* culture of Celosia spp. using low cost media in which there was a shoot regeneration recorded on the low cost media supplemented with 70 mL/L of young coconut water plus corn flour, rice flour, cassava flour, and potato starch without addition of MS although the low cost media supplemented with ½ MS showed more effects on shoots regeneration compared to the medium without MS (Daud *et al.*, 2011). However, this experiment showed no shoot multiplication even at the highest concentration of coconut water of Matag variety which is 100 mL/L and could be due to the presence of other precipitates in the coconut water which can hindered the action of endogenous cytokinin in the coconut water.

The Effect of Different Concentrations of Coconut Water and Different Types of Gelling Agents on the Mean Shoot Height of Cassava in Low Cost Media

For the mean shoot heights of cassava Putih variety, there was a significant difference ($p<0.05$) on the mean shoot height produced where the highest mean shoot height was obtained from the control treatment, T1 (1.0 mg/L BAP + 0.01 mg/L NAA) which was 6.97±0.13 (Table 1).

For the low cost media, the highest mean shoot height was recorded on the low cost media supplemented with the highest concentration of coconut water which was T11 (agar-agar strip + corn flour with 100 mL coconut water) which produced the mean shoot heights of 6.87±0.10 (Table 1). This findings was in agreement with the findings of Buah and Agu-Asare (2014) on their study of using coconut water from fresh and dry fruit as an alternative to BAP on Dwarf Cavendish Banana where the plant cultured on medium supplemented with fresh coconut water produced the highest shoot height compared to the plant cultured on medium supplemented with BAP. Throughout all treatments of low cost media, the highest mean shoot height was recorded on low cost media supplemented with 20 g/L corn flour. This indicates that the presence of coconut water at the highest concentration on the low cost media with the addition of agar-agar strip with corn flour can produce the best shoot height. This result was in agreement with the finding obtained by Mohamed et al. (2010) on the uses of corn starch and potato starch as an agar alternative to Solanum tuberosum in which the uses of corn starch and potato starch has no significant effect in plantlet height but produce the significant effect on the number of shoots over the control treatment with agar. The use of commercial starch or flour as the alternative gelling agents in tissue culture media are due to high amount of starch, vitamin C and carbon sources and low amount of other minerals (Daud *et al.*, 2011).

The Effect of Different Concentrations of Coconut Water and Different Types of Gelling Agents on the Mean Number of Leaves of Cassava in Low Cost Media

For the number of leaves of cassava Putih variety, there was a significant difference ($p<0.05$) on the mean number of leaves produced where the highest mean number of leaves was obtained from the control treatment, T1 (1.0 mg/L BAP + 0.01 mg/L NAA) which was 16.23±0.73 (Table 1). For the low cost media, the highest mean number of leaves was recorded on T11 (agar-agar strip + corn flour with 100 mL coconut water) which produced the mean number of leaves of 5.77±0.43 (Table 1). The result was in agreement with the findings obtained from Lalitha *et al.* (2013) on the effect of plant derived gelling agents on micropopagation of mulberry. From their experiment, the highest number of nodes and number of leaves were obtained from the MS medium gelled with corn flour (22 g/L) in combination with 3.5 g/L agar compared to other plant derived gelling agents.

From this experiment, the lowest mean shoot height, mean number of nodes and mean number of leaves were recorded from the low cost media of 14 g/L agar-agar strip only or 14 g/L agar-agar strip with combination of 20 g/L corn flour without addition of coconut water (Table 1). This showed that coconut water is needed in the growth of shoot and the use of Maxigreen50 liquid fertilizer only cannot induce the shoot growth in the low cost media. This could be due to absence of important vitamins in Maxigreen50 liquid fertilizer needed for plant growth in vitro. Thus, the addition of coconut water can help to supply the vitamins or nutrient sources for the growth of plantlets as the use of young coconut water can acts as a plant growth regulators that gives a better response on plant tissue culture (Daud *et al.*, 2011).

IV. CONCLUSION

The best low cost media for the induction of shoot height and number of leaves for cassava Putih variety was the low cost media of 2 mL/L Maxigreen50 liquid fertilizer, 30 g/L table sugar, 14 g/L agar-agar strip + 20 g/L corn flour supplemented with 100 mL coconut water. However, there was no shoot multiplication recorded on the low cost media of 2 mL/L Maxigreen50 liquid fertilizer, 30 g/L table sugar, 14 g/L agar-agar strip + 20 g/L corn flour supplemented with 100 mL coconut water.

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