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Effects of Different Organic Additives on *in vitro* Shoot Regeneration of *Celosia* sp.

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Abstract: Nowadays, many researches were conducted in minimizing tissue culture technology due to the overhead of cost needed. The purpose of this study was to investigate the effects of using five kinds of organic additives at four level concentrations responsive to the number of shoots produced for eight weeks in culture. Stem segment explants of *Celosia* sp. were cultured on MS medium that have been supplemented with different kinds of extract juice that serve as organic additives which are mature coconut, young coconut, papaya, banana and tomato at 20, 30, 50 and 70 ml L^{-1}. The numbers of shoot on each explant were recorded and the mean of ten replicates explants were calculated. Among the media used, young coconut water at 70 ml L^{-1} induced the highest shoot regeneration (14.21±8.26), followed by mature coconut water at 50 ml L^{-1} (13.14±10.33). Banana and tomato juice promote highest shoot regeneration of stem segments at 50 ml L^{-1} that produced 9.57±4.68 and 9.28±5.82 shoots per explants, respectively. While the lowest concentration which at 20 ml L^{-1} of papaya juice showed highest shoot regeneration (10.50±3.45) produced among the three other concentration tested. Statistical results showed that there were significant differences interactions effects ($p<0.05$) in terms of number of shoot regenerated between the types of extracts juices determined by ANOVA test. Comparing number of shoots regenerated that were cultured in control media, it showed higher than all of experimental medium composition. There were no big different in cost required in preparation of control media and the experimental media. Applications of five kinds of local fruit in tissue culture media should be considered since it responsive in shoot regeneration.

Key words: Organic additives, banana juice, papaya juice, tomato juice, coconut water

INTRODUCTION

Various factors should be considered in developing plant tissue culture technique. For example in physical components include the equipment and buildings with preparation room, transfer room, culture or growth room, hardening and weaning area, soil-growing area (greenhouses, plastic tunnels), packaging and shipping area and related facilities office and store for chemicals, containers and supplies (Ahloowalia and Prakash, 2002). Besides that, modification of composition of culture media actively studied in order to reduce cost required and provides this technique capable to all users. Previous study from Raghu *et al*. (2007) managed to reduce costs by using household sugar instead of laboratory grade sucrose and tap water instead of double distilled water. Low cost options adopted should be effective in lowering cost of production without compromising the quality of produced plants (Savangikar, 2002). Many researchers conducted to substitute any items needed in media culture preparation such as gelling agents, additive compounds and sucrose, as objectively to minimize the cost operational.

For most developing countries like Ghana to benefit from the direct use of tissue cultured material, the cost of commercial micro propagation has to be drastically reduced without compromising the quality of micro propagules (Demo *et al*., 2008). This can be done through identifying cheaper alternatives to some of the expensive media composition. A large number of complex additives like coconut water, banana pulp, peptone, tomato juice, slap honey and beef extract can be very effective in providing undefined mixture of organic nutrients and growth factors (Aktar *et al*., 2007). From the previous study, Rahman *et al*. (2004) demonstrated that the addition of potato, corn and papaya extract at various

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concentrations able to enhanced the growth of the calli of the *Doritaenopsis* orchid and promoted the regeneration of Protocorm-like Bodies (PLBs) from the calli. While Prakash *et al*. (2002) stated plant extracts such as coconut milk, banana extract and tomato juice can be very effective in providing undefined mixture of organic nutrients and growth factors.

Celosia sp. are available in various colors include yellow, gold, red, pink, orange and wine. *Celosia* offers large flower clusters on top of green or reddish leaves. *Celosia* sp. considered as shrub since its heights range from 6 to 36 inches. Velvety crested types (cockscomb) are rippled whereas others are shaped like plumes or spikes. The seeds of this flower are located at the bottom or base of the stems. The seeds are many causes it has been selected for plant tissue culture technique which need around ten days culturing (Ong, 2006).

The aim of this study was to identify the type and concentration level of five different juices that are mature coconut, young coconut, papaya, banana and tomato which response for high shoot regeneration. Modification or replacement of culture media composition is important to reduce the cost and facilitate the work culture by using readily available materials.

MATERIALS AND METHODS

Preparation of stock plants: All experiments were conducted at Laboratory B2.5, Institute of Biological Sciences, Faculty of Sciences, University of Malaya, Kuala Lumpur, Malaysia in year 2010. In these experiments, explants used were seed of the *Celosia* sp. Preparation of stock plant was done in a clean and sterile condition. Seeds of *Celosia* sp. washed 3 times using distilled water to remove impurities and microorganism on the seed surface. Next, the seeds shake with 100% Clorox solution (sodium hypochlorite) and add with two drops of Tween-20 for 5 min. The next step was shaking the seeds in 70% Clorox solution, 50, 30 and 20% for 2 to 3 min. Then, the seeds were rinsed using distilled water and 70% alcohol for 1 min. The last washing stage used sterile distilled water for 4 to 5 times. *Celosia* sp. sterile seeds were cultured in basic MS medium alone. After a period of 6-8 (2 months old) of *Celosia* sp. weeks for growth, explants were randomly selected for use as stock plantlets. In carrying out this study, the stem segments of *Celosia* sp. were used as explants.

Preparation of juice/extract: About 100 g of banana, tomato and papaya used to extract the juice according to the required concentration. The fruit used properly washed before cut. Fruit that has been cut so finely ground using a blander. The juice extraction is filtered and then stored at -20°C for future use. For the coconut water, samples were taken directly from the coconut palm maturity and the young coconut. Coconut water filtered to remove impurities from palm kernel, coconut bark or coconut husk.

Preparation of culture media with the addition of juices or extracts from fruits: Media materials used are basic MS medium but was added to the five type of fruit juice that had been prepared beforehand. There were four concentration extract juices levels used which begin by 20, 30, 50 and 70 ml L^{-1}. Preparation of media as the provision of basic MS medium by adding 30 g L^{-1} of sucrose daily and 8 g L^{-1} for Oxoid technical agar. Extract juice was added to the solution of basic MS medium and then adjusted to pH 5.8.

Culture conditions: In each vial which was filled with culture media, 2 cm explants of stem segment were placed for the inoculation of *Celosia* sp. the culture within 8 weeks were controlled under room condition which are light intensity (1000 μmol/m^2/sec), temperature at 25±1°C and 70-80% relative humidity and 16/8 h light/darks of photoperiod.

Experimental design: Eight weeks is a period of cultural growth in this study. After eight weeks of culturing, the numbers of shoot on each explant were recorded. The mean of ten replicates explants were calculated. Statistical significance was determined using two way Analysis of Variances (ANOVA) by SPSS version 16 statistical package. The values with $p<0.05$ considered as significant. The differences mean number of shoot regeneration was contrasted using Tukey's test.

RESULTS AND DISCUSSION

Six types of extract juice have been used as culture media for eight weeks to obtain the number of shoots produced. The results shown on Table 1 indicate the Mean±SD of 14 replicates for each treatment concentration. The differences in the number of regenerated shoots did not differ significantly. Young coconut water at concentration 70 ml L^{-1} was noted as a medium that has resulted in the highest number of shoots produced (14.21±8.26) as shown in Table 1 and Fig. 1a. The next best of shoots regeneration was recorded on mature coconut water at concentration 50 ml L^{-1} (13.14±10.33) (Fig. 1e) followed by the same extract but in matured condition at concentration 30 ml L^{-1} (11.57±8.82). Banana extract juice at concentration

Table 1: Mean number of *in vitro* shoots regeneration derived from stem segment explants after eight weeks cultured in media MS containing organic additives with different concentration

Concentration (ml L^{-1})	No. of shoots±SD produced				
	Mature coconut	Young coconut	Papaya	Banana	Tomato
20	8.62±5.58^{ab}	9.42±5.55^{ab}	10.50±3.45^{ab}	8.43±3.56^{ab}	8.50±4.21^{a}
30	11.57±8.82^{ab}	8.64±4.60^{ab}	10.21±3.25^{ab}	7.00±5.42^{a}	7.50±2.82^{ab}
50	13.14±10.33^{ab}	10.71±7.82^{ab}	9.35±4.78^{ab}	9.57±4.68^{ab}	9.28±5.82^{ab}
70	11.14±5.61^{ab}	14.21±8.26^{ab}	7.71±6.29^{a}	8.71±4.99^{ab}	7.64±6.05^{ab}

Values are Means±SD (n = 14) followed by the same letters are not significantly different at 5% level by Tukey's test

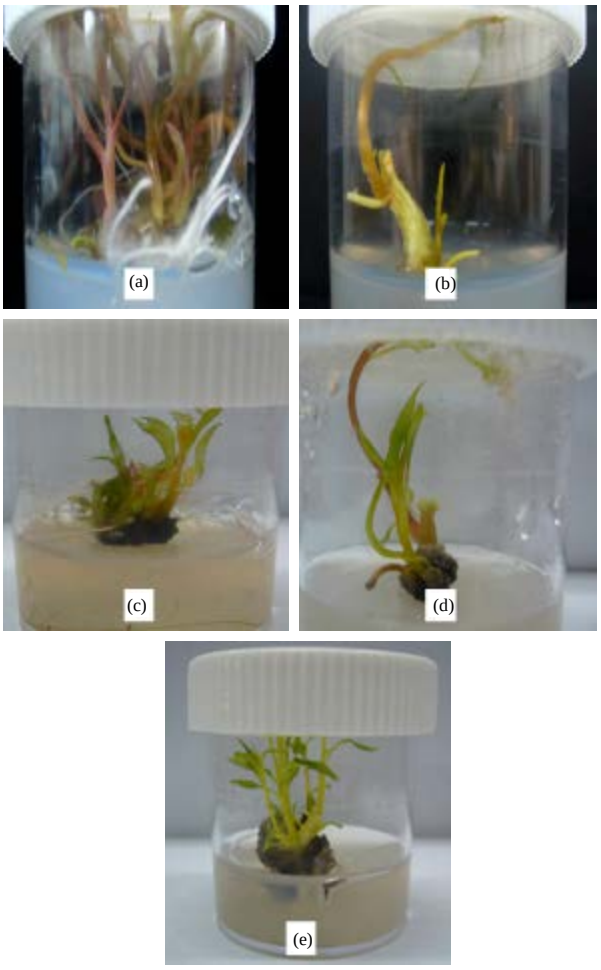

Fig. 1: The regenerated shoots *in vitro* from stem segments explants of *Celosia* sp. cultured in MS medium containing different juices at different concentration; (a) 70 ml L^{-1} of young coconut water, (b) 50 ml L^{-1} of banana juice, (c) 50 ml L^{-1} of tomato juice, (d) 20 ml L^{-1} of papaya juice and (e) 50 ml L^{-1} of matured coconut water

30 ml L^{-1} were have less additive ability in promoting shoots regeneration of 7.00±5.42 numbers of shoot in culturing within eight weeks averagely. There was a difference in the number of shoots production might influenced by the nutritional content in each of the organic material is not the same. For example, inorganic component contained in tomato include 292 mg of potassium, 30 mg of phosphorus, 14 mg of magnesium and 12 g of calcium. Differ to papaya that contains 360, 7, 14 and 34 mg of potassium, phosphorus, magnesium and

calcium, respectively. The number of shoots regeneration of *Celosia* sp. experienced differences since the nutritional values differ to each others.

Numbers of shoot obtain using old coconut show the same result with previous study by Al-Khayri (2010) that culturing embryo of date palm (*Phoenix dactylifera* L.). The numbers of shoots were significantly influence by coconut water concentration. As the concentration of coconut water increase, the number of resultant shoots also increased, reaching a maximum (50 ml L^{-1}). Increasing coconut water concentration up to 70 ml L^{-1} resulted in inhibition of shoots regeneration. This inhibition pattern shows the same result with Al-Khayri (2010).

According to George (1993), coconut water composed of many amino acids, nitrogenous compounds, inorganic compounds, organic acids, carbon sources, vitamins and growth regulators such as cytokinin and auxin. Research conducted by Yong *et al.* (2009) confirms that 94% of coconut water contains growth inducing compounds as specified as George (1993) can influence *in vitro* cultures. In number of plant species, regeneration improvement was achieved by augmenting culture medium with coconut water (Maddock *et al.*, 1983; Mathias and Simpson, 1986; Al-Khayri *et al.*, 1992; Boase *et al.*, 1993; Nasib *et al.*, 2008).

On the other hand, reduction numbers shoot regeneration occurred in 70 ml L^{-1} of mature coconut water from 13 to 11 as shown in Table 1 influenced by browning condition of the culture. Brown color appears slowly in agar show the plantlet secrete phenolic compound and oxidized. Browning is a restriction in *in vitro* culture due to the phenolic compound blocked enzyme activities and promoting death of the culture (Sukendah *et al.*, 2008).

Papaya is a major fruit crop worldwide that is primarily consumed as fresh fruit. Papaya fruits consist mostly of water and carbohydrate, low in calories and rich in natural vitamins and minerals, particularly in vitamins A and C, ascorbic acid and potassium (Da Silva *et al.*, 2007). In this present study, papaya extracts juice promised shoots regeneration in *Celosia* sp. stem segment cultivation as captured in Fig. 1d that supported by growth promoting substances as specified above. But the numbers shoot regeneration showed reduction since there was increasing in concentration of extract juices. This phenomenon occurred might caused by degradation of ascorbic acid that function as anti-browning in culture. During this study, the increasing of brown thickening on agar can be observed as increasing concentration extract juice concentration. Naturally, ascorbic acid rapidly decays in plant tissue culture media. Study by Elmore *et al.* (1990) stated within 50 min to 3 h after preparing 100 mM solutions, ascorbic acid was destroyed. Role as anti-browning and antioxtidant in plant cell growth that remained in papaya extract juice influents the shoot regeneration of *Celosia* sp. But the less concentration of the ascorbic acid caused the growth retardation occurred. In general, antioxidant compounds can facilitate *in vitro* cultures by their protective effect against oxidative stress.

Culture media are often supplemented with a variety of organic extracts which have constituents of an undefined nature. Banana extract juice or scientifically known as *Musa sapientum* were analysed for minerals contents by Anhwange *et al.* (2009). The result of mineral content indicate the concentrations (mg g^{-1}) of potassium, calcium, sodium, iron, manganese, bromine, rubidium, strontium, zirconium and niobium to be 78.10, 19.20, 24.30, 0.61, 76.20, 0.04, 0.21, 0.03, 0.02 and 0.02, respectively. High concentration of potassium which is one of the macronutrient needed in plant tissue culture support the shoots regeneration of *Celosia* sp. as proofed in Fig. 1b.

Tomato extract juice that has been added to the culture medium cannot be optimally utilized by the *Celosia* sp shoot primordial as in Fig. 1c. In Table 1 indicates increasing the concentration of tomato juice extract causes reduction of number of shoots regeneration. The shoot primordial experienced the growth retardation might be caused by effect of unsuitable pH in media culture. Since, the high content of acidic compound in tomato, it causes the changes in pH value to more acidic. Previous study by Cheng-Fan and Williams (1997) that using *Rosa canina* as experimental fruit. *Rosa canina* compatible with this present study that used tomato since it was in same berry organic fruit as tomato. Both of them are souring tested and cause pH changes in media. They conclude from their study that changes in pH occurred steadily during the start explants were cultured until it reaches equilibriums. However, the value and type of minerals found in the juice extract not changed at all. But what promising the growth retardation caused by the solubility and dissociation of minerals occurred.

The number of shoots production was not significantly influenced by different typed extract juices at all concentrations. The insignificantly represented by the number of shoots produced were about the same to each other (Table 1). Besides that, statistical results showed that there were not significant differences interactions effects ($p<0.05$) in terms of number of shoot regenerated between the types of extract juices used (mature and young coconut water, papaya, bananas and tomatoes). All extract juices contained its own nutrients and vitamins that support the shoots regeneration of

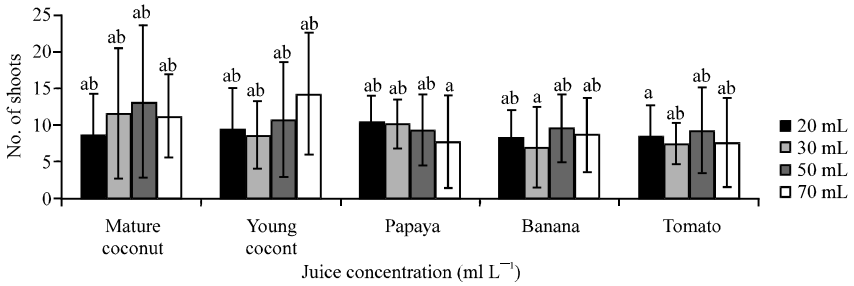

Fig. 2: Effect of different extract juices and concentrations on the shoot regeneration of *Celosia* sp. The results indicate the mean, standard deviation and mean differences for each treatment concentration

Table 2: Cost of alternative organic additives in preparation 1 liter of medium

Source (s)	Organic substances	Price (RM)*	Uses	Cost (RM)*
Mature coconut	Coconut water	4.00/each	100 mL	1.00
Young coconut	Coconut water	4.00/each	100 mL	1.00
Papaya	Papaya juice	2.00/kg	100 g	0.20
Banana	Banana juice	2.00/kg	100 g	0.20
Tomato	Tomato juice	2.50/kg	100 g	0.25

*RM-Ringgit Malaysia

Table 3: Comparison of costs to preparing 1 liter of medium

	Medium		
Comparison	1	2	3 (control)
Combination	Coconut water**+ MS powder+Agar+Sucrose	Fruit juice *** +MS powder + Agar+Sucrose	MS powder+2.0 mg L^{-1} BAP+1.0 mg L^{-1} NAA+vitamins+ Agar+Sucrose
Cost (RM) *	1.00+33.00+4.48 +2.70 = 41.18	0.22+33.00+4.48 +2.70 =40.40	33.00+0.24+0.003+0.28+4.48 +2.70 = 40.70
Number of shoots regenerated	(i) mature coconut water = 13.14±10.33 (ii) young coconut water = 14.21±8.26	(I) Papaya juice = 10.50±3.45 (ii) Banana juice = 9.57±4.68 (iii) Tomato juice = 9.28±5.82	15.50±1.67

*RM-Ringgit Malaysia, **Means cost of coconut water: RM 1.00, ***Means cost of papaya, banana and tomato juice: RM 0.22

Celosia sp. The mean differences contrasted using Tukey's test. Different letters on Fig. 2 indicate values are not significantly different ($p<0.05$).

The detail price of every extract juices that were used in this present study stated in Table 2. With assumption that, consumption of coconut water was worth RM 1.00, while the use of other extract juices were valuable only RM 0.20. Cost comparison media culture preparation shown in Table 3. Costs preparation using local extract juices as in media 1 and 2 were very small difference compared to the control medium (media 3) as used the MS basal medium. The cost differences between medium 3 and medium 1 was only RM 0.48 while between medium 3 and medium 2 was RM 0.30 for preparing one liter of medium. Since there was a large cost difference, the extract juice can be used as alternative items.

CONCLUSION

The five types of extract juices namely; mature coconut, young coconut, papaya, banana and tomato tested their responsibility in shoots regeneration stem segment on *Celosia* sp. The experiment result showed all five types of extract juice successful in promoting growth. This offer new possibilities of using raw materials as organic alternatives will attract more people to try plant tissue culture techniques.

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