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Influence of different type of Culture Media and activated charcoal on Callus Induction and ShootMultiplication of *Cadaminelyrata*

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Cadaminelyrata, aquatic plant, is use for decoration on a small aquarium. The present study was to increase the number of them by tissue culture. Shoot explant were excised and cultured on solid, liquid and semi-solid Murashige and Skoog (MS) medium supplemented with 0.5 mg/l NAA (α-naphthaleneacetic acid) and 2 mg/l BA (N_6-benzyladenine) with or without 0.2% activated charcoal (AC). The cultures were placed under light conditions at 14 h photoperiod, 27±1 ^oC to initiate callus induction and plant regeneration for 3 months. The result revealed that semi-solid MS medium supplemented with0.5 mg/l NAA and 2 mg/l BA without 0.2% AC gave the callus induction (100%)and size of callus at 1.06 centimeter better than another type of culture media.Liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA with or without 0.2% AC, gave the highest average number of shoot at 68.80/explant and shoot length at 10.69 centimeter,significant different with another type of culture media. This result showed that AC and type of culture media is improper for callus induction and shoot number.

Keywords: *Cadaminelyrata*, Type of Culture Media, Shoot Multiplication

Introduction

Cardaminelyrata, known commonly as Japanese cress and Chinese ivy, is a species of aquatic plant in the mustard family. It is native to the marshes of eastern China and Siberia, as well as Korea and Japan.The species is cultivated as an aquarium ornamental.Some fish will nibble on the leaves. (Wikipedia,2558).It is use for decoration on a small aquarium. *In vitro* tissue culture is a powerful alternative tool for propagation and plant conservation.Plant growth regulators are important in plant tissue culture since they play vital roles instem elongation, tropism, and apical dominance (Abobkar*et al*., 2012).Auxin and Cytokinin are the major phytohormones to regulating plant growth, development and classic phytohormone involved in cell division, growth, and organogenesis. There is enough residual cytokinin present in shoots therefore, little or no

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cytokinin is required in rooting medium (Hu and Wang, 1983). Several workers have reported multiple shoot induction with cytokinins in the growth medium (Tanveer*et al*., 2010; Joshi *et al*., 2012). Normally culture media for plant cell tissue culture used in form of solidify but some plant can growth with different type of media such as liquid and semi-solid medium.

Activated charcoal (AC) is commonly used intissue culture media. It may have either beneficial orharmful effects on the culture, depending upon themedium, and tissue used. Activated charcoal can be added to culture media that compounds are able to reduce the oxidation of phenolic compounds that can prevent death due to explant browning and increase shoot regeneration(Praveen *et al*., 2009).Activated charcoal is an essential component of plant tissue culture media. It is a strong adsorbent that can absorb toxic substances. It is an essential component of many plant tissue culture media, which prevents browning of cultured tissues and mediaby adsorption of toxic compounds like polyphenolsreleased by cultured tissues (Thomas, 2008).

Thus,present study aimed were (1) to compare solid, semi-solid and liquid cultures for *in vitro* multiplication of shoots (in order to achieve higher biomass production for induced mutation and plant conservation in the further); and (2) to analyze the content of activated charcoal on culture media for callus induction and *in vitro* grown shoots of *Cadaminelyrata*.

Materials and methods

The plant material was cultured on solid-MS free medium for 3 months. Shoots were excised with sterile scalpel blade, then it were inoculated on 6 types of culture media; solid, semi-solid and liquid used for the shoot bud induction was Murashige and Skoog (MS) medium supplemented with 0.5 mg/l NAA, 2 mg/l BA, 0.75% (w/v) agar, 3% (w/v) sucrose with or without 0.2% activated charcoal. The pH was adjusted to 5.7 with 1 N NaOH or HCl and the media were autoclaved at 121 ◦C at 15 p.s.i. (1.04 kg cmi2) pressure for 15 min. The cultures were maintained at 27±1 ◦C under a 14-h photoperiod of 50 μmol m^{-2} s^{-1} irradiance provided by cool white fluorescent. All the explants were placed horizontally on the medium in contact with the medium. Observations were recorded every month of culture. Factorial in completely randomized design with 10 replicates (each replicate consists of 10 shoots) was designed. The number of cultures producing shoot and shoot length were recorded and compared among those culture media.

Results

After 1 week of culture, explants showed the growth response in different type of culture medium. The axillary buds of nodal explants began

to grow within 5 days of cultured. After 2-3 weeks of cultured on solid and semi-solid medium without activated charcoal callus were occurred. For callus induction the result shown that only solid and semi-solid MS medium without 0.2%activated charcoal gave the callus induction to 100% (Table 1). Average size of callus and plant regeneration it was showed that solid MS medium gave the highest size of callus at 1.06 cm (Table 1), followed by semi-solid MS medium (1.03 cm). These types of culture media are not analysis. In this study, the significant influence of activated charcoal and liquid medium.

Table 1 Effect of different type of MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA on callus formation and average size of callus of *Cadaminelyrata* after cultured for3 months.

Culture media	Callus induction (%)		Size of callus (cm.)	
	with AC	without AC	with AC	without AC
1. solid MS medium	0.00	80	0.00	1.03[a]
2. semi-solid MS medium	0.00	100	0.00	1.06[a]
3. liquid MS medium	0.00	0	0.00	0[b]
F-test	-	-	-	**
CV.	-	-	-	8.18

- = Data not analysis.

liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA gave the highest average number of shoots at 68.80 shoots/explant, followed by semi-solid MS medium (41.71 shoots/explant) and solid MS medium (28.60 shoots/explant), respectively (Table 2). Culture media without activated charcoal gave the better number of shoot than culture media with activated charcoal (54.67 and 38.07 shoots/explant). The combination of type of culture media supplemented with or without activated charcoal the result revealed that liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BAwithout activated charcoalgave the best result on number of shoot at 71.10 shoots/explant, followed by the same culture medium with activated charcoal (66.50 shoots/explant) and semi-solid MS medium without activated charcoal (51.50 shoots/explant), respectively.

Table 2 Effect of different type of MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA on No. of shoot of *Cadaminelyrata* after cultured for 3 months.

Culture media	No. of shoot (shoot/explant)		Average[1] culture media
	with AC	without AC	
1. solid MS medium	15.80[f]	41.40[e]	28.60C
2. semi-solid MS medium	31.90[d]	51.50[c]	41.70B
3. liquid MS medium	66.50[b]	71.10[a]	68.80A
Average[2with or without AC]	38.07B	54.67A	**
			CV.(%)10.92

** = Significant difference at p≤0.01 level.
[1/] = Value followed by different letter are significantly different according to DMRT.

For shoot length, liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA gave the highest average of shoots lengthat 10.69 centimeter, followed by semi-solid MS medium (7.39 centimeter) and solid MS medium (3.90centimeter), respectively (Table 3). Culture media without activated charcoalgave the shoot length are not significant different with culture media with activated charcoal (7.68 and 6.97centimeter). The combination of type of culture media supplemented with or without activated charcoal the result revealed that liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA with activated charcoal gave the best result on shoot length at 11.27centimeter, followed by the same culture medium withoutactivated charcoal (10.11centimeter) and semi-solid MS medium without activated charcoal (8.65 centimeter), respectively.The characteristics of multiple shoots (Fig. 1) and shoot length (Fig. 2) of *Cadaminelyrata*were cultured on different culture media.

Table 3 Effect of different type of MS medium supplemented with 0.5 mg/l NAA and 2mg/l BA on Shoot length of *Cadaminelyrata* after cultured for 3 months.

Culture media	Shoot length (cm)		Average[1] culture media
	with AC	without AC	
1. solid MS medium	3.51d	4.29d	3.90c
2. semi-solid MS medium	6.12c	8.65b	7.39b
3. liquid MS medium	11.27a	10.11a	10.69a
Average[2with or without AC]	6.97a	7.68a	**
			CV.(%) 20.33

** = Significant difference at p≤0.01 level.
[1/] = Value followed by different letter are significantly different according to DMRT.

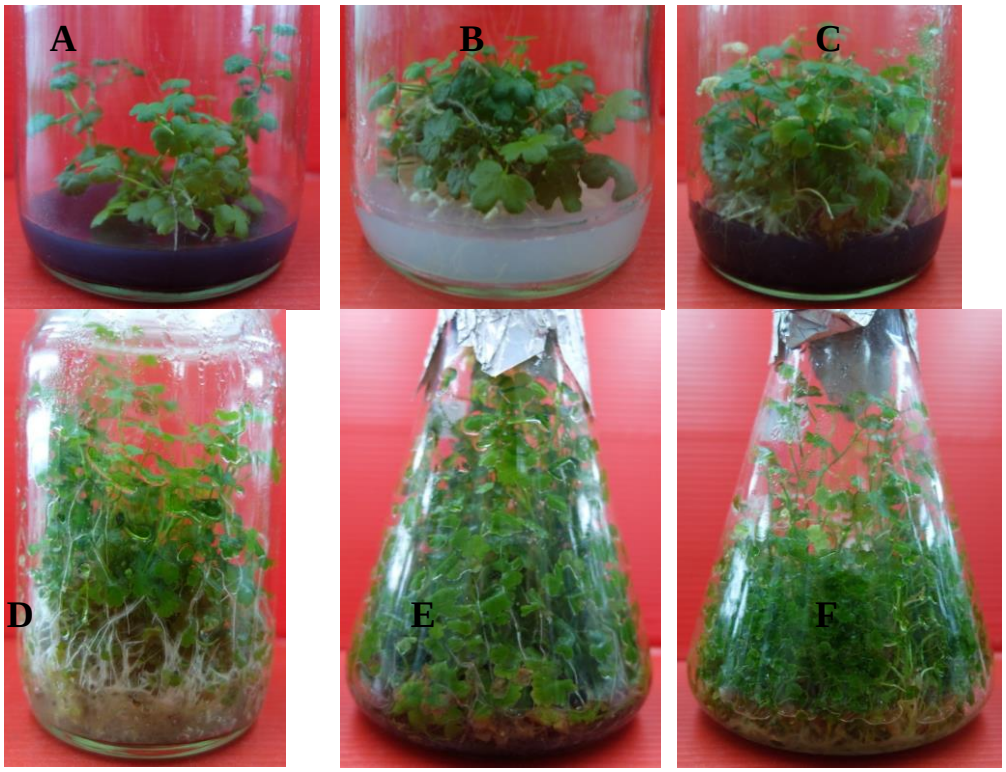

Fig. 1 Characteristics of multiple shoots of *Cadaminelyrata*after cultured on MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA for 3 months of *Cadaminelyrata*(bar = 0.5 cm).

A: Solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA with 0.2%AC.

B: Solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA without 0.2%AC.

C: Semi-solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA with 0.2%AC.

D: Semi-solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA and without 0.2%AC.

E: Liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA and with 0.2%AC.

F: Liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA and without 0.2%AC.

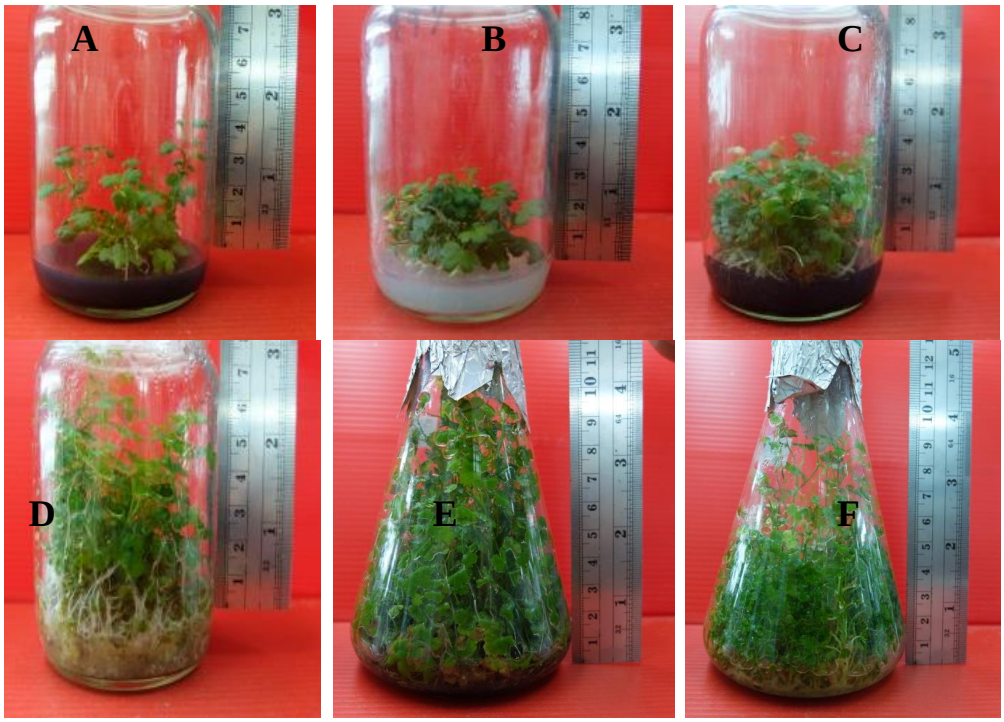

Fig. 2 Characteristics of shoots and shoot length after cultured on MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA for 3 months of *Cadaminelyrata.*

A: Solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA with 0.2%AC.

B: Solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA without 0.2%AC

C: Semi-solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA with 0.2%AC.

D: Semi-solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA and without 0.2%AC.

E: Liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA and with 0.2%AC.

F: Liquid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA and without 0.2%AC.

Discussions

The beneficiary effects ofactivated charcoal on tissue responses *in vitro* could beattributed to (a) establishing polarity by darkening the medium (Dumas and Monteuuis (1995); (b) adsorption of inhibitory substances, produced by either the media or explant(Fridborg and Eriksson, 1975; Fridborg*et al*., 1978); (c) adsorption of plant growth regulators and other organic compounds (Constantin*et al*., 1977; Weatherhead*et al*., 1978); or (d) the releaseof substances naturally present in or adsorbed by activated charcoal (Johansson *et al*., 1990). Itwas either beneficial or harmful effects on the culture, depending upon the medium, and tissue used like Pan and Staden (1999). The adsorptive capacities of

activated charcoal have also been shown to affect the composition of the media in a selective manner; thiamine HCl and nicotinic acid were removed from media by activated charcoal, whereas inositol and sucrose were not and activated charcoal gave the effected on callus growth and shoot organogenesis in tobacco (Constantin*et al*., 1977)as we find in our investigationbut gave the *in vitro* generated healthy shoots. The development of an activatedcharcoal-free media is an alternative. Activated charcoalis commonly increases the roots growth and improves the in vitro morphogenic response of tissues in several ways(Dharishini*et al*., 2015).

Data shown that shoot explants of *Cadaminelyrata* were cultured on liquid MS mediumsupplemented with 0.5 mg/l NAA and 2 mg/l BA gave the highest number of shoots like *in vitro* regeneration of brahmishoots (Praveen *et al*., 2009) but different from NeerBrahmi that reported by Dharishini*et al*. (2015).

Conclusions

Semi-solid MS medium supplemented with 0.5 mg/l NAA and 2 mg/l BA without 0.2% activated charcoal gave the callus induction (100%) and size of callus (1.06 centimeter). Liquid MS medium supplemented with the same plant growth regulator with or without 0.2% activated charcoal gave the highest average number of shoot (68.80/explant) and shoot length (10.69 centimeter). Activated charcoal and different type of culture media is improper for callus induction and shoot multiplication of *Cadaminelyrata*.

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