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Full Length Research Paper

Optimization of callus induction medium for *Hymenocallis littoralis* (Melong kecil) using root and bulb explants

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Callus was initiated using bulb and root explants of *Hymenocallis littoralis* on the Murashige and Skoog (MS) basal media supplemented with various auxins, namely 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, picloram, napthaleneacetic acid (NAA) and indole-3-acetic acid (IAA) at various concentrations. Degree of callus formation based on weight was found highest (+++++) in NAA treatment at 2.0 mg/L (93.33%). Dicamba produced the lowest (+) number of callus formation at 3.0 mg/L (13.33%). In terms of physical appearance, callus induced from NAA at 2.0 mg/L produced white yellowish and globular callus and Dicamba at 3.0 mg/L produced white and hard callus. Callus induction from bulb is possible except with IAA. Bulb explants grown on 2.0 mg/L 2,4-D and Dicamba formed callus after 11 days, followed by 3.0 mg/L picloram, as well but only 9 days for 2.0 mg/L NAA. Degree of callus formation was found to be the highest (+++++) in 2,4-D at 2.0 mg/L (93.33%). Picloram and NAA produced the lowest (++) callus formation at 3.0 and 2.0 mg/L (40.0% each). In terms of physical appearance, callus induced from 2, 4-D at 2.0 mg/L showed yellowish, and soft globular callus. Picloram at 3.0 mg/L produced white yellowish and soft callus; meanwhile, NAA at 3.0 mg/L produced small callus appearance of white and yellowish colour. The callus induction protocol developed in this study provides a fundamental investigation of bioactive constituents from the *H. littoralis* medicinal plant.

Key words: *Hymenocallis littoralis* plants, callus induction, auxins, bulb explants, root explants.

INTRODUCTION

Plants are a valuable source of a vast array of chemical compounds, and they synthesize and accumulate extractable organic substances in quantities sufficient to be economically useful as raw materials for various commercial applications (Rashida and Rabia, 2007). Plant cell and tissue cultures provide an alternative approach to plants which are difficult to cultivate, or has a long cultivation period, or has a low yield, product yield by cell culture may significantly produce a higher yield than those obtained from the parents (Hippolyte et al., 1992; Zhong et al., 1994). Plant cell cultures are generally more desirable than a solid medium because of higher growth rates resulting from high medium to tissue contact (Rashida and Rabia, 2007). However, plant cell cultures have been used for producing valuable biochemicals, such as drugs, flavourings, pesticides and fragrances (Nagamori et al., 2001). Cultured plant cells and tissues are widely recognized as promising alternatives for the production of valuable secondary metabolites (Wu et al., 2003; Rosli et al., 2009; Maziah et al., 2010). *Hymenocallis littoralis* (Melong kecil) commonly known as 'spider lily' is a bulbous, herbaceous plant from the family of Amaryllidaceae (Rafael and Michael, 2009). The plant is distributed by the sea and in swamps in tropical, sub-tropical, and temperate regions throughout the world

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(Ji and Merow, 1985). Throughout the history of *H. littoralis*, several alkaloids had been discovered from its bulb. The first alkaloid lycorine was proven to have antineoplastic, cytotoxic and antiviral properties (Renard-Noiaki et al., 1989, Lin et al., 1995, Abou-Donia et al., 2008).

The present investigation was carried out to study callus induction from the bulb and the root explants of *H. littoralis*. The objectives of present study were to test various concentrations of phytohormone for callus induction of root and bulb of *H. littoralis* wild plant.

MATERIALS AND METHODS

Plant source

H. littoralis plants used in this study was obtained from Penang Botanical Gardens, Penang, Malaysia.

Sterilization of explants

The explants collected from *H. littoralis* plants were washed under running tap water for 45 min. The explants then were place into the laminar flow and were soaked in the 75% ethanol within five minutes. Then 75% ethanol used was discarded and three drops of Tween-20 (Sigma) were added as a wetting agent into 15% (v/v) of commercial Clorox solution (5.2% $HgCl_2$) and the explants were soaked in the sterile solution for 20 min. The solution used then was discarded. The explants then were soaked in the 75% ethanol within five minutes, and then the explants were then rinsed for 5, 10, and 20 min with 200 ml of sterile distilled water. The sterilized explants were then cut into 1.5 × 0.2 cm for roots and 0.6 cm × 0.7 cm × 0.8 cm for bulbs.

Initiation of callus culture

The sterilized explants, namely bulb and root of *H. littoralis,* were cultured in the vial containing 10 ml of MS on the (Murashige and Skoog, 1962) basal media containing vitamins, 3% (w/v) sucrose and 0.25% (w/v) Gelrite supplemented with various auxins, namely 2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, picloram, NAA and IAA at concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L. The pH of the medium was adjusted to 5.7 to 5.8, prior autoclaving at 106 kPa, 121°C. Cultures were incubated at 25 ± 2°C in the dark for 4 weeks to determine the best condition for the initiation and maximum production of callus culture using bulb and root explants. The cultures were observed daily for one month to determine the time, amount of initiation and formation of the callus. The experiment was conducted in ten replicates.

Statistical analysis

The data were compared by one-way ANOVA following by a Tukey to compare means of the sample.

RESULTS AND DISCUSSION

In the first part of the study, various concentrations of phytohormones were used to select the suitable hormone for induction and development of callus cultures. Different parts of young explant such as root and bulb were cultured in basal MS medium supplemented with auxins. Callus was first initiated along the cut edges of culture, depending on the different auxin concentrations in the culture medium.

Tables 1 and 2 showed the duration for callus formation based on number of days, percentage of callus induction (C_{ip}), nature of response and degree of callus formation of callus induced from root and bulb explants in the induction media supplemented with different auxins. For root explants, results obtained revealed that all of the five auxins, the first callus could induce callus within 8 days grown on 1.0 mg/L of 2,4-D, followed by 9 days for picloram (2.0 and 3.0 mg/L), NAA (1.0, 2.0, 3.0 and 4.0 mg/L) and IAA (3.0 mg/L). Similar observation was reported on callus induction from root explants of the *Eurycoma longifolia* plants within 8 days (Maziah et al., 2010). However, degree of callus formation based on weight was found to be the highest (+++++) in NAA treatments at 2.0 mg/L (93.33%) followed by 2,4-D (+++) at 2.0 mg/L (70.0%), picloram and IAA (++) at 3.0 mg/L (70.0 and 73.33%).

On the other hand, dicamba produced the lowest (+) number of callus formation at 3.0 mg/L (13.33%). This result showed a significant increase in the induction of the callus (Table 1). In addition, no callus was formed from the treatment without any hormone. In terms of physical appearance (Figure 1), callus induced from NAA at 2.0 mg/L produced white yellowish, and globular callus, followed by 2, 4-D at 1.0 mg/L with white and soft callus. Meanwhile, Dicamba at 3.0 mg/L produced white and hard callus. Additional roots were formed in medium containing IAA at 3.0, 4.0 and 5.0 mg/L.

Meanwhile, callus induction for bulb results obtained revealed that four auxin (except IAA) could produced callus (Table 2). Bulb explants grown on 1.0, 2.0, 3.0 and 5.0 mg/L 2,4-D and 1.0, 2.0, 3.0 and 4.0 mg/L dicamba formed callus after 11 days of culture followed by 2.0 and 3.0 mg/L picloram. Degree of callus formation was found to be the highest (+++++) in 2, 4-D at 2.0 mg/L (93.33%) followed by dicamba (+++) at 2.0 mg/L (63.33%). Picloram and NAA produced the lowest (++) at 3.0 and 2.0 mg/L (40.0% each) callus formation. Similar observations were reported on callus induction in *Tabernaemontana pandacaqui* (Sierra et al., 1991), *Miscanthus* x *ogiformis* Honda 'Giganteus' (Holme and Petersen, 1996), *Withania somnifera* (Manickam et al. 2000), maize (Zacchini et al., 2000), *Genista* plants (Luczkiewicz and Glod, 2003), and *Asiatic salsola* species (Stefaniak et al., 2003).

No callus was formed from the treatment of Murashige and Skoog (1962) basal media supplemented without phytohormone. In addition, the bulb explants turned to green hard structure and shoots were formed on media without auxin. In terms of physical appearance (Figure 2), callus induced from 2,4-D at 2.0 mg/L produced yellowish, and soft globular callus, followed by dicamba

Table 1. Callus Induction from root explants of *Hymenocallis littoralis* in MS basal medium + MS vitamins, 3% (w/v) sucrose, 0.25% (w/v) Gelrite agar supplemented with different hormones concentration (0, 1, 2, 3, 4, 5 mg/L).

Hormones and concentration (mg/L)		Period to form callus (day)	% of callus induction (Cip)	Nature of response	Degree of callus formation
2,4-D	0.0	NoC	NoC	Explant turned to brown	-
	1.0	8	40.0^{ab}	White soft callus form	+++
	2.0	9	70.0^{ab}	White soft callus form	+++
	3.0	9	56.67^{b}	White soft callus form, little roots were grown	+++
	4.0	9	46.67^{ab}	White soft callus form	+++
	5.0	10	3.33^{ab}	White yellowish, nodular callus	++
Dicamba	0.0	NoC	NoC	Explant turned to green and hard structured, shoots formed	-
	1.0	NoC	NoC^{ab}	Explant turned to brown	-
	2.0	NoC	NoC^{ab}	Explant turned to brown	-
	3.0	11	13.33^{b}	White hard callus form. Explant turned to brownish	+
	4.0	NoC	NoC^{ab}	Explant turned to brown	-
	5.0	NoC	NoC^{ab}	Explant turned to brown	-
Picloram	0.0	NoC	NoC	Explant turned to green and hard structured, shoots formed	-
	1.0	11	6.67^{ab}	Explant turned to brown. Small white soft callus formed	+
	2.0	9	63.33^{ab}	Explant turned to small soft white callus	+++
	3.0	9	70.0^{b}	Explant turned to small soft white callus	++
	4.0	10	50.0^{ab}	Explant turned to small soft white callus	++
	5.0	9	46.67^{ab}	Explant turned to small soft white callus	++
NAA	0.0	NoC	NoC	Explant turned to brown	-
	1.0	9	33.33^{ab}	Explant turned to green	++
	2.0	9	93.33^{ab}	White yellowish globular callus	+++++
	3.0	9	73.33^{b}	White yellowish globular callus	+++++
	4.0	9	73.33^{ab}	White yellowish soft callus	+++++
	5.0	10	53.33^{ab}	White yellowish soft watery	++++
	5.0	10	16.67^{ab}	White hard callus formed	+
IAA	0.0	-	NoC	Explant turned to green and hard structured, shoots form	-
	1.0	10	30.0^{ab}	Small soft callus, Explant turn to white and green	++
	2.0	10	36.67^{ab}	Small soft callus, Explant turn light brown	++

Table 1. Contd.

	3.0	9	73.33[b]	Small white hard callus. Explant turns to white hard structure	+
	4.0	10	20.0[ab]	Explant turns to brown. Large and long root formed	+
	5.0	10	16.67[ab]	White hard callus formed	+

- = no callus formed, + = very few callus formation, ++ = minor callus formation, +++ = slight callus formation, ++++ = moderate callus formation, +++++ = profuse callus formation, Means within the column having the same letter were not significantly different by the Turkey HSD test ($p > 0.05$), NoC=No callus.

Table 2. Callus induction from bulb explants of *Hymenocallis littoralis* in MS basal medium + MS vitamins, 3% (w/v) sucrose, 0.25% (w/v) Gelrite agar supplemented with different hormones concentration (0, 1, 2, 3, 4, 5 mg/L).

Hormones and concentration (mg/L)		Period to form callus (day)	% of callus induction(C_{ip})	Nature of response	Degree of callus formation
2,4-D	0.0	NoC	NoC	Explant turned to green and hard structured, shoots formed	-
	1.0	11	23.33[ab]	White soft callus, shoot formed	++
	2.0	11	93.33[b]	Yellowish, soft globular callus	+++++
	3.0	11	73.33[ab]	Yellowish, soft globular callus	+++
	4.0	15	23.33[ab]	White- yellowish, nodular callus	++
	5.0	11	20.00[ab]	White -yellowish, nodular callus	++
Dicamba	0.0	NoC	NoC	Explant turned to green and hard structured, shoots formed	-
	1.0	11	10.00[ab]	White-yellowish, small globular callus	+
	2.0	11	63.33[b]	Yellowish-white, soft and compact callus, torpedo shape	+++
	3.0	11	40.00[ab]	Yellowish-white compact callus	++
	4.0	11	46.67[ab]	Yellowish, soft and compact callus	++
	5.0	13	10.00[ab]	White yellowish, not all callus produced from the explant	+
Picloram	0.0	NoC	NoC	Explant turned to green and hard structured, shoots formed	-
	1.0	13	NoC[ab]	Explant turned to expand hard white and red in colour	-
	2.0	11	10.00[b]	Explant turned to small globular callus	+
	3.0	11	40.00[ab]	White yellowish callus, soft callus	++
	4.0	13	13.33[ab]	Small white callus	+
	5.0	11	10.00[ab]	White soft callus	+
NAA	0.0	NoC	NoC	Explant turned to green and hard structured, shoots formed	-
	1.0	10	10.00[ab]	White soft, watery callus	+
	2.0	9	40.00[b]	White, yellow small callus	++
	3.0	9	23.33[ab]	White soft watery and friable	+

Table 2. Contd.

4.0	13	10.00[ab]	White soft watery and friable	+
5.0	13	NoC[ab]	Explant turned to hard white structure	-
5.0	NoC	NoC[ab]	Explant turn to white and green hard structure	-

NoC=No callus.

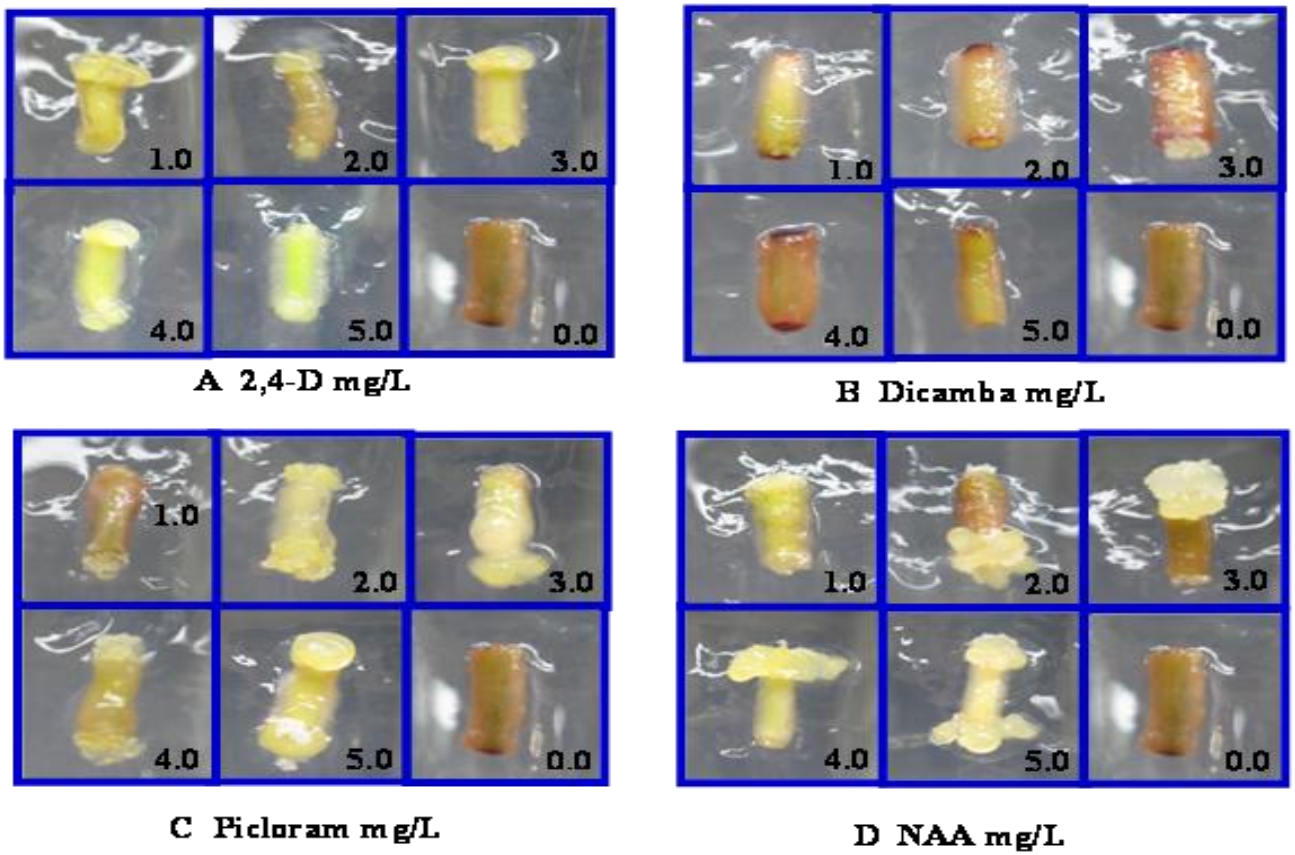

Figure 1. Induction of *Hymenocallis littoralis* callus cultures derived from root explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) Gelrite agar supplemented with different hormones concentration (0, 1, 2, 3, 4, 5 mg/L) after 30 days of culture. A (2,4-D), B (dicamba), C (picloram), D (NAA), E (IAA). The scale (1 cm = 0.5 cm) representing the explants above.

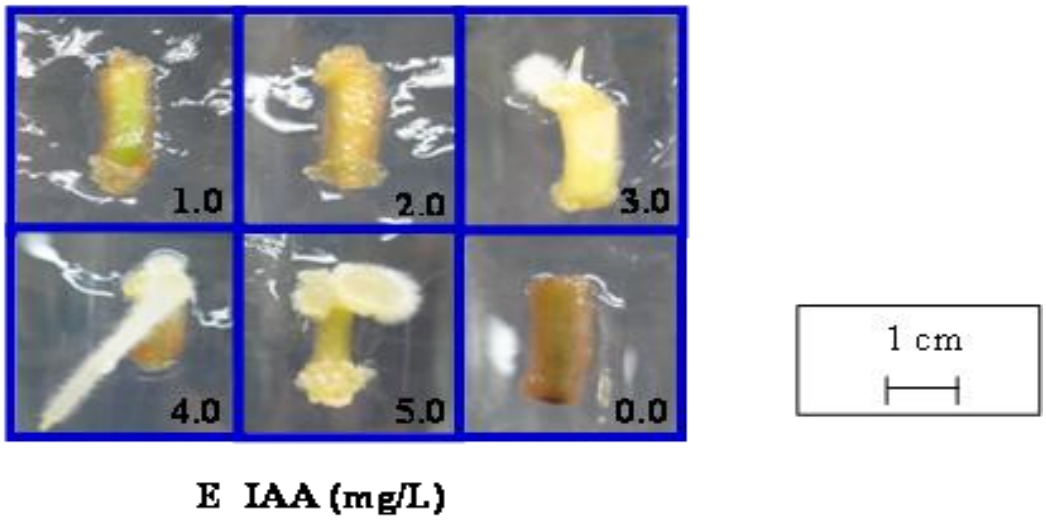

Figure 1. Contd.

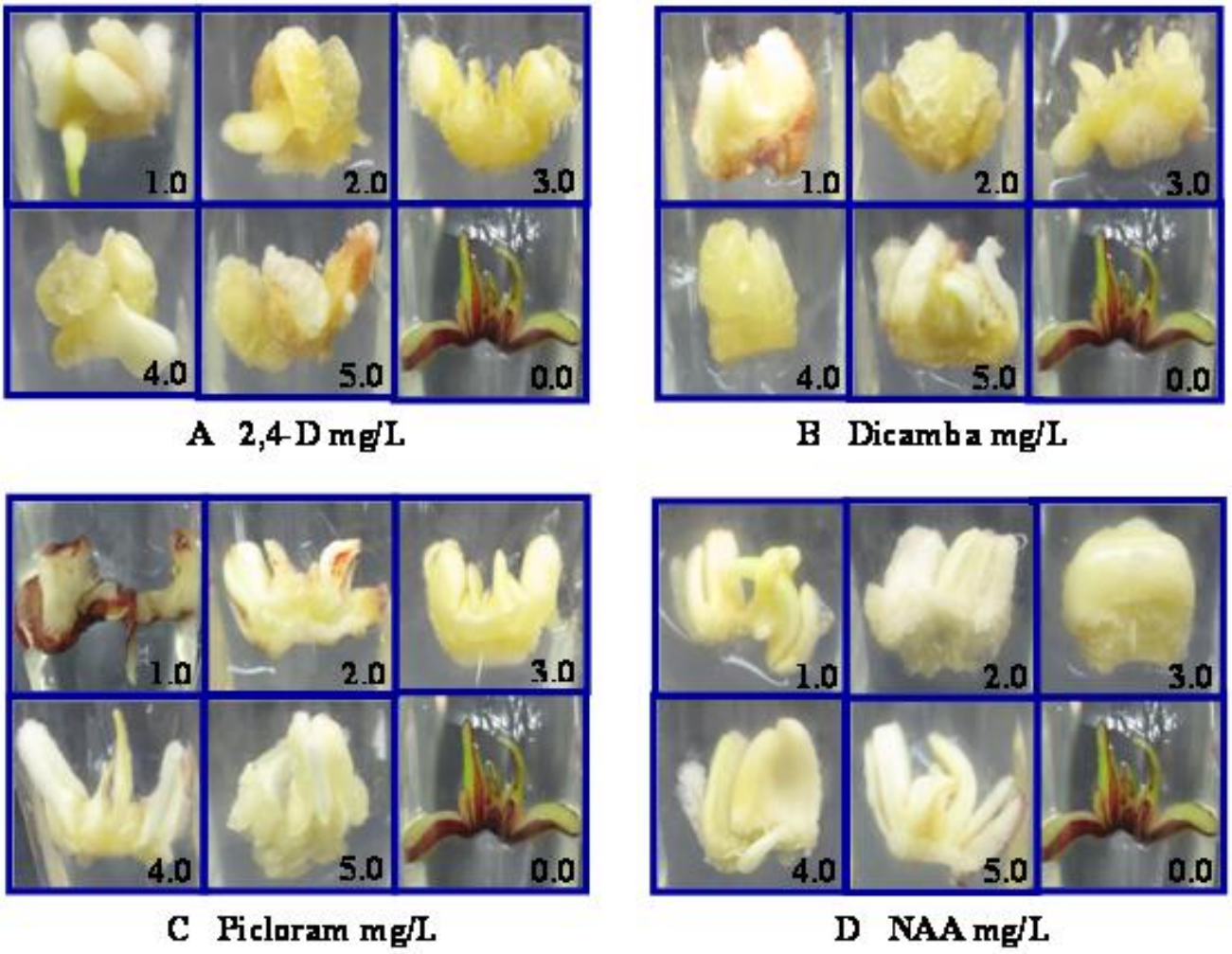

Figure 2. Induction of *Hymenocallis littoralis* callus cultures derived from bulb explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) Gelrite agar supplemented with different hormones concentration (0, 1, 2, 3, 4, 5 mg/L) after 30 days of culture. A (2,4-D), B (dicamba), C (picloram), D (NAA), E (IAA). The scale (1 cm = 0.5 cm) representing the explants above.

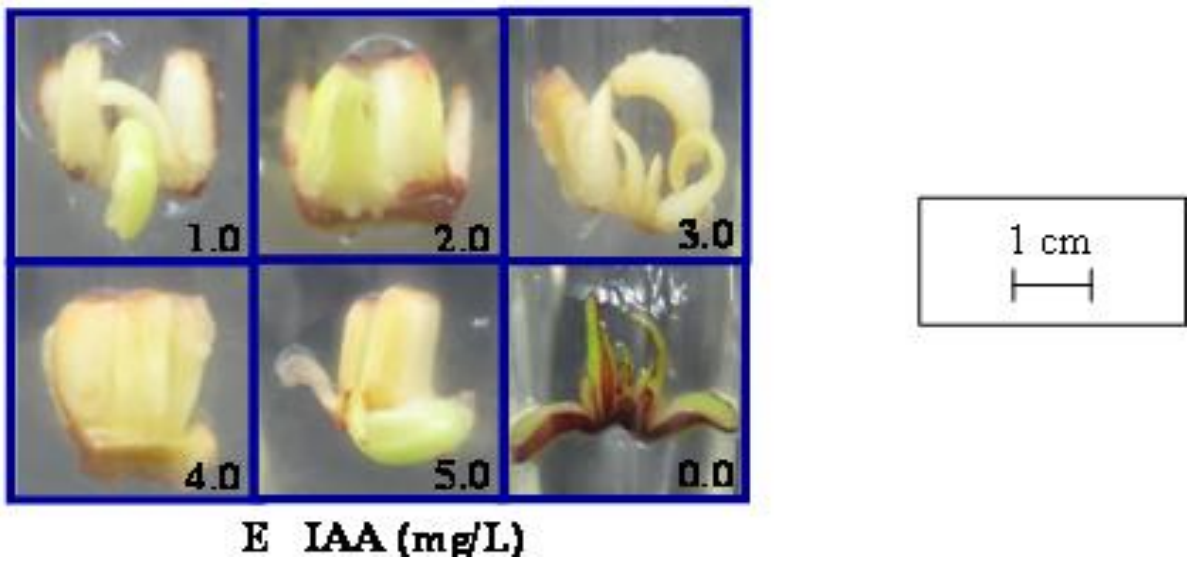

Figure 2. Contd.

at 2.0 mg/L yellowish-white, soft, compact callus and torpedo in shape. Meanwhile, picloram at 3.0 mg/L produced white yellowish callus and soft callus appearance. NAA at 3.0 mg/L produced small callus appearance of white and yellowish in colour.

No response was observed in the medium containing IAA, even the cultures were kept for prolonged eriod. A similar result was obtained by Das et al. (1995). The percentages of callus induction using various types of explants were found to be increased significantly by using selected types of auxin in *Eurycoma longifolia* plants.

Conclusion

The best auxin used for callus induction from root explants of *H. littoralis* was NAA at 2.0 mg/L followed by 2,4-D at 2.0 mg/L, picloram at 2.0 mg/L, IAA at 2.0 mg/L, and dicamba at 3.0 mg/L. Meanwhile, the best auxin used for callus induction from bulb explants of *H. littoralis* (Melong kecil) was 2,4-D at 2.0 mg/L, followed by Dicamba at 2.0 mg/L, picloram at 3.0 mg/L, and NAA at 2.0 mg/L.

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