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Full Length Research Paper

Optimization of suitable auxin application in a recalcitrant woody forest plant of *Eurycoma longifolia* (Tongkat Ali) for callus induction

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A study was carried out to determine and optimize suitable auxin for callus induction in *Eurycoma longifolia*. The induction of callus cultures using leaf, petiole, rachis, stem, tap root, fibrous root, cotyledon and embryo segments were successfully achieved by using various auxins such as 2,4-D, IAA, NAA, picloram and dicamba. The cultures were observed daily for one month. The overall results from the experiments showed that the presence of 2,4-D was the most suitable auxin for the highest callus induction and growth performance rate by using various explants types of *E. longifolia*. The callus initiation in leaf explant was the highest using 1.0 mgL^{-1} of 2,4-D (81.67%), followed by picloram (78.33% at 3.0 mgL^{-1}), dicamba (73.33% at 2.0 mgL^{-1}) and NAA (61.67% at 4.0 mgL^{-1}). Auxin, 2,4-D and picloram at 4.0 mgL^{-1} were found to be the most effective for callus induction (78.33%) using petiole explant. It was followed by using dicamba (73.33% at 2.0 mgL^{-1}), NAA (73.33% at 3.0 mgL^{-1}) and IAA (61.67 % at 3.0 mgL^{-1}), respectively. In addition, the percentage of callus induction from rachis was obtained at highest rate by using picloram (86.67 % at 4.0 mgL^{-1}), followed by 2,4-D (81.67% at 4.0 mgL^{-1}), dicamba (78.33% at 2.0 mgL^{-1}), NAA (58.33% at 2.0 mgL^{-1}) and IAA (45% at 3.0 mgL^{-1}). Meanwhile, for stem explant, it has been noticed that highest callus induction at 88.33% could be obtained from medium containing 2,4-D at 2.0 mgL^{-1}, followed by picloram (80% at 5.0 mgL^{-1}), dicamba (85% at 5.0 mgL^{-1}), NAA (76.67% at 1.0 mgL^{-1}) and IAA (63.33% at 4.0 mgL^{-1}). For tap root explant, the highest amount of callus was formed at 3.0 mgL^{-1} 2,4-D and 1.0 mgL^{-1} picloram (81.67%), followed by dicamba (78.33% at 1.0 mgL^{-1}). For fibrous root explant, the highest callus was produced in 2,4-D at 4.0 mgL^{-1} (86.67%), picloram (78.33% at 1.0 mgL^{-1}) and dicamba (78.33% at 2.0 mgL^{-1}). The highest scoring of callus induction from cotyledon was determined by using 2,4-D at 4.0 mgL^{-1} (85%), followed by picloram (83.33% at 1.0 mgL^{-1}), dicamba (78.33% at 2.0 mgL^{-1}), NAA (56.67% at 1.0 mgL^{-1}) and IAA (36.67% at 3.0 mgL^{-1}). Finally, the highest scoring for callus induction from embryo was produced in 2, 4-D and picloram at 2.0 and 5.0 mgL^{-1} (both 85%) followed by NAA and IAA 83.33% at 1.0 and 4.0 mgL^{-1}, respectively, and dicamba, 81.67% at 5.0 mgL^{-1}. The percentages of callus induction using various types of explants were found to be increased significantly by using selected types of auxins in *E. longifolia* plants.

Key words: *Eurycoma longifolia,* Callus induction, auxin.

INTRODUCTION

Simaroubaceae family is a large family comprising of 30 genera and 200 species, of which eight genera and 10 species are found in Malaysia (Ang et al., 2002). *Eurycoma longifolia* Jack is a Malaysian plant belonging to Simaroubaceae family. It is known as '*Tongkat Ali*' by the local folks. It is a tall, slender shrub-tree that can be commonly found as an under storey in the lowland forest at up to 500 m above sea level and also along the hilly

jungle slopes of Malaysia (Ang et al., 1995; 2002).

E. longifolia is a single-stemmed slow growing tree from the rainforests of Southeast Asia. It is also found in other countries of the South-East Asia region and widely distributed in primary and secondary forests in Burma, Indochina, Thailand, Sumatra, Borneo, and Philippines (Kuo et al., 2003). 'Tongkat' Ali is also known as '*Pasak Bumi*' in Indonesia and '*Bidara Pahit*' in some regions of Jawa, Indonesia. In Vietnam it is called '*cay ba binh*', which means a tree that can cure hundred of diseases (Chan et al., 1999). Nurhanan et al. (2005) reported that extracts obtained from the root of this plant for its possible cytotoxic effect against various cancer cell lines such as KB, DU-145, RD, MCF-7, CaOV-3 and MDBK. Similarly, the cytotoxic effects of *E. longifolia* have also been reported by Abd Razak et al. (2007).

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). Recently, we have reported on the successful of enhancement production of 9-methoxycanthin-6-one in callus cultures of *E. longifolia* (Rosli et al., 2009). In addition, a large number of literature reviews on the production of secondary metabolite from callus cultures have been reported (Sierra et al., 1991; Luczkiewicz and Glod, 2003, Elizabete et al., 2009, Zsuzsanna and Anja, 2009). The objective of the present study is to determine and optimize suitable auxin for callus induction in *E. longifolia.*

MATERIALS AND METHODS

Plant materials

Tongkat Ali plants (7 years old) used was grown at the Laboratory of Natural Product Discovery, Institute of Bioscience, Universiti Putra Malaysia (UPM), Serdang, Selangor. Different parts of the plants, leaves (0.7cm x 0.7cm), petioles (0.5 cm), rachises (0.5 cm), stems (0.5 cm), tap roots (0.3 cm), fibrous roots (0.3 cm), cotyledons (0.4 cm) and embryos (0.2 cm), were used as plant materials for this study.

Sterilization of explants

The explants collected from Tongkat Ali plant were washed under running tap water for 30 min. Two drops of Tween-20 (Sigma) were added as wetting agent into 15% commercial Clorox (Sodium hypochlorite 5.25%) solution and the explants were soaked in the sterile solution for 30 minutes. The explants were then rinsed for 5, 10, and 20 minutes with 100 ml of sterile distilled water. The sterilized explants were then cut into small pieces and then transferred into vials (8.4cm x 2.4cm).

Callus Induction

The explant was cultured in the vial containing 10 mL of MS basal medium, 3% (w/v) sucrose and 0.25% (w/v) Gelrite. Various types and concentrations of auxins (2,4-D, dicamba, picloram, NAA and IAA) different (0, 1.0, 2.0, 3.0, 4.0 and 5.0 mgL^{-1}) were tested. The pH of the medium was adjusted to 5.7 prior to adding Gelrite and autoclaving (106 kPa, 121°C, 15 min). The experiment was conducted in ten replicates. The cultures were incubated at 25 ± 2°C in the dark. The cultures were observed daily for one month to determine the amount of callus initiated and formation of the callus.

Statistical analysis

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

RESULTS AND DISCUSSION

Induction of *E. longifolia* callus cultures from different explants

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 leaf, petiole, rachis, stem, tap root, fibrous root, cotyledon and embryo, and were cultured in basal MS medium supplemented with auxins. The auxins used were 2,4-D, picloram, dicamba, NAA and IAA (1, 2, 3, 4, and 5 mgL^{-1}). Callus was first initiated along the cut edges of culture, depending on the different auxin concentrations in the culture medium. Table 1 to 4 showed the percentage of callus induced from leaf, petiole, rachis, stem, tap root, fibrous root, cotyledon and embryo explants in the induction media supplemented with different auxins.

For leaf explant, the first callus was induced after 7 days of culturing in medium containing 2,4-D, followed by picloram, dicamba and NAA treatments. The calluses were induced at 8, 9 and 10 days of culture, respectively. No response was observed in the medium containing IAA. As shown in Table 1, the callus initiation was the highest at 1.0 mgL^{-1} 2,4-D (81.67%) followed by picloram (78.33% at 3.0 mgL^{-1}), dicamba (73.33% at 2.0 mgL^{-1}) and NAA (61.67% at 4.0 mgL^{-1}). This result showed a significant increase in the induction of the callus (Table 1).

Callus was induced from petioles after 8 days of culture in medium containing 2,4-D, picloram and IAA. While in medium containing dicamba and NAA, the callus was induced after 9 and 10 days of culture, respectively. Auxin 2,4-D and picloram at 4.0 mgL^{-1} were found to be the most effective auxin (78.33%) for callus induction

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Abbrevaitions: MS, Murashige and Skoog medium; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **IAA,** indole-3-acetic acid; **NAA,** 1-naphthaleneacetic; **PGR,** plant growth regulator.

Table 1. Percentage of callus induced from leaves and petiole explants in the induction media (MS) supplemented with different auxins

Hormones and Concentrations (mg l^{-1})	C_{ip} (%) explants	
	Leaf	Petiole
Control	No^C	No^C
2,4-D		
1.0	81.67 ^{a}	11.67 ^{c}
2.0	68.33 ^{a,b}	25.0 ^{c}
3.0	41.67 ^{b}	45.0 ^{b}
4.0	41.67 ^{b}	78.33 ^{a}
5.0	23.33 ^{c}	56.67 ^{b}
Picloram		
1.0	41.67 ^{c}	13.33 ^{c}
2.0	58.33 ^{b}	21.67 ^{b,c}
3.0	78.33 ^{a}	58.33 ^{a}
4.0	18.33 ^{d}	78.33 ^{a}
5.0	13.33 ^{d}	35.0 ^{b}
Dicamba		
1.0	21.67 ^{d}	58.33 ^{b}
2.0	73.33 ^{a}	78.33 ^{a}
3.0	56.67 ^{b}	41.67 ^{c}
4.0	41.67 ^{c}	20.0 ^{d}
5.0	23.33 ^{d}	11.67 ^{d}
NAA		
1.0	18.33 ^{c}	36.67 ^{c}
2.0	40.0 ^{b}	61.67 ^{b}
3.0	61.67 ^{a}	73.33 ^{a}
4.0	56.67 ^{a}	23.33 ^{d}
5.0	18.33 $_{c}$	13.33 ^{d}
IAA		
1.0	No^C	43.33 ^{c}
2.0	No^C	50.0 ^{b}
3.0	No^C	61.67 ^{a}
4.0	No^C	15.0 ^{d}
5.0	No^C	11.67 ^{d}

No^C = No callus induced; **C_{ip}** = The percentage of callus induction. Analysis of variance (ANOVA) was applied to angular transformed data and mean separation was performed by Tukey' test ($p<0.05$). Each treatment was conducted in 10 replicates.

Table 2. Percentage of callus induced from rachis and stem explants in the induction media (MS) supplemented with different auxins.

Hormones and Concentrations (mg l^{-1})	C_{ip} (%) explants	
	Rachis	Stem
Control	No^C	No^C
2,4-D		
1.0	11.67 ^{d}	18.33 ^{d}
2.0	21.67 ^{d}	88.33 ^{a}
3.0	40.0 ^{c}	55.0 ^{b}
4.0	81.67 ^{a}	41.67 ^{c}
5.0	61.67 ^{b}	13.33 ^{d}
Picloram		
1.0	38.33 ^{c}	11.67 ^{c}
2.0	43.33 ^{c}	21.67 ^{c}
3.0	65.0 ^{b}	45.0 ^{b}
4.0	86.67 ^{a}	58.33 ^{b}
5.0	26.67 ^{c}	80.0 ^{a}
Dicamba		
1.0	58.33 ^{b}	11.67 ^{c}
2.0	78.33 ^{a}	20.0 ^{c}
3.0	45.0 ^{b}	48.33 ^{b}
4.0	15.0 ^{c}	63.33 ^{b}
5.0	11.67 ^{c}	85.0 ^{a}
NAA		
1.0	36.67 ^{c}	76.67 ^{a}
2.0	58.33 ^{b}	61.67 ^{a,b}
3.0	80.0 ^{a}	41.67 ^{b,c}
4.0	16.67 ^{d}	20.0 ^{c,d}
5.0	11.67 ^{d}	11.67 ^{d}
IAA		
1.0	16.67 ^{c}	13.33 ^{b}
2.0	31.67 ^{b}	21.67 ^{b}
3.0	45.0 ^{a}	63.33 ^{a}
4.0	18.33 ^{c}	78.33 ^{a}
5.0	13.33 ^{c}	30.0 ^{b}

NoC = No callus induced; Cip = The percentage of callus induction. Analysis of variance (ANOVA) was applied to angular transformed data and mean separation was performed by Tukey' test ($p<0.05$). Each treatment was conducted in 10 replicates.

from petioles explant. It was followed by dicamba (73.33% at 2.0 mgL^{-1}), NAA (73.33% at 3.0 mgL^{-1}) and IAA (61.67% at 3.0 mgL^{-1}) (Table 1). Significant increase was found from the results of percentage of callus induction (Table 1). The callus of rachis was initiated after 8 days growing in MS medium that contained 2,4-D. For NAA and IAA, dicamba and picloram treatments, the callus was induced at 11, 12 and 13 days of culture, respectively. The results showed that the percentage of callus induction from rachis was the highest in picloram (86.67% at 4.0 mgL^{-1}) followed by 2,4-D (81.67% at 4.0 mgL^{-1}), dicamba (78.33% at 2.0 mgL^{-1}), NAA (58.33% at 2.0 mgL^{-1}) and IAA (45% at 3.0 mgL^{-1}) (Table 2). However the medium supplemented with IAA showed little response to the callus initiation, but the percentage of callus induction was significantly increased (Table 2).

Among the plant growth regulators (PGRs) used, 2,4-D showed the highest callus initiated for stem explant of *E. longifolia*. In MS basal medium with 2,4-D, the callus was initiated after NAA supplementation; the initiation time for callusing was shorter that was 7 days. Meanwhile, medium containing IAA was longer for the initiation time

Table 3. Percentage of callus induced from tap root and fibrous root explants in the induction media (MS) supplemented with different auxins.

Hormones and Concentrations (mg l^{-1})	C_{ip} (%) explants	
	Tap root	Fibrous root
Control	**No^{C}**	**No^{C}**
2,4-D		
1.0	35.0 ^{c}	38.33 ^{c}
2.0	56.67 ^{b}	48.33 ^{c}
3.0	81.67 ^{a}	65.0 ^{b}
4.0	18.33 ^{d}	86.67 ^{a}
5.0	11.67 ^{d}	18.33 ^{d}
Picloram		
1.0	81.67 ^{a}	78.33 ^{a}
2.0	56.67 ^{b}	63.33 ^{a}
3.0	35.0 ^{c}	45.0 ^{b}
4.0	18.33 ^{d}	36.67 ^{b}
5.0	13.33 ^{d}	16.67 ^{a}
Dicamba		
1.0	78.33 ^{a}	73.33 ^{a}
2.0	56.67 ^{b}	78.33 ^{a}
3.0	35.0 ^{c}	58.33 ^{b}
4.0	18.33 ^{d}	41.67 ^{c}
5.0	11.67 ^{d}	13.33 ^{d}
NAA		
1.0	No^{C}	81.67 ^{a}
2.0	No^{C}	66.67 ^{a,b}
3.0	No^{C}	65.0 ^{a,b}
4.0	11.67 ^{a}	56.67 ^{b,c}
5.0	No^{C}	38.33 ^{c}
IAA		
1.0	No^{C}	23.33 ^{b}
2.0	13.33 ^{a}	35.0 ^{a}
3.0	No^{C}	13.33 ^{b,c}
4.0	No^{C}	11.67 ^{c}
5.0	No^{C}	11.67 ^{c}

NoC = No callus induced; Cip = The percentage of callus induction. Analysis of variance (ANOVA) was applied to angular transformed data and mean separation was performed by Tukey' test ($p<0.05$). Each treatment was conducted in 10 replicates.

Table 4. Percentage of callus induced from cotyledon and embryo explants in the induction media (MS) supplemented with different auxins.

Hormones and Concentrations (mg l^{-1})	C_{ip} (%) explants	
	Cotyledon	Embryo
Control	**No^{C}**	**No^{C}**
2,4-D		
1.0	11.67 ^{c}	23.33 ^{d}
2.0	48.33 ^{b}	85.0 ^{a}
3.0	60.0 ^{b}	58.33 ^{b}
4.0	85.0 ^{a}	43.33 ^{c}
5.0	21.67 ^{c}	13.33 ^{d}
Picloram		
1.0	83.33 ^{a}	11.67 ^{d}
2.0	66.67 ^{b}	21.67 ^{d}
3.0	48.33 ^{c}	40.0 ^{c}
4.0	18.33 ^{d}	63.33 ^{b}
5.0	13.33 ^{d}	85.0 ^{a}
Dicamba		
1.0	66.67 ^{a,b}	11.67 ^{c}
2.0	78.33 ^{a}	21.67 ^{c}
3.0	41.67 ^{b,c}	41.67 ^{b}
4.0	25.0 ^{c,d}	63.33 ^{a}
5.0	11.67 ^{d}	81.67 ^{a}
NAA		
1.0	11.67 ^{c}	83.33 ^{a}
2.0	30.0 ^{b}	61.67 ^{b}
3.0	56.67 ^{a}	45.0 ^{b}
4.0	No^{C} ^{d}	25.0 ^{c}
5.0	No^{C} ^{d}	11.67 ^{c}
IAA		
1.0	11.67 ^{c}	11.67 ^{c}
2.0	25.0 ^{b}	25.0 ^{b,c}
3.0	36.67 ^{a}	68.33 a
4.0	No^{C} ^{d}	83.33 ^{a}
5.0	No^{C} ^{d}	40.0 ^{b}

NoC = No callus induced; Cip = The percentage of callus induction. Analysis of variance (ANOVA) was applied to angular transformed data and mean separation was performed by Tukey' test ($p<0.05$). Each treatment was conducted in 10 replicates.

for callusing which was 10 days. Referring to the Table 2, 88.33% was obtained from medium containing 2,4-D at 2.0 mgL^{-1} 2,4-D, followed by picloram (80% at 5.0 mgL^{-1}) ,dicamba (85% at 5.0 mgL^{-1}), NAA (76.67% at 1.0 mgL^{-1}) and IAA (63.33% at 4.0 mgL^{-1}). All auxins showed a significant increase except for NAA (Table 2).

For tap root explant, callus was induced after 14 days of culturing in media containing 2, 4-D and dicamba. Callus induction in medium with picloram required 15 incubation days. However, the medium supplemented with NAA and IAA showed little or no response to callus induction even after 30 days of incubation period. The highest amount of callus was formed at 3.0 mgL^{-1} 2,4-D and 1.0 mgL^{-1} picloram (81.67%) followed by dicamba (78.33% at 1.0 mgL^{-1}) (Table 3). The percentage of callus induction was significantly increased (Table 3). The initiation time for fibrous root callusing was 9 days. It was shorter if compared with the tap root. Table 3 showed that the highest callus was produced in 2,4-D at 4.0 mgL^{-1} (86.67%), picloram (78.33% at 1.0 mgL^{-1}) and dicamba (78.33% at 2.0 mgL^{-1}). Observation showed a significant increase in callus induction using all auxins except NAA and IAA (Table 3).

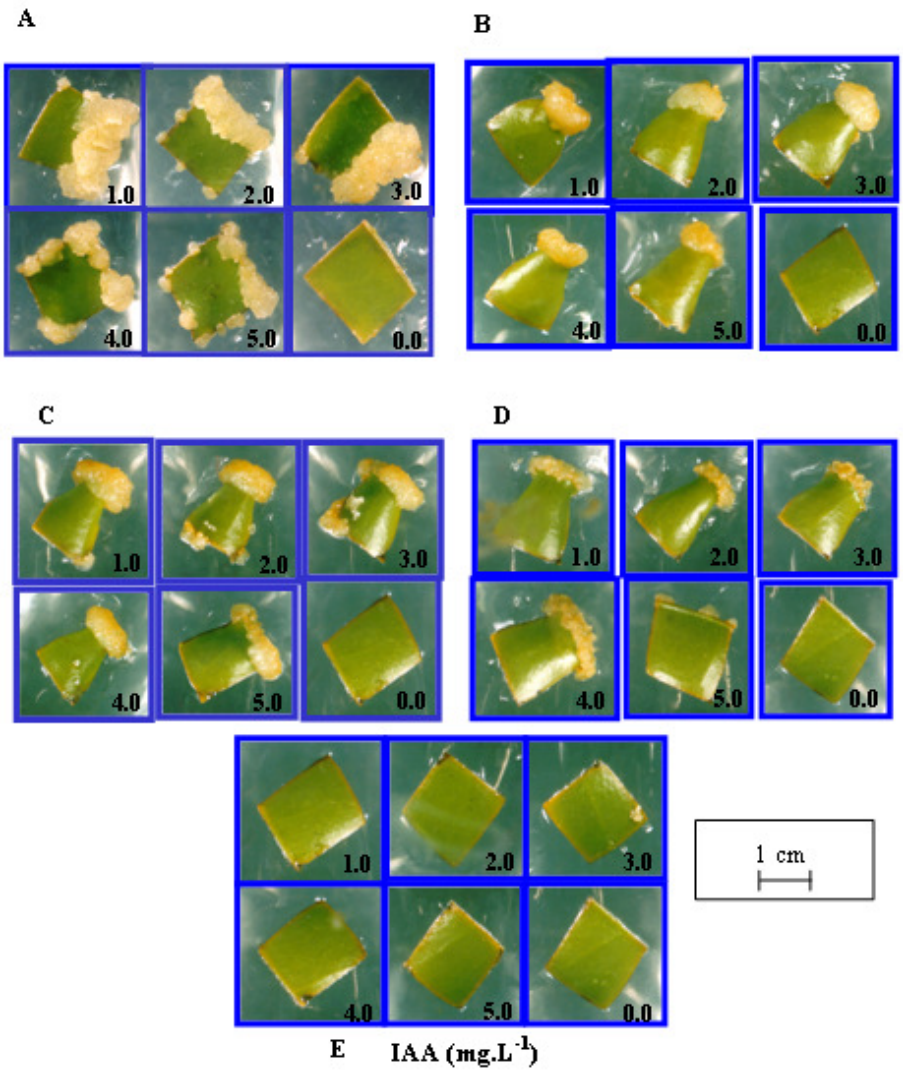

Figure 1. Induction of *Eurycoma longifolia* callus cultures derived from leaf explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) gelrite supplemented with different hormones concentration (0, 1, 2, 3, 4, and 5 mgL^{-1}). A (2,4-D), B (dicamba), C (picloram), D (NAA), and E (IAA). The scale bar (1 cm = 0.7 cm) representing the explants above.

The callus initiation time of the cotyledon was as long as that of the leaves and petioles hence, the cotyledon derived for callus was induced at 6, 7 and 8 days of culture in picloram, 2,4-D, dicamba, NAA and IAA, respectively. Results in Table 4 showed that the highest scoring of callus induction from cotyledon was produced in 2,4-D at 4.0 mgL^{-1} (85%) followed by picloram (83.33% at 1.0 mgL^{-1}), dicamba (78.33% at 2.0 mgL^{-1}), NAA (56.67% at 1.0 mgL^{-1}) and IAA (36.67% at 3.0 mgL^{-1}). The result showed a significant increase for all the auxins except dicamba (Table 4).

Callus from embryo explant was initiated after 6 and 7 days of incubation in medium containing 2,4-D, picloram and dicamba; followed by NAA and IAA. However, the medium supplemented with NAA and IAA showed little or no response to the callus initiation even only roots were grown from the explant. The highest scoring for callus induction from embryo was produced in 2,4-D and picloram at 2.0 mgL^{-1} and 5.0 mgL^{-1} (both 85%) followed by NAA and IAA 83.33% at 1.0 $mgL^{-1}$and 4.0 mgL^{-1} respectively, and dicamba, 81.67 % at 5.0 mgL^{-1} (Table 4). As a conclusion, the percentage of callus inducted increased significantly for all the auxins (Table 4).

Differences in physical appearance of callus from leaves were also observed. Callus morphology obtained from 2,4-D, picloram and dicamba treatments was friable and yellowish in colour. On the other hand, both the compact-yellowish callus and the compact-white yellowish callus were observed in medium containing NAA (Figure 1). Callus morphology obtained from petioles was

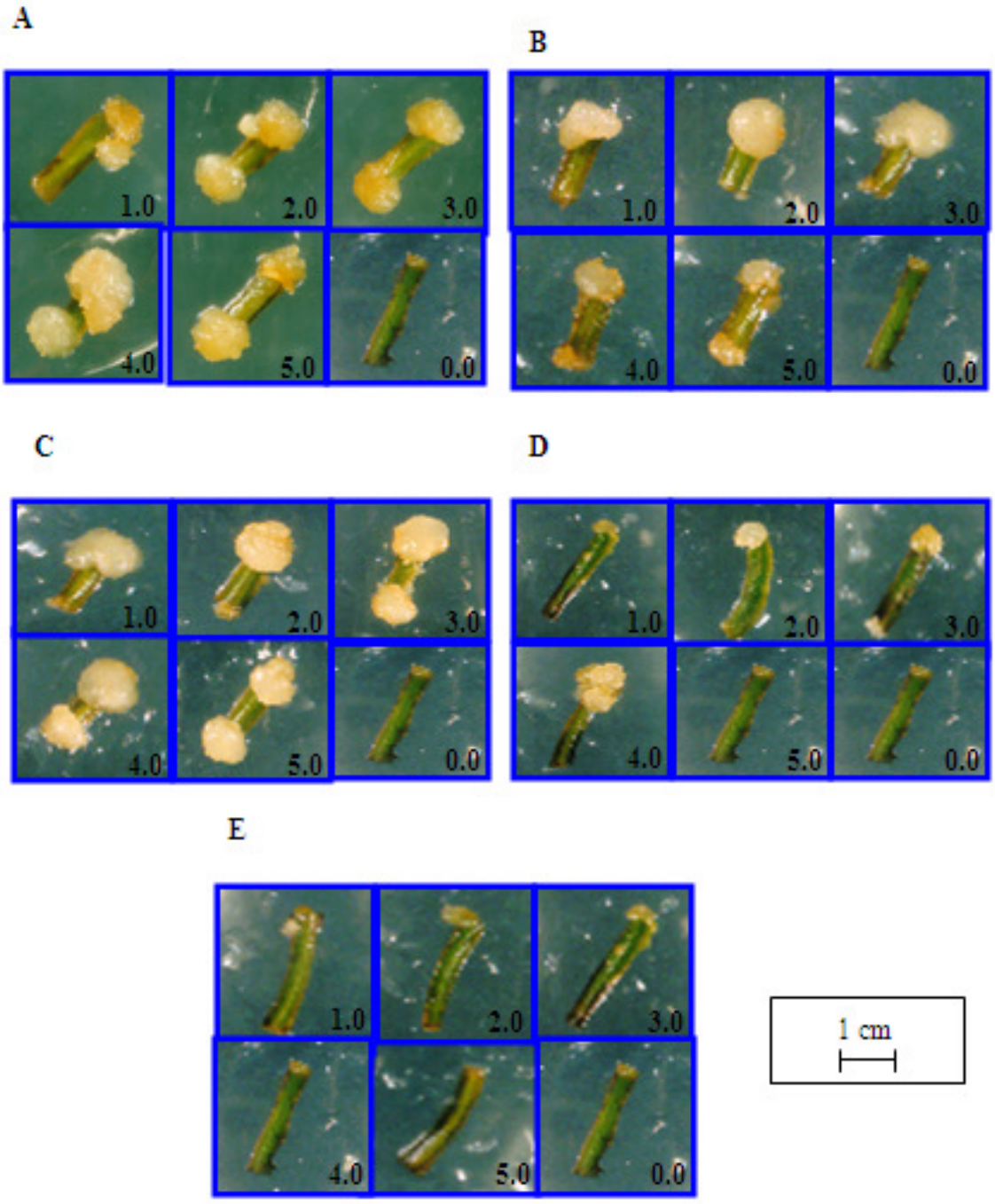

Figure 2. Induction of *Eurycoma longifolia* callus cultures derived from petiole explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) gelrite supplemented with different hormones concentration (0, 1, 2, 3, 4, and 5 mgL^{-1}). A (2,4-D), B (dicamba), C (picloram), D (NAA), and E (IAA). The scale (1 cm = 0.5 cm) representing the explants above.

generally friable and yellowish in colour as observed in leaf derived callus (Figure 2). Physical appearance of callus obtained from rachis was friable and yellowish (Figure 3). Observations showed that the stem explants cultured in 2,4-D, picloram and dicamba produced yellowish-white friable callus. In some stem explants, the callus formed in NAA and IAA containing media appeared as brownish and friable but still yellowish-white and friable callus that formed in 4.0 and 5.0 mgL^{-1} IAA (Figure 4). Callus morphology obtained from 2,4-D, picloram and dicamba treatments was friable and yellowish in tap root (Figure 5). Soft and white callus induced from fibrous root explants was obtained in all treatments. After subsequent sub-culturing, the soft and white callus turned into friable

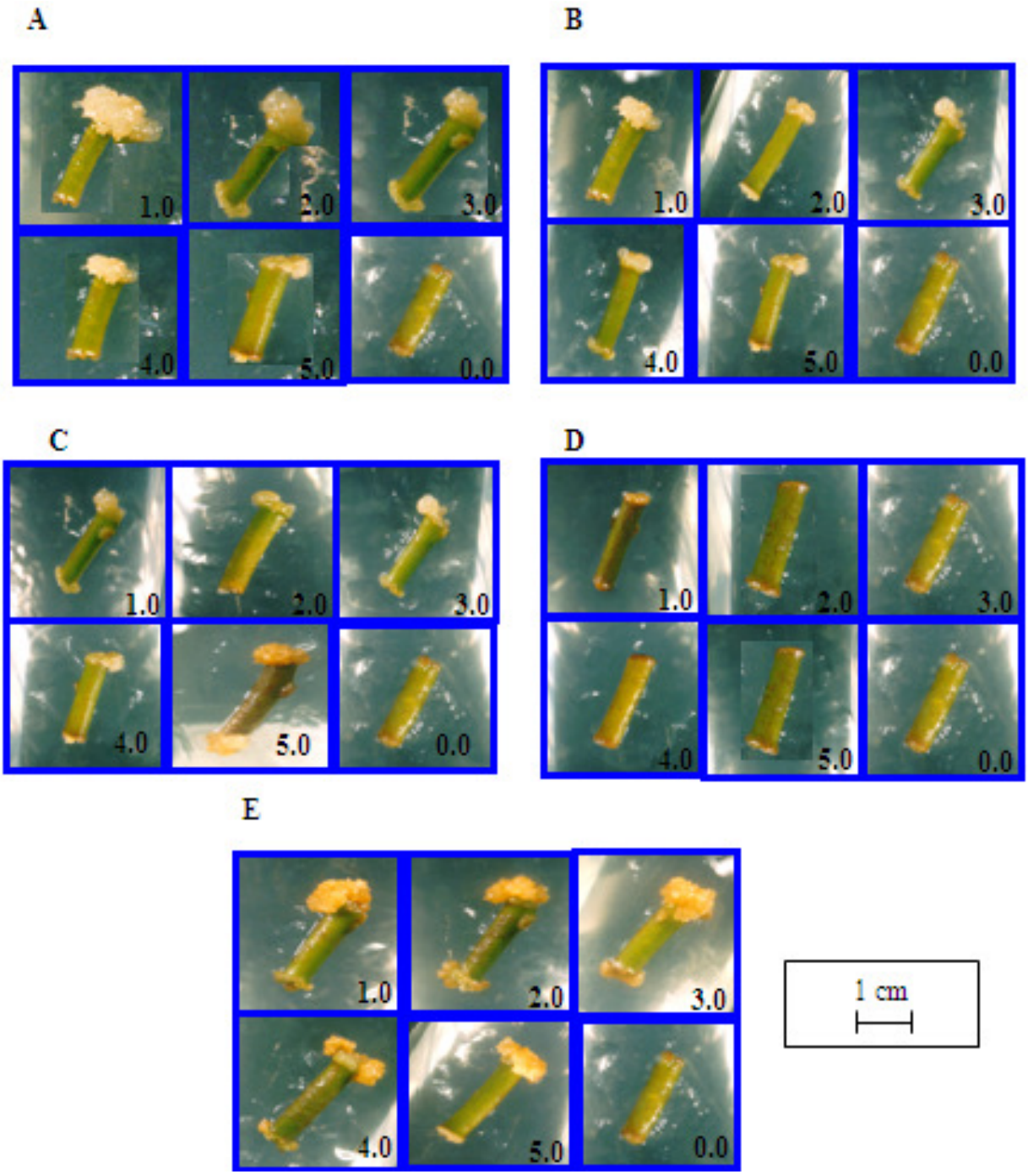

Figure 3. Induction of *Eurycoma longifolia* callus cultures derived from rachis explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) gelrite supplemented with different hormones concentration (0, 1, 2, 3, 4, and 5 mg. L^{-1}). A (2,4-D), B (dicamba), C (picloram), D (NAA), and E (IAA). The scale (1 cm = 0.5 cm) representing the explants above.

and yellowish tissue. However, little roots were grown from the cut end in medium containing IAA (Figure 6). For cotyledon, friable and yellowish callus was formed in medium containing 2,4-D, picloram and dicamba. However, friable and brownish callus was also formed in medium containing NAA and IAA. No callusing was observed in medium supplemented with 4.0 and 5.0 mgL^{-1} NAA and IAA. Roots were formed in medium containing NAA and IAA (Figure 7). For embryo, friable and yellowish callus was formed in medium containing 2,4-D, picloram and dicamba. However, friable and brownish callus was also formed in medium containing NAA and IAA (Figure 7).

The results from the above experiments indicated that

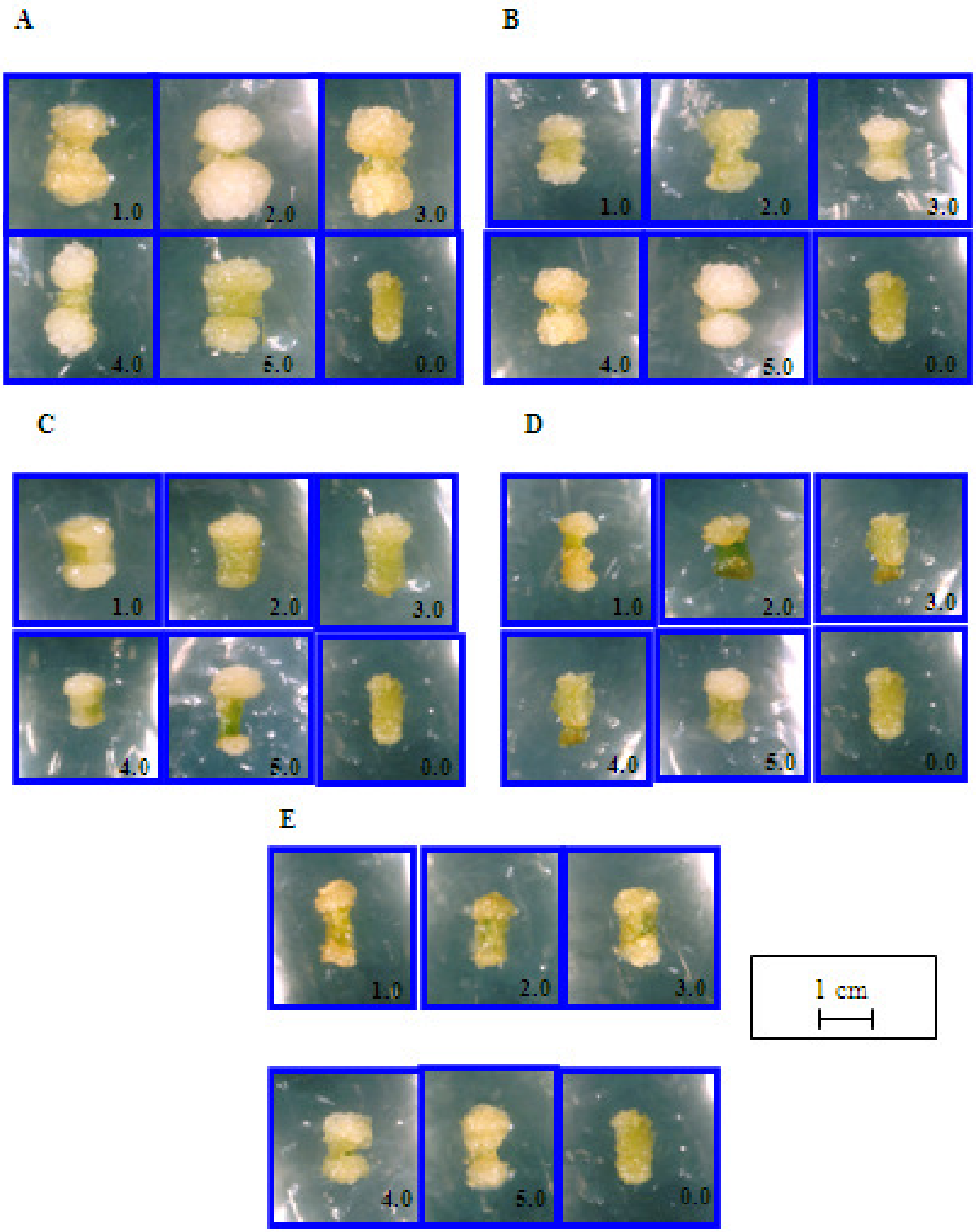

Figure 4. Induction of *Eurycoma longifolia* callus cultures derived from stem explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) gelrite supplemented with different hormones concentration (0, 1, 2, 3, 4, and 5 mgL^{1}). A (2,4-D), B (dicamba), C (picloram), D (NAA), and E (IAA). The scale (1 cm = 0.5 cm) representing the explants above.

2,4-D, picloram and dicamba were suitable for callus induction for every explant types. However, 2,4-D was the major auxin for callus induction in all explants of *E. longifolia* studied. Similar observations were reported on callus induction in *Genista* plants (Luczkiewicz and Glod 2003), Asiatic *Salsola* species (Stefaniak et al., 2003),

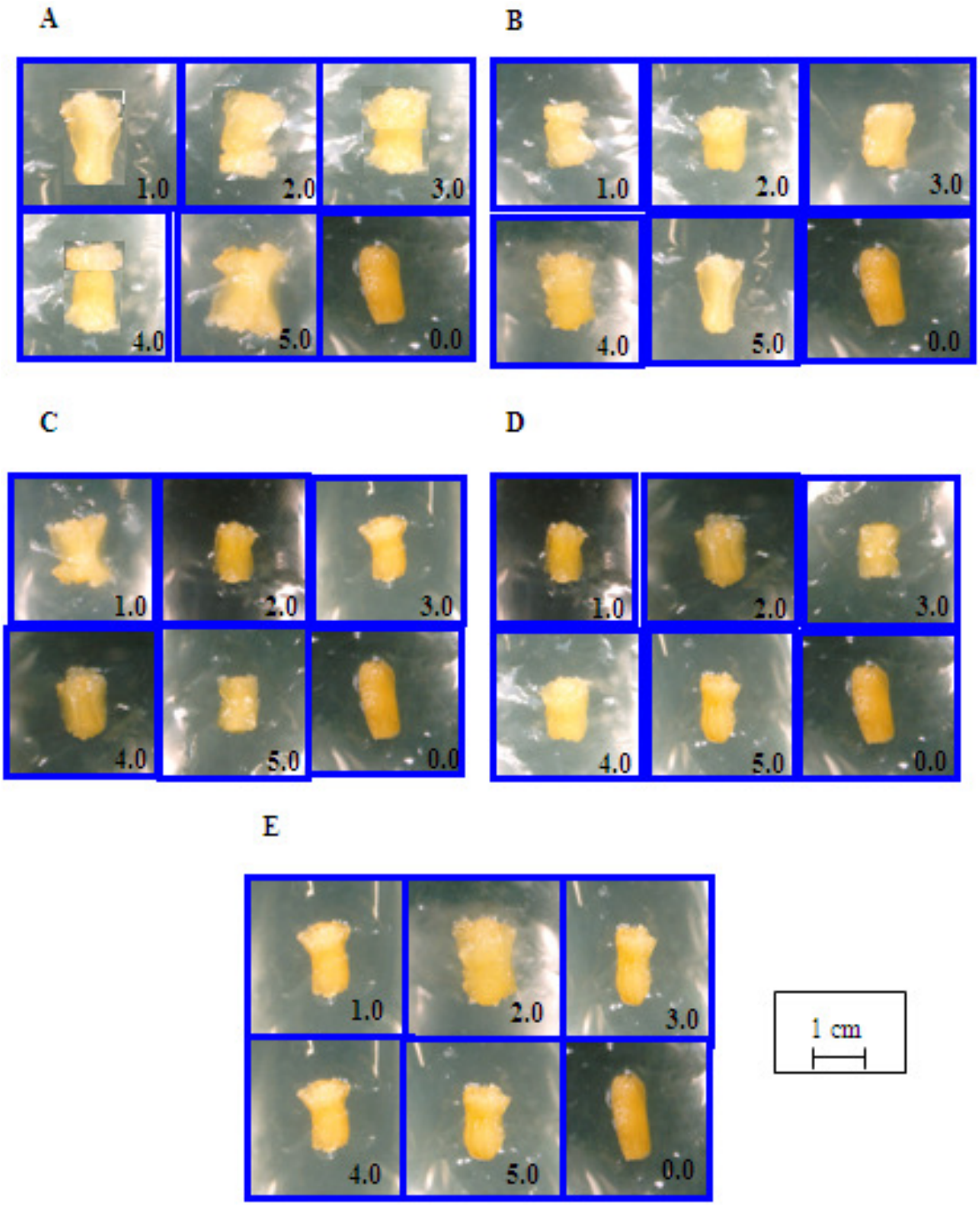

Figure 5. Induction of *Eurycoma longifolia* callus cultures derived from tap root explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) gelrite supplemented with different hormones concentration (0, 1, 2, 3, 4 and 5 mg. L^{-1}). A (2,4-D), B (dicamba), C (picloram), D (NAA), and E (IAA). The scale (1 cm = 0.3 cm) representing the explants above.

maize plant (Zacchini et al., 2000), *Withania somnifera* (Manickam et al., 2000), *Miscanthus* x *ogiformis* Honda '*Giganteus*' (Holme and Petersen, 1999), *Tabernaemontana pandacaqui* (Sierra et al., 1991) and *Oryza sativa* (Syaiful et al., 2009). According to Borejsza and Hrazdin (1994), the occurrence of callus was observed on the surface of leaf explants. MS medium alone failed to induce callus, even when the cultures

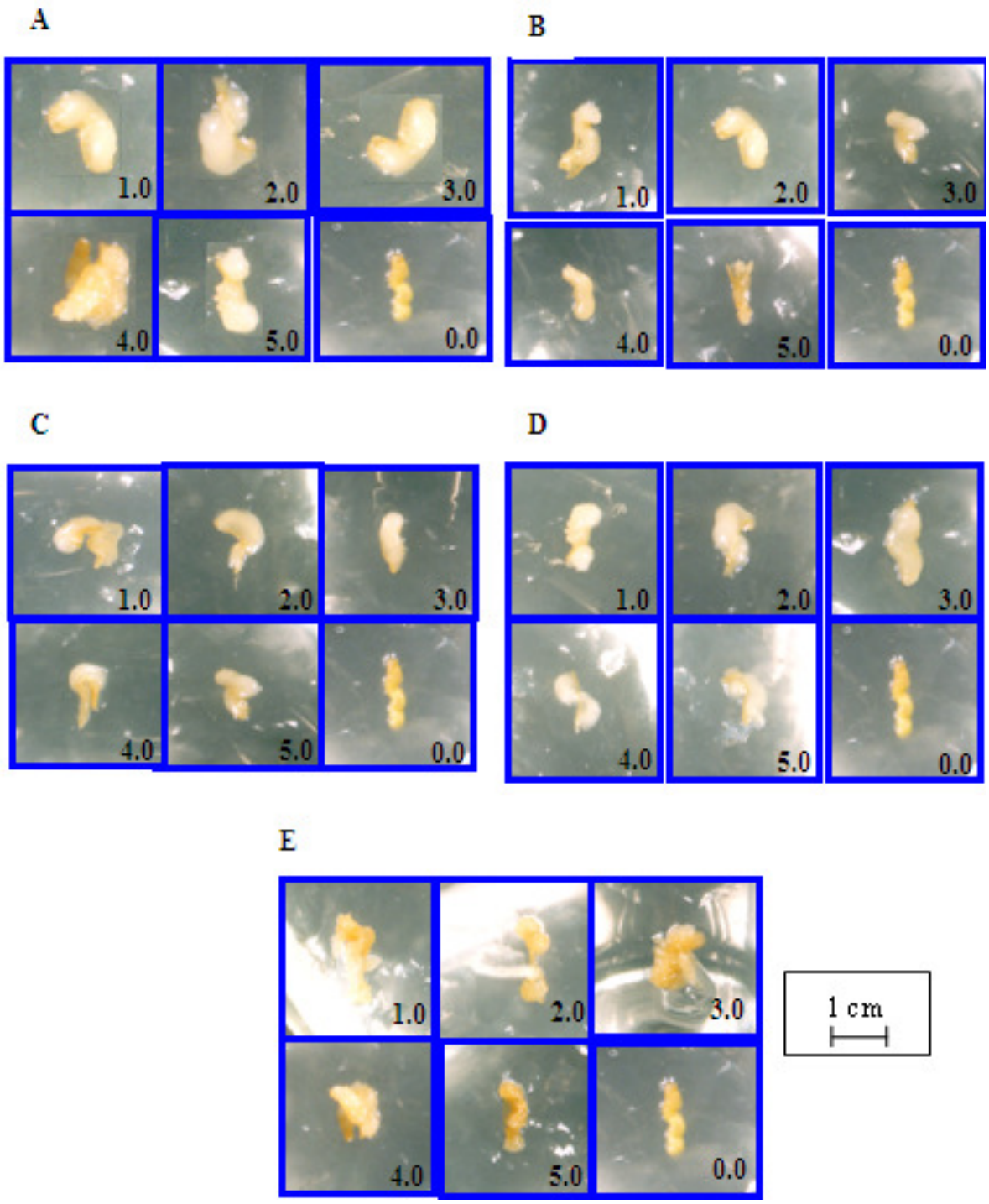

Figure 6. Induction of *Eurycoma longifolia* callus cultures derived from fibrous root explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) gelrite supplemented with different hormones concentration (0, 1, 2, 3, 4, and 5 mgL^{-1}). A (2,4-D), B (dicamba), C (picloram), D (NAA), and E (IAA). The scale (1 cm = 0.3 cm) representing the explants above.

were kept for prolonged period.

Therefore, the induction of callus cultures from leaf, petiole, rachis, stem, tap root, fibrous root, and cotyledon and embryo segments were achieved by using various auxins. These phytohormones were suitable for callus induction for every explant types. The results from the experiments indicated that 2, 4-D was the best major auxin for callus induction for all explants types of

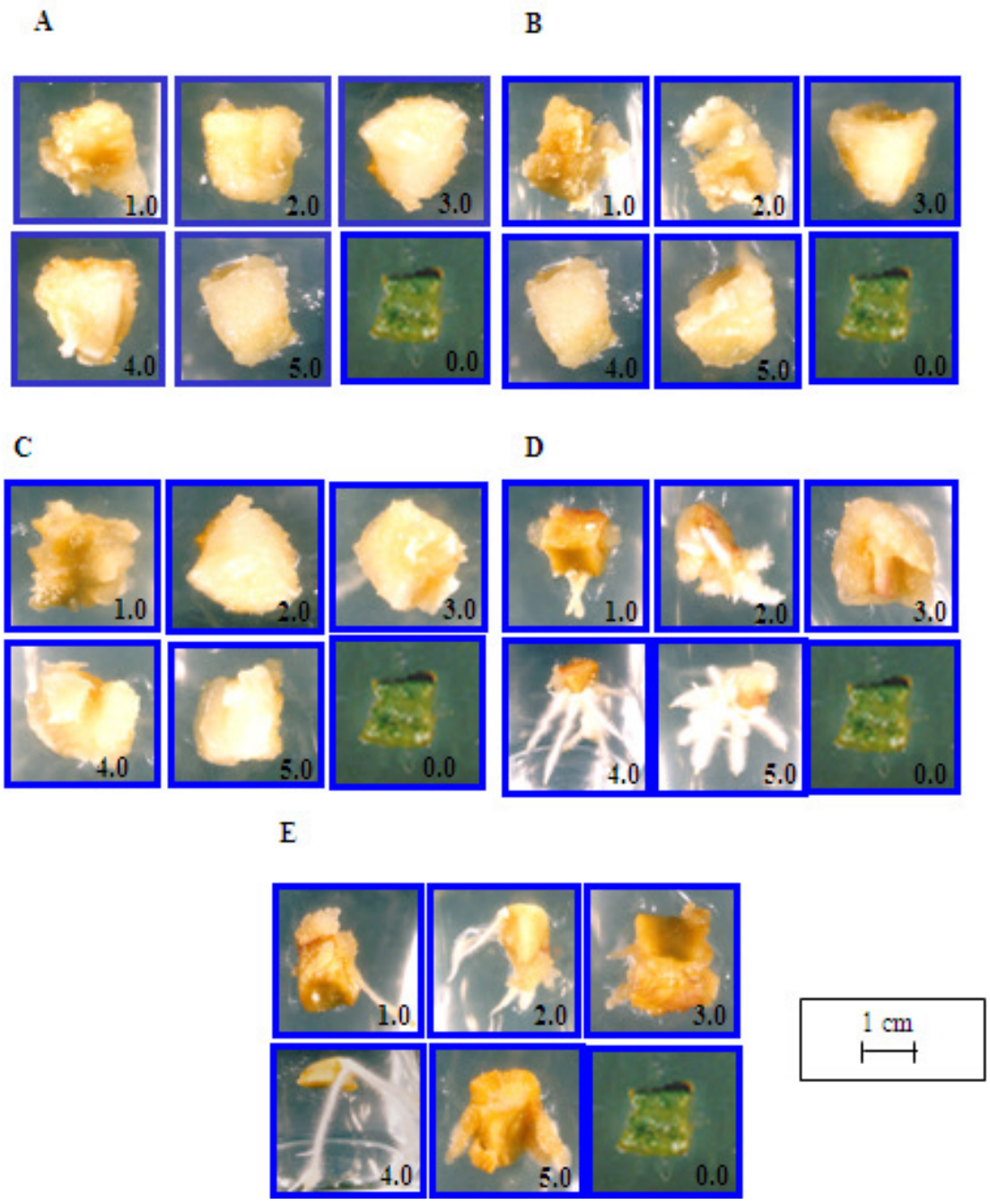

Figure 7. Induction of *Eurycoma longifolia* callus cultures derived from cotyledon explants in MS basal medium + B5 vitamins, 3% (w/v) sucrose, 0.25% (w/v) gelrite supplemented with different hormones concentration (0, 1, 2, 3, 4, and 5 mg. L^{-1}). A (2,4-D), B (dicamba), C (picloram), D (NAA), and E (IAA). The scale (1 cm = 0.4 cm) representing the explants above.

Eurycoma longifolia.

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