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Article

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THE ROLE OF PLANT GROWTH REGULATORS IN THE DEVELOPMENT OF *in vitro* FLOWERING, HISTOLOGY AND ULTRASTRUCTURAL STUDIES IN *Impatiens balsamina* CV. DWARF BUSH

*O Papel dos Reguladores de Crescimento de Plantas no Desenvolvimento dos Estudos **in vitro** de Floração, Histologia e Ultrastruturais de **Impatiens balsamina** cv. Dwarf Bush*

ABSTRACT - An efficient protocol for *in vitro* flowering was successfully established for *Impatiens balsamina* cv Dwarf Bush, an important medicinal plant, through tissue culture techniques. Shoot, stem and petiole explants obtained from 4 week-old aseptic seedlings cultured on MS medium supplemented with different concentrations of plant growth regulator (PGR) were used for *in vitro* flower induction. Gibberellic acid (GA_3), benzylaminopurine (BAP) and kinetin (Kin) treatment singly applied in MS media (pH 5.8), could all stimulate flowering at 23-26 °C with photoperiod of 16 hours light and 8 hours dark. It was observed that shoot explants were more responsive than stem explants in floral formation. Regeneration was achieved via direct organogenesis. For shoot explants, the treatment that induced the highest rate of *in vitro* flowering (7.30 ± 0.16 flowers per plantlet) was 1.0 mg L^{-1} GA_3. Ultrastructural and histological analysis of *in vivo* and *in vitro* flowers were done to discover any somaclonal variation. This research described a simple protocol for rapid *in vitro* flowering that will be very
beneficial for further breeding, cytological and molecular biology research.

Keywords: *in vitro* regeneration, ultrastructural, histology, gibberellic acid, flowering response.

*RESUMO - Um protocolo eficiente para a floração **in vitro** foi estabelecido com sucesso para **Impatiens balsamina** cv. Dwarf Bush, uma planta medicinal importante, através de técnicas de cultura de tecidos. Foram utilizados explantes com broto, talo e pecíolo, extraídos de mudas assépticas com quatro semanas de vida, cultivadas em meio MS, suplementadas com diferentes concentrações de reguladores de crescimento de plantas (RCP), para indução de floração **in vitro**. O tratamento com ácido giberélico (GA3), benzilaminopurina (BAP) e cinetina (Kin), aplicado isoladamente em meio MS (pH 5,8), conseguiu estimular a floração a 23-26 °C, com fotoperíodo de 16 horas com luz e 8 horas sem. Observou-se que os explantes de broto eram mais responsivos que os explantes de talo na formação floral. A regeneração foi obtida via organogênese direta. Para explantes de broto, o tratamento que induziu a maior taxa de floração **in vitro** (7,30 ± 0,16 flores por plântula) foi o de 1,0 mg L^{-1} GA3. Análises ultraestruturais e histológicas de flores **in vivo** e **in vitro** foram realizadas para detectar qualquer variação somaclonal. Esta pesquisa descreveu um protocolo simples para a rápida floração **in vitro**, que será muito benéfico para futuras pesquisas em genética, citologia e biologia molecular.*

Palavras-chave: regeneração *in vitro*, ultraestrutural, histologia, ácido giberélico, resposta da floração.

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INTRODUCTION

Impatiens balsamina is an important medicinal plant that belongs to the family Balsaminaceae. The flower of *I. balsamina* is very attractive for research, because its flowering process requires short day conditions, and flower reversion can be obtained in an expected way after conversion to long day conditions (Pouteau et al., 1998). The flowers have been exploited to cool burning skin or cool fever (Taha et al., 2009). *In vitro* flowering acts as an essential tool in examining flower induction, initiation, and the floral developmental process through plant growth regulators, such as cytokinins, gibberellins, and auxins (Ziv and Noar, 2006). Furthermore, *in vitro* flowering provides an ideal experimental system, preferable to *in vivo* grown plants, for studying the biological mechanism of flowering (Zhang, 2007).

In vitro flowering can also reduce external factors that affect the flowering process by allowing researchers to control environmental factors and use exogenous plant growth regulators (Zhang et al., 2008). *In vitro* flower formation can provide a model system for studying flower induction and development, allowing a means for conducting microbreeding, and a source of biochemicals and pharmaceuticals (Tisserat et al., 1990). In many plants, *in vitro* flowering was normally achieved by the application of exogenous hormones to the culture medium (Taha, 1997; Jana and Sekhawat, 2011). In tissue culture, *in vitro* flowering serves as an important tool for many reasons. One of the most important is that it shortens the life cycle of plants for breeding programs. Another is that it aims to include studying flower induction and initiation, and floral development.

Explants that have been used for regeneration in *Impatiens* include cotyledons of immature ovules of *I. platypetala* (Kyungkul, 1993) and shoot tips from *Impatiens* hybrids (Kyungkul and Stephens, 1987). *Impatiens* most commonly propagate by seed, but this method has many limitations, such as low rate of germination, especially in Malaysia. Breeding cycle can be shortened to generate better quality of plant varieties for crop improvement to meet market demand. In addition, the product has potential for commercial scientific handicraft, futuristic gifs, and for miniature garden. This present study highlights the effort to develop an effective system for inducing *in vitro* flowering for one of Malaysia's native balsams, *I. balsamina*. *In vitro* flowering for *I. balsamina* was developed using shoot, petiole, and stem explants derived from aseptic seedlings. Ultrastructural and histological studies were also conducted to compare variations, if any, which might occurr as a result of *in vitro* flowering.

MATERIALS AND METHODS

In vitro flowering of *I. balsamina* cv. Dwarf Bush

The seeds of *I. balsamina* were collected from the garden at the Institute of Biological Sciences, University of Malaya, and washed carefully under running tap water for 30 min. The seeds were then washed with Dettol detergent and two drops of Tween 20 for 5 min., followed by rinsing three times with distilled water. Seeds were surface sterilized with different concentrations (70%, 50% and 30%) of sodium hypochlorite solution for 5 min. each and washed thoroughly with distilled water. Finally, the seeds were treated with 70% ethanol for 1 min. and rinsed with sterile distilled water, once again in a laminar airflow cabinet. Seeds were cultured aseptically on basal medium (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose and solidified with 0.25% (w/v) gelrite. The germination of seeds in tissue culture tend to grow very fast compared of being sowed in traditional method.

The pH of the medium was adjusted to 5.8 using NaOH and HCl. The media were autoclaved at 121 °C for 20 min after adjusting the pH. Seeds were germinated aseptically. Young stem, shoot, and petiole obtained from aseptic seedlings were used as source of explants. Stem, shoot, and petiole explants were excised from 4 week-old aseptic seedlings into 5 x 5 mm, then cultured onto the experimental media supplemented with different plant growth regulators for inducing *in vitro* flowering. The hormones used were gibberellic acid (GA_3), kinetin, and benzylaminopurine (BAP) at various concentrations and the cultures were maintained at temperature of 23-26 °C with 16 h of light and 60 µmol m^2 s^{-1} of photosynthetic photon flux density, and 8 h of dark. Thirty replicates were used for each treatment. The number of flowers per explant, number of adventitious shoots, and plant height were measured weekly for 8 weeks.

Histological and ultrastructural analysis

In vitro and *in vivo* flowers were used and both samples were sliced and fixed in 3% (w/v) paraformaldehyde and 2% (v/v) glutaraldehyde in 0.2 M phosphate buffer at pH 7.2 for 24 h at room temperature. Samples were dehydrated in a graded ethanol series (30-100%). Later, the samples were infiltrated and embedded in basic resin (Technovit 7100) for one-week retention time and cut into cross section. Embedded samples were sectioned into 3.5 µm thick segments with a microtome (RM 2135 Leica, Germany). Sections were double stained with periodic acid shchiff (PAS) and naphthol blue black. The samples were then viewed under an Axioskop Zeiss (Germany) microscope attached to an AxioCamMRc video camera and were then analyzed using the AxioVision 4.7 software. Simultaneously, ultrastructural examination of *in vitro* and *in vivo* flowers were carried out and compared. Ultrastructural features were viewed by Field-Emission Scanning Electron Microscopy (FESEM), Quanta™ 450FEG. FESEM was used to check for any abnormalities and differences between *in vivo* and *in vitro* flower buds of *I. balsamina.*

Statistical analysis

Data analysis was conducted through analysis of variance (ANOVA) for completely randomized design. Duncan's multiple range test (DMRT) and 5% significant level was used to compare means.

RESULTS AND DISCUSSION

***In vitro* flowering of *I. balsamina* cv. Dwarf Bush**

Figure 1A shows *I. balsamina* as a control plant. Stem, shoot, and petiole explants from 4-week-old aseptic seedlings were used to induce *in vitro* flowering (Figure 1B). *In vitro* ûowering was successfully induced from all explants after 8 weeks of culture (Figure 1C). The *in vitro* flower buds began to bloom on week 4 of culture. The control consisted of all similar types of explants cultured on MS media without PGRs. The application of exogenous PGRs has significantly reduced the time of flowering. It seems that a period of 8 weeks in culture was appropriate for flowering in this present study. Table 1 shows the highest rate of *in vitro* flowering, which was induced from shoot explants (7.30 ± 0.16 flowers per plantlet) cultured on MS medium supplemented with 1.0 mg L^{-1} GA_3. Meanwhile, the highest number of shoots formed (12.83 ± 0.10) and the tallest plantlets (10.80 ± 0.10 cm) were also observed in this treatment.

Table 2 shows the effects of different concentrations of PGRs on stem explants. However, the rate of *in vitro* flowering was less for stem explants than for shoot explants. The highest rate of *in vitro* flowering was 4.30 ± 0.15 flowers per plantlet. Meanwhile, the highest number of shoots per explant (8.60 ± 0.15) and the highest height of plantlets (8.70 ± 0.17 cm) were observed in the 0.5 mg L^{-1} kinetin treatment group. Table 3 shows the effects of different concentrations of PGRs on petiole explants. The petiole explants started to induce shoot after 2 weeks of culture. As with shoot explants, the 1.0 mg L^{-1} GA_3 treatment also resulted in the highest number of *in vitro* flowers (3.20 ± 0.17), while 0.5 mg L^{-1} kinetin induced the highest number of shoots per explant (6.57 ± 0.30) and the highest height of plantlets (8.47 ± 0.16 cm), as noted with stem explants. Therefore, GA_3 was found to be the most effective PGR for inducing flowering *in vitro*. The number of shoots and plant height were related to the rate of *in vitro* flowering. This study has also found that shoot explants are more responsive compared to stem and petiole explants.

Histological and ultrastructural analysis

Longitudinal sections were prepared from *in vivo* and *in vitro* flowers. *In vitro* flowers of *I. balsamina* were collected from 2-month-old plantlets. Meanwhile, *in vivo* flowers were collected from 3 month-old intact plants. Histological analysis of *in vivo* carpels showed 5 different tissues, namely, abaxial surface, ovule, funiculus, placenta, and stamen (Figure 2A). The ovules attached to a thickened region of the ovary wall termed as the placenta. Each ovule is connected at its base to the placenta via the funiculus. Histological analysis showed that sexual reproduction

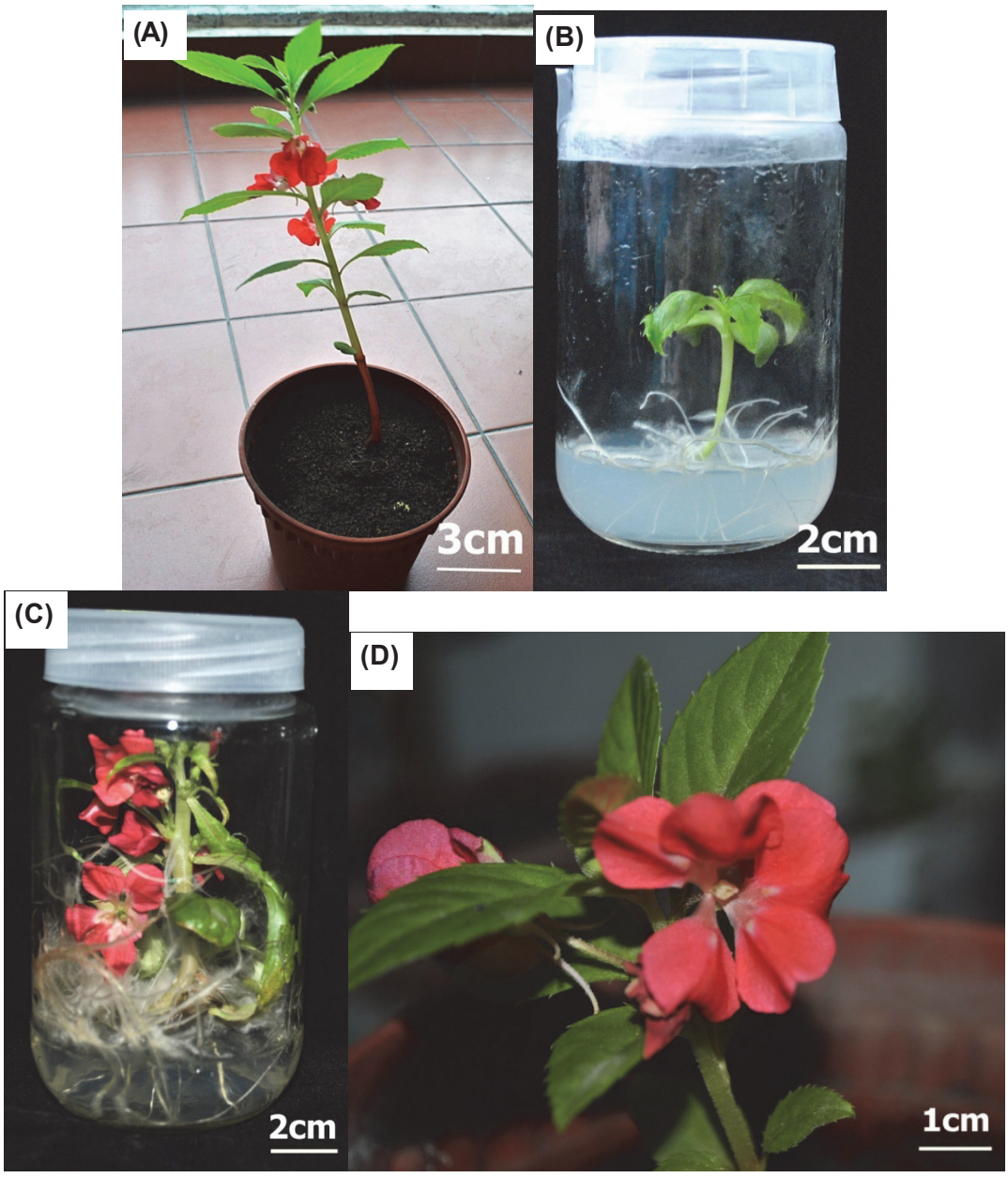

Figure 1 - (A) Four-month-old *in vivo* flowering of *Impatiens balsamina* (B) Aseptic seedlings (C) *In vitro* flowering of *Impatiens balsamina* cultured on MS supplemented with 1.0 mg L^{-1} GA_3 after 8 weeks. (D) *In vitro* flowering after 3 months.

was already fully developed in *in vivo* flowers compared to *in vitro* flowers. Meanwhile, histological analysis of *in vitro* flowers showed that there are 4 different tissues, namely, capitulum, sepal, petal, and ovary (Figure 2B). The abaxial surface was thick compared to *in vivo* flowers. Cell structures were not fully developed, perhaps due to the age of *in vitro* explants, which was just 8 weeks. Nonetheless, morphologically, the flowers were the same. In the early development of most flowers the primordia arises in centripetal sequence, so that the perianth is initiated first and the gynoecium last. Field Emission Scanning Electron Microscopic (FESEM) examinations were performed on *in vitro* and *in vivo* flower buds of 2 week-old *I. balsamina*. FESEM of *in vitro* *I. balsamina*. flower buds of, treated with 1.0 mg L^{-1} $GA_{3,}$ showed structures more clearly compared to *in vivo* flower buds (Figure 3A, B). This substantiates the premise that tissue culture promoted faster growth of the plantlets, allowing them to reach maturity at least about 2-3 weeks earlier. Outer surface of *in vivo* flower bud also showed more trichomes compared to outer surface of *in vitro* flower bud (Figure 3C, D). This may be due to the controlled environment with less trichomes found in *in vitro* plantlet.

Table 1 - Effects of different concentrations of plant growth regulators on shoot explants: mean number of shoots per explant, mean height of plantlet, mean (%) explants that produced shoots/callus and mean number of flowers per explant

MS + PGR (mg L^{-1})	Mean number of shoots per explant	Mean height of plantlet (cm)	Mean (%) explants produced shoots	Mean (%) explants produced flowers	Mean number of flowers per explant
Control	1.27±0.10 g	4.87±0.19 e	100.00±0.00 a	100.00±0.00 a	1.60±0.23 e
0.5 GA_3	8.00±0.17 c	7.53±0.34 b	100.00±0.00 a	100.00±0.00 a	3.73±0.13 c
1.0 GA_3	12.83±0.10 a	10.80±0.10 a	100.00±0.00 a	100.00±0.00 a	7.30±0.16 a
1.5 GA_3	8.30±0.27 c	8.00±0.30 b	100.00±0.00 a	100.00±0.00 a	5.30±0.25 b
2.0 GA_3	6.80±0.23 d	6.73±0.26 c	100.00±0.00 a	100.00±0.00 a	4.33±0.15 c
0.5 BAP	5.73±0.21 e	7.20±0.19 bc	100.00±0.00 a	100.00±0.00 a	4.10±0.17 c
1.0 BAP	5.86±0.35 e	6.60±0.21 c	100.00±0.00 a	100.00±0.00 a	3.80±0.11 c
1.5 BAP	4.60±0.16 f	7.83±0.16 b	100.00±0.00 a	100.00±0.00 a	3.50±0.16 c
2.0 BAP	6.40±0.21 d	4.50±0.22 c	100.00±0.00 a	100.00±0.00 a	2.50±0.08 d
0.5 KIN	5.30±0.29 e	5.80±0.34 d	100.00±0.00 a	100.00±0.00 a	3.00±0.03 cd
1.0 KIN	10.20±0.26 b	8.50±0.32 b	100.00±0.00 a	100.00±0.00 a	5.50±0.03 b
1.5 KIN	6.60±0.30 d	7.20±0.28 bc	100.00±0.00 a	100.00±0.00 a	4.15±0.11 c
2.0 KIN	5.80±0.10 e	7.00±0.19 bc	100.00±0.00 a	100.00±0.00 a	3.40±0.13 c

Data represents mean value ± standard error (SE) with 30 explants in each treatment. Means with different letters in the same column are significantly different at $p<0.05$, by ANOVA and Duncan's multiple range test (DMRT)

Table 2 - Effects of different concentrations of plant growth regulators on stem explants: mean number of shoots per explant, mean height of plantlet, mean (%) explants that produced shoot/callus and mean number of flowers per explant

MS + PGR (mg L^{-1})	Mean number of shoots per explant	Mean height of plantlet (cm)	Mean (%) explants produced shoots	Mean (%) explants produced flowers	Mean number of flowers per explant
Control	1.00±0.10 e	4.50±0.19 c	90.00±0.05 b	85.00±0.00 b	0.90±0.18 d
0.5 GA_3	6.80±0.18 b	7.20±0.25 b	100.00±0.00 a	95.00±0.07 a	3.50±0.17 ab
1.0 GA_3	7.20±0.15 ab	7.50±0.17 ab	100.00±0.00 a	95.00±0.05 a	4.30±0.15 a
1.5 GA_3	7.80±0.25 a	8.00±0.20 a	100.00±0.00 a	90.00±0.10 a	4.00±0.15 a
2.0 GA_3	6.50±0.13 b	8.00±0.25 a	100.00±0.00 a	90.00±0.08 a	3.53±0.16 ab
0.5 BAP	6.90±0.10 b	8.47±0.16 a	100.00±0.00 a	85.00±0.06 b	2.00±0.23 c
1.0 BAP	4.97±0.17 c	8.13±0.18 a	100.00±0.00 a	85.00±0.08 b	2.50±0.21 c
1.5 BAP	4.03±0.11 c	6.53±0.14 b	100.00±0.00 a	90.00±0.10 a	3.20±0.18 b
2.0BAP	3.30±0.16 d	6.00±0.12 b	100.00±0.00 a	95.00±0.09 a	3.70±0.09 ab
0.5 KIN	8.60±0.15 a	8.70±0.17 a	100.00±0.00 a	90.00±0.08 a	3.80±0.05 ab
1.0 KIN	8.10±0.36 a	7.53±0.15 ab	100.00±0.00 a	92.00±0.10 a	4.00±0.07 a
1.5 KIN	4.80±0.34 c	7.50±0.16 ab	100.00±0.00 a	88.00±0.08 b	4.00±0.18 a
2.0 KIN	3.83±0.19 d	6.10±0.11 b	100.00±0.00 a	85.00±0.06 a	3.00±0.19 b

Data represents mean value ± standard error (SE) with 30 explants in each treatment. Means with different letters in the same column are significantly different at $p < 0.05$, by ANOVA and Duncan's multiple range test (DMRT)

In vitro flowering has great importance for selective hybridization, especially in the case of using pollen from rare stocks, and may be the first step towards the possibility of recombining genetic material via *in vitro* fertilization (Murthy et al., 2012). *In vitro* flowering is presumably the most obscure of all the *in vitro* plant developmental processes. GA_3 comprise hundreds of compounds, some of which regulate different aspects of plant growth and development, including seed germination, stem elongation, leaf expansion, and flower and seed development. They may act alone or in association with other hormones (Weiss and Ori, 2007). Early *in vitro* flowering of micropropagated plantlets can shorten their breeding cycle to generate better quality of plant varieties to meet market demand (Haque and Ghosh, 2013). In this study, we have induced *in vitro* flowering and promoted adventitious shoot formation of *I. balsamina* GA_3 was found to promote earlier *in vitro* flowering in *I. balsamina* GA_3 has previously been reported to promote

Table 3 - Effects of different concentrations of plant growth regulators on petiole explants: mean number of shoots per explant, mean height of plantlet, mean (%) explants that produced shoot/callus and mean number of flowers per explant

MS + PGR (mg L^{-1})	Mean number of shoots per explant	Mean height of plantlet (cm)	Mean (%) explants produced shoots	Mean (%) explants produced flowers	Mean numberof flowers per explant
Control	0.98±0.12 d	4.00±0.20 d	85.00±0.08 b	70.00±0.10 b	0.80±0.08 c
0.5 GA_3	4.80±0.15 b	6.50±0.15 b	90.00±0.09 a	85.00±0.06 a	2.50±0.18 a
1.0 GA_3	5.40±0.16 b	6.80±0.18 b	95.00±0.14 a	85.00±0.15 a	3.20±0.17 a
1.5 GA_3	5.00±0.15 b	6.00±0.20 b	95.00±0.11 a	82.00±0.11 a	3.00±0.15 a
2.0 GA_3	5.00±0.17 b	5.50±0.15 c	90.00±0.18 a	80.00±0.05 a	2.03±0.19 b
0.5 BAP	3.93±0.08 bc	5.50±0.24 c	88.00±0.09 ab	80.00±0.05 a	2.00±0.20 b
1.0 BAP	3.50±0.16 c	6.40±0.16 b	85.00±0.11 b	82.00±0.04 a	2.20±0.19 ab
1.5 BAP	3.00±0.11 c	5.90±0.09 c	90.00±0.02 a	80.00±0.11 a	2.50±0.16 a
2.0BAP	4.27±0.08 b	5.60±0.11 c	89.00±0.06 ab	83.00±0.15 a	1.50±0.10 bc
0.5 KIN	6.57±0.30 a	8.47±0.16 a	90.00±0.14 a	85.00±0.04 a	2.80±0.20 a
1.0 KIN	4.97±0.16 b	6.97±0.13 b	95.00±0.09 a	85.00±0.16 a	3.00±0.17 a
1.5 KIN	4.13±0.06 b	6.40±0.14 b	90.00±0.10 a	80.00±0.20 a	2.00±0.16 b
2.0 KIN	3.23±0.18 c	5.50±0.16 c	90.00±0.17 a	80.00±0.04 a	2.00±0.23 b

Data represents mean value ± standard error (SE) with 30 explants in each treatment. Means with different letters in the same column are significantly different at $p<0.05$, by ANOVA and Duncan's multiple range test (DMRT).

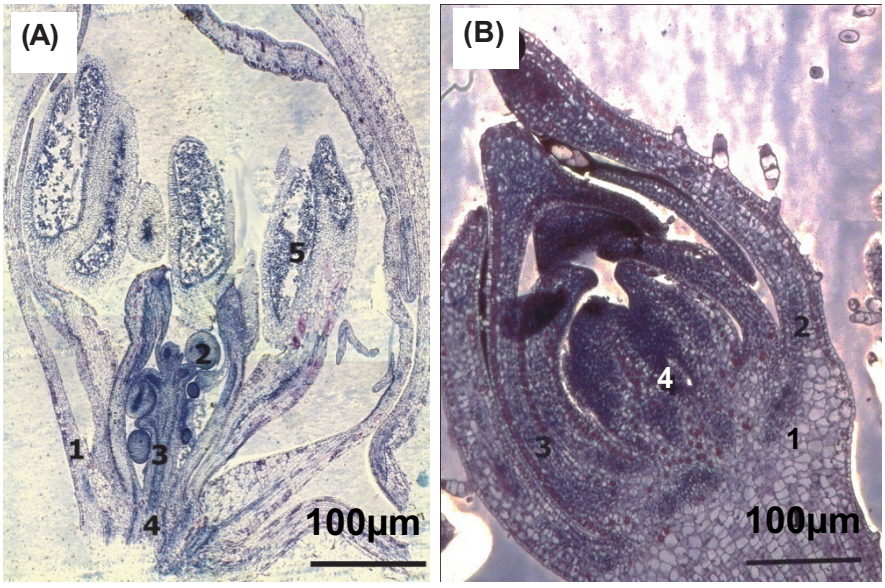

Figure 2 - Longitudinal sections of flower of *Impatiens balsamina* (A) *In vivo* flower showing the presence of (1) abaxial surface (2) ovule (3) funiculus (4) placenta (5) stamen (B) *in vitro* flower showing the presence of (1) capitulum (2) sepal (3) petal (4) ovary.

flowering in long day ornamental plants, such as *Zantedeschia* (Kozlowska et al., 2007) and a facultative long day plant, *Brunonia* (Wahyuni et al., 2011).

The most effective plant growth regulator for inducing *in vitro* flowering in our study was GA_3, followed by BAP, and kinetin. This result agrees with reports on the application of GA_3 to *Henckelia humboldtianus,* which also encouraged earlier flowering (Sumanasiri et al., 2013). The effect on some species is greater than that on others. The effectiveness of GA_3 in inducing *in vitro* flowering was also observed in many other plant species such as *Phalaenopsis hybrida* (Su et al., 2001). GA_3 are a group of key hormones regulating many aspects of plant growth and development (Yamaguchi, 2008).

The most effective concentration of GA_3 was 1.0 mg L^{-1}, which strongly affected the maximum production of *in vitro* flowering in *I. balsamina*. Meanwhile, BAP was also found to promote *in vitro* flowering, as observed in *Perilla frutescens* (Zhang, 2007) and *Boerhaavia diffusa* (Sudarshana et al., 2008). This is similar to our results, in which BAP also induced *in vitro* flowering. Moreover, kinetin applied singly also induced *in vitro* flowering of *I. balsamina* as well. Saha and Ghosh (2014) reported that kinetin could induce *in vitro* flowering in *Luffa acutangula*. Miles and Hagen Jr (1968) reported that there are flavones in flower petals of *I. balsamina*.

Silva et al. (2014) reported that level of plant growth regulators (PGRs), genotype, and culture conditions are strongly associated with the initiation and development of floral organs from buds or callus tissue of *Dendrobium*. This may be due to the level of flavones in the flower petals that are affected by plant growth regulators. There are enormous physical and chemical aspects that affect the *in vitro* flowering mechanism. The *in vitro* flowering processes are under the influence of signals, including variations in endogenous levels (Campos and Kerbauy, 2004). However, only few studies have addressed the effect of PGRs in *in vitro* flowering. Therefore, the observations reported hereby could render opportunities for further studies on the molecular physiology of flowering, particularly to understand how exogenous hormones regulate flower color in *I. balsamina* under controlled *in vitro* conditions.

The *in vivo* and *in vitro* morphology of the *I. balsamina* flowers were similar although histology analysis revealed that cell structures in *in vitro* flowers were not fully developed, possibly due to the environmental cultural conditions. *In vitro* flowering exhibited miscellaneous pollination biology. Numerous species of the genus *Impatiens* have diverse pollination biology (Schoen et al., 1994). The carpels in *Impatiens* enclose the ovules and provide the style and stigma, as has been reported for some other species (Sattler and Lacroix, 1988). The carpel column connections in *Impatiens* provide evidence to support the theory of how the primordial carpel fused to form the

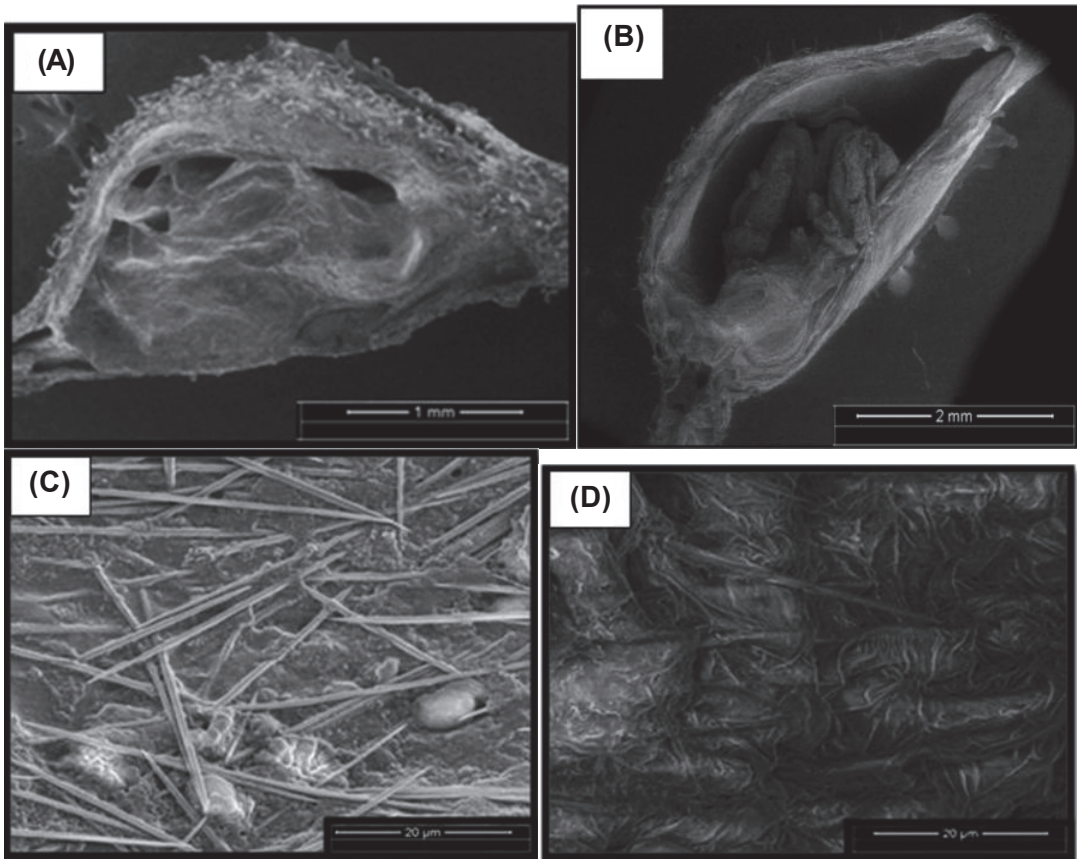

Figure 3 - Field Emission Scanning Electron Micrographs (FESEM) of 2-week-old *in vivo* and *in vitro Impatiens balsamina* flower bud (A) *In vivo* flower bud (B) *In vitro* flower bud from plantlet cultured in MS medium with 1.0 mg L^{-1} GA_3 (C) outer surface of *in vivo* flower bud (D) outer surface of *in vitro* flower bud.

placenta in angiosperm evolution (Muller et al., 2001). The *Impatiens* placenta is consequently best described as a near-cylindrical terminal extension of the floral apex, whose base is linked to the adjacent carpels (Chiurugwi et al., 2007). Field Emission Scanning Electron Microscopic (FESEM) examination was performed on 2-week old *in vitro* and *in vivo* flower buds of *I. balsamina*. FESEM of *in vitro* flower buds of *I. balsamina* treated with 1.0 mg L^{-1} GA_3 showed internal structures more clearly compared to *in vivo* flower buds. Ovule structure could be clearly seen on *in vitro* flower buds. This proved that tissue culture promoted faster growth of plantlet.

The *in vitro* flowering process is very useful for large-scale production of plants. Moreover, it also can be used to study the physiological, biochemical, and molecular basis of the complex process of flowering. Effective protocols must be developed for mass propagation to conserve germplasm and to get uniform plants of a selected genotype. It is also helpful for *I. balsamina*, since it is very important to increase *in vitro* flowering frequency for genetic transformation studies, breeding programs, and even for scientific decorations or handicrafts.

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