

Kalanchoë Regeneration from Flower Buds and Leaves *in vitro*

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Summary

Flower buds from 2nd level of *Kalanchoë blossfeldiana* ‘Beacon’ and ‘Sunrise cyme inflorescence were used as explants. Explants cultured on medium containing half strength MS combined 1mg^l⁻¹ BA and 0.5mg^l⁻¹ NAA proliferated more shoots. Marginal leaf explants of ‘Beacon’ cultured on the foregoing medium as well as leaf vein explants of ‘Sunrise’ cultured on the ½ MS medium supplemented with 2 mg^l⁻¹ BA and 0.5 mg^l⁻¹ NAA regenerated more shoots. New shoots began to initiate roots within 2-3 week after transferring to the ½ medium without plant growth regulator. All rooted plantlets survived after transplanting.

Introduction

Kalanchoë blossfeldiana Poelln. is one of the most important potted plants because of their ease of propagation and low water requirement, moreover wide colour range of flowers and bright attractive green leaves (Hernández *et al.*, 2004; Hwang *et al.*, 2008). The micropropagation of Kalanchoe had been developed by using shoot tip, node, internode, and leaf as a source of explants (Smith and Nightingale, 1979; Dicken and van Staden, 1988; Frello *et al.*, 2002; Jaiswal and Sawhney, 2006; Sanikhani *et al.*, 2006).

Using floral organs as explants had been recognized as potent source for micropropagation. The advantages were ease of sterilization, low rate of contamination, and conservation of the mother plant which used in initiation culture (Tomsone *et al.* 2004; Tunjit, 2008). In addition, explants of floral organ exhibited efficient for shoot regeneration e.g. flower bud of carnation

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(Miller *et al.*, 1991) and *Hylotelephium sieboldii* (Nakano *et al.*, 2005), flower of rhododendrons (Tomsone *et al.*, 2004), scape of gerbera (Chu *et al.*, 2006), florets with inflorescence of *Euphobia milli* (Tanjit, 2008) and *Ponerorchis graminifolia* Rchb. (Mitsukuri *et al.*, 2009), and branch of inflorescence of *Limonium* (Topoonyanont *et al.*, 2000).

Materials and Methods

Pot flowers of *K. blossfeldiana* cv. Beacon and cv. Sunrise were grown in the greenhouse of the National Chung Hsing University. The levels of double cymes inflorescences were described as Fig. 1a. While leaf explants were obtained from the 4th or 5th leaf pairs from shoot tip. Flower buds from levels of inflorescences or leaf pieces from various leaf positions (Fig. 1b) of both cultivars were used as explants.

All explants were harvested and surface sterilized with 0.5 % (w/v) sodium hypochlorite for 5 minutes for flower buds, or 8 minutes for leaf explants, respectively, then, rinsed three times with sterile water. Flower bud explants were placed vertically into the medium, while leaf explants from different parts were placed horizontally as well.

Shoot regeneration via flower bud explants

In order to determine the effect of explant position on the response to *in vitro* culture, only young flower buds at the level of 2nd, 3rd, 4th or 5th of “Beacon” were cultured in the medium consisted of 4.4g l⁻¹ MS salts (Sigma Ltd., Min. U.S.A.), 30g l⁻¹ sucrose, 7g l⁻¹ Bacto-agar, 1mg l⁻¹ BA and 1mg l⁻¹ NAA. The pH of medium was adjusted to 5.7 prior to sterilization at 121 °C for 15 minutes.

Flower buds of both cultivars, Beacon and Sunrise, at 2nd level were used as explants. The medium of MS salts at half strength supplemented with the combination of BA at 0, 0.5, 1.0, 2.0 or 4.0 mg l⁻¹ and NAA at 0, 0.5 or 1.0 mg l⁻¹, respectively.

Shoot regeneration via leaf explants

Leaf explants of lamina with midrib segment (P.1), margin part of the leaf (P.2), or leaf blade between vein and margin (P.3) were cut into 5 mm x5 mm pieces. The medium for leaf explants were the same as ½ MS basal media that described above, but supplemented with combinations of BA at 0, 1, 2 or 4 mg l⁻¹ and NAA at 0, 0.5 or 1 mg l⁻¹.

The culture environment was maintained at 25±2 °C, 35 μmol m⁻²s⁻¹ PPFD from cool white fluorescent lamps (East Asia, FL40D/38, Taiwan), 16-h photoperiod. After 2 months of culture, new shoots regenerated from each explant were counted. New shoots were cut and placed in test tube filled with 10 ml ½ MS salt medium for shoot elongation and rooting. After

3-4 weeks, plantlets with well developed roots were transplanted to 128 cell tray with BVB substrate (Bas Van Buuren No.4 medium, Visser Co., the Netherlands). Young plants were placed on mist bench with covered by saran sheet from directed sunlight for 2-3 weeks. The plants were shifted into to 2 inch pots and then 3.5 inch pots until flowering.

Experiments were conducted according to completely randomized design (CRD), ten explants as ten replicates for each treatment and repeated 3 times. Data were analysed with SPSS version 16.0 using one way or two analyses of variance (2-ways ANOVA), according to Turkey's test, at $p \leq 0.05$.

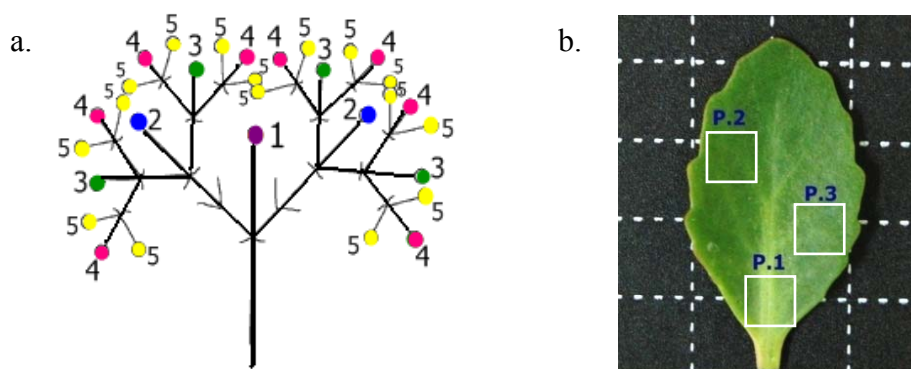


Fig. 1. The flower buds explants from different levels of a cyme inflorescence (a); leaf explants used in the experiments, midrib (P.1), leaf margin (P.2), and leaf blade between vein and margin (P.3) (b).

Results

Flower buds from different levels of *K. blossfeldiana* cv. 'Beacon' were found browning (data not shown) when cultured on MS medium supplemented with 1 mg l^{-1} BA and 1 mg l^{-1} NAA. There was no any significance between flower buds from different levels of cyme inflorescence (Table 1). The bud of *K. blossfeldiana* cv. 'Beacon' proliferated 1.6~3.4 shoots.

Unfolded flowers from 2nd level were cultured on 1/2 medium containing BA at 0, 0.5, 1, 2, or 4 mg l^{-1} , and NAA at 0, 0.5, 1, 2, or 4 mg l^{-1} , respectively. Flowers buds of 'Beacon' were no significant response to BA combined with NAA. There were some regenerated shoots from buds cultured on medium contained BA at 1 mg l^{-1} combined with NAA at 0.5 or 2 mg l^{-1} , or NAA at 1 mg l^{-1} supplemented with BA at 2 or 4 mg l^{-1} (Table 2). Flower buds explants of *K.*

blossfeldiana ‘Sunrise’ cultured on the medium containing BA at 1 mg^l⁻¹ combined NAA at 0.5 mg^l⁻¹ proliferated more shoots as 3.2 (Table 2).

There were few regenerated shoots from cv. Beacon leaf explant. The proliferation rate was not significant difference among treatments. Each explants regenerated 0~1.8 shoots only, and the highest was from marginal leaf explant cultured on media supplemented with 1 mg^l⁻¹ BA combined 0.5 mg^l⁻¹ NAA. In addition, leaf vein explant (P.1) did not regenerated while cultured on medium containing both BA and NAA at 1 mg^l⁻¹. Leaf margin explants (P.2) did not regenerate when cultured on medium containing higher concentration of BA (Table 3). Also, explants from leaf blade between vein and margin (P.3) showed the same tendency. The increase of BA and NAA concentration resulted the lower regeneration rate (Table 3).

The treatment of ‘Sunrise’ leaf experiment indicated that supplement of higher concentration of BA or addition of NAA from 0.5 to 1 mg^l⁻¹ in medium at the same BA concentration did not increase the number of regenerated shoots, except marginal leaf explants only. The maximum shoots amount as 5.3 was obtained from leaf vein explant cultured on media supplemented 2 mg^l⁻¹ BA and 0.5 mg^l⁻¹ NAA, however, only explants cultured on media containing 4 mg^l⁻¹ BA combined 0.5 mg^l⁻¹ NAA had significantly more shoots regeneration than leaf explants from other positions.

Most shoots began to initiate roots within 2-3 week of transfer to free from plant growth regulator medium. The rooted plantlets were successfully transplant to BVB substrate with a survival rate of 100%.

Table 1. Effect of floret levels of the cyme inflorescence on shoot regeneration

Level on inflorescence	Shoots/explant ¹
2 nd	3.4 a
3 rd	2.5 a
4 th	1.6 a
5 th	2.6 a

¹ Means in a column followed by the same letter are not significantly different according to ANOVA, Turkey’s test at 5% level.

Explants cultured on MS medium containing 1 mg^l⁻¹ BA and 1 mg^l⁻¹ NAA.

Table 2. Effect of BA and NAA on shoot regeneration from flower buds at 2nd level.

BA (mg/l)	NAA(mg/l)	'Beacon'	'Sunrise'
0	0	0 a ^z	0 b
0.5	1	0 a	0.5 ab
1	0.5	1.7 a	3.2 a
1	1	0 a	0 b
1	2	0.8 a	0.8 ab
1	4	0 a	0.8 ab
2	1	0.3 a	0.8 ab
4	1	1.5 a	0.5 ab

^z Mean separation within each column followed by Turkey's test at $P \leq 0.05$. Data collected after 2 months of culture.

^y Explants cultured on half-strength MS medium containing different BA and NAA concentration.

Table 3. Effect of BA and NAA on shoot regeneration of 'Beacon' leaf.

BA (mg/l) ^y	NAA(mg/l)	Leaf vein ^z	Leaf margin	Between leaf
0	0	0	0	0
1	0.5	0.4	1.8	0.8
1	1	0	0.1	0.2
2	0.5	0.8	0	0.3
2	1	0.2	0.1	0.2
4	0.5	0.2	0	0.1
4	1	0.2	0	0.1

^z NS: Non significant. Data collected after 2 months of culture.

^x Explants cultured on half-strength MS medium containing different BA and NAA concentration.

Table 4. Effect of BA and NAA on shoot regeneration of ‘Sunrise’ leaf.

BA (mg/l) ^X	NAA(mg/l)	Leaf vein ^Z	Leaf margin	Between leaf
0	0	0 a ^Z , A ^Y	0 b, A	0 a, A
1	0.5	3.9 a, A	2.3 ab, A	3.1 a, A
1	1	3.8 a, A	3.5 a, A	2.8 a, A
2	0.5	5.3 a, A	1.4 ab, A	1.3 a, A
2	1	1.3 a, A	1.5 ab, A	1.8 a, A
4	0.5	3.0 a, A	0.5 ab, B	0.7 a, B
4	1	2.4 a, A	1.6 ab, A	2.4 a, A

^Z Small letters mean separation within columns according to Turkey’s test at $P \leq 0.05$.

^Y Capital letters mean separation within rows according to Turkey’s test at $P \leq 0.05$.

Data collected after 2 months of culture.

^X Explants cultured on half-strength MS medium containing different BA and NAA concentration.

Discussion

It was found that the regenerated shoots from the petiole or leaf explants increased greatly when NAA was added together with BA (Yang *et al.*, 1995; Modgil *et al.*, 1999; Kantia and Kothari, 2002; Guo *et al.*, 2005; Zhang *et al.*, 2000). Furthermore, the combination of these plant growth regulators were also efficient for shoots regeneration from flower bud explants (Miller *et al.*, 1991; Nakano *et al.*, 2005; Mitsukuri *et al.*, 2005). In this study, whatever flower buds or leaf explants from *K. blossfeldiana* cv. Beacon or Sunrise were cultured on half strength MS medium without growth regulators were incapable of proliferation (Table 2, 3, and 4).

Miller *et al.* (1991) found that the adventitious shoots arisen from sub-epidermal cells of carnation petal base. Kakeni (1979) assumed that the regeneration area of flower bud explant was associated with the zone of cell elongation in that area. On *Kalanchoë*, shoots only proliferated at the proximal region near the receptacle (data not shown). Moreover, the floret culture of *Ponerorchis graminifolia* Rchb. (Mitsukuri *et al.*, 2009) and *Doritaenopsis* (Vendarme *et al.*, 2007) showed floret from the lower position had higher frequency of bud formation and promoted more survival rate than the lower level of floret explant. In this study,

flower from flower bud at 2nd level of inflorescence also had higher regeneration rate (Table 1 and 2).

In terms of using leaf as explant, shoot regeneration mainly occurred from the basal mid-vein explant. There were many researches about using lamina segment with petiole or midrib of leaf as explant induced higher frequency of shoot regeneration (Tiwari *et al.*, 1998; Modgil *et al.*, 1999; Kantia and Kothari, 2002; Papafotiou and Martini, 2009). Papafotiou and Martini (2009) expected that the explant with petiole or midrib from the basal part of leaf probably had more plant growth regulators and had sufficiency of nutrients through the mid vein. However, more shoots proliferated from leaf margin explant of 'Beacon' (Table3). Many species of *Kalanchoë* were known about their ability to produce new plants on the leaf margin via embryogenesis (Jaiswal and Sawhney, 2006; Garces *et al.*, 2007; Kulka, 2008). *In vitro*, the most factor associated with continuous proliferation of embryogenic cells was auxin (Merkle *et al.*, 1995). The global embryo formation required auxin, but it was transferred to medium without any plant growth regulator for further development. Early studies of Wetherell and Dougall (1979) on carrot embryogenic culture supported that the most normal differentiation to adventitious shoot was usually achieved through the complete removal of exogenous auxin from the medium. The margin of leaf had few of endogenous hormones compare to the vascular bundles tissue (Jaiswal and Sawhney, 2008). It was suggested that loosing of apical dominance of sufficient auxin induced shoot proliferation from leaf margin area.

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長壽花花芽及葉芽瓶內再生

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關鍵字: 芽體再生、花芽培植體、葉培植體

摘要：本試驗以長壽花‘燈塔’和‘日出’兩品種的二級小花蕾為培植體，培養在 $1/2$ MS 鹽類濃度、添加 1mg l^{-1} BA 和 0.5mg l^{-1} NAA 的培養基中有較佳的芽體增殖率。以‘燈塔’的葉緣培植體培養在上述的培養基時有較佳的增殖率；而‘日出’的葉中肋培植體，培養在 $1/2$ MS 鹽類濃度、添加 2mg l^{-1} BA 和 0.5mg l^{-1} NAA 培養基時，有較佳的增殖率。培植體移至不含植物生長調節劑的培養基，2~3 週後芽體開始發根，發根的小苗移出瓶外後皆可成活。

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