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# The Effect of Different Concentrations of Plant Growth Regulators on Micropropagation of *Kalanchoe blossfeldiana* cv. White

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Abstract

Shoot tips from actively growing, greenhouse maintained plants of *Kalanchoe blossfeldiana* were cultured *in vitro* for shoot proliferation and root initiation on Murashige and Skoog (MS) basal medium supplemented with NAA and BA, both in concentrations of 0.00, 0.50, 1.00 and 2.00 mg $l^{-1}$. Results showed that the maximum plantlets height (7.012 cm), node number (4.516), root number (8.860) and root length (10.160 cm) were obtained in MS medium containing 1 mg $l^{-1}$ BA + 1 mg $l^{-1}$ NAA. Maximum shoot number (5.886), leaf number (8.980) and proliferation index (1.791) were calculated in medium supplemented with 1 mg $l^{-1}$ BA + 0.5 mg $l^{-1}$ NAA. Minimum plantlets height (1.988 cm), node number (1.283), root number (2.720), root length (3.016 cm), shoot number (1.221), leaf number (2.015) and proliferation index (0.405) were obtained in medium without BA and NAA (control). Fresh and dry weights of plantlets were calculated, too. About 85% of the micropropagated plantlets were established successfully in acclimatization medium containing peat, perlite and sand (1:1:1). Regenerated plantlets were morphologically identical with mother plants.

**Keywords:** Crassulaceae, *In vitro* propagation, Plant growth regulators, Proliferation index, Shoot explants.

**Abbreviations:** BA_benzyladenine; NAA_naphtaleneacetic acid; MS_Murashige and Skoog.


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## INTRODUTION

*Kalanchoe* is used as ornamental potted plant around the world and contains medicinal values (Ofokansi *et al*., 2005; Nahar *et al*., 2008). *Kalanchoe* is very famous for its antimicrobial, antiinflammatory, antidiabetic and antitumor activity (Torres-Santos *et al*., 2003; Tadeg *et al*., 2005). Genus Kalanchoe consists of about 130 species of annual and perennial shrubs, climbers and small trees. Usually, it is cultivated as garden ornamental in rock and sand gardens with a medium humidity. Since, *Kalanchoe* is a slow growing plant therefore it is extremely necessary to develop a tissue culture system for rapid production of plant for commercial and medicinal purposes (Khan *et al*., 2006). Because of its medicinal importance and potential to produce value added secondary metabolites in tissue culture, it is of great interest to develop biotechnological methods to improve the production of this plant *in vitro* (Khan *et al*., 2006). One of the most extensive tissue culture techniques is micropropagation. Micropropagation is an effective technique for propagation of pathogen-free ornamental plants. *In vitro* propagation could be a valuable alternative to propagation by seeds or cuttings. Studies on micropropagation of *Kalanchoe blossfeldiana*, as an ornamental plant, are relatively low. Some studies on in vitro proliferation of *Kalanchoe* have been done by several researchers (Frello *et al.*, 2002; Khan *et al.*, 2006; Sanikhani *et al.*, 2006). Micropropagation of some Kalanchoe species were obtained on a hormone free medium (Khan *et al.*, 2006). Also, some studies on the micropropagation of Kalanchoe reported the use of different plant growth regulators like IAA, 2, 4-D, NAA and TDZ (Dickens and Staden, 1990; Ioannou and Ioannou, 1992; Frello *et al.,* 2002, Kordi *et al*., 2013). The aim of the present study was the effect of different concentrations of BA and NAA on micropropagation of *Kalanchoe blossfeldiana*, an ornamental plant.

## MATERIAL AND METHODS

Mother plants of *Kalanchoe blossfeldiana* cv. White were prepared from a commercial greenhouse in Karaj city, Alborz province, Iran. Micro-cuttings, apical buds containing two young leaves, were isolated from the mother plants and used as primary explants. Apical buds were washed thoroughly under running tap water for 20 min and disinfected with a 20% (v/v) NaOCl aqueous solution for 15 min then rinsed three times in sterile distilled water (10 min each). At the end, apical buds were sterilized for 3 min in 70% ethanol followed by three times rinses with sterile distilled water (15 min each). Shoot tips were excised from apical buds using binocular and used as final explants. Shoot tips were cultivated on MS (Murashige and Skoog, 1962) basal medium supplemented with 0, 0.5, 1 and 2 mg $l^{-1}$ of BA and 0, 0.5, 1 and 2 mg $l^{-1}$ of NAA. The media were adjusted to pH 5.7-5.8 and solidified with 7 g $l^{-1}$ Agar-agar. The media were pH adjusted before autoclaving at 121°C, 1 atm. for 20 min. Five shoot tips were cultivated in culture flasks. The cultures were incubated in growth chamber whose environmental conditions were adjusted to 26 ± 1°C and 75-80% relative humidity, under a photosynthetic photon density flux 50 µmol/$m^2$/s with a photoperiod of 16 h per day. Plant height, shoot number, node number, leaf number, root number, root length, fresh weight, dry weight and proliferation index were measured 5 wk after shoots tips culture. For determination of dry weight, plantlets produced in vitro were dried in Oven at 105°C for 24 h, following obtaining of fresh weight. Proliferation index was calculated via shoot number divided by explants number. The experimental design was R.C.B.D. Each experiment was carried out in three replicates and each replicate includes five specimens. Analysis of variance (ANOVA) was done using SPSS statistical software and means were compared using LSD at 0.05 level of probability.

## RESULT AND DISCUSSION

An effective micropropagation method was done for the *in vitro* plant regeneration of *Kalanchoe blossfeldiana*. For establishing a plant regeneration protocol, current study investigated the effect of different concentrations of BA and NAA on the efficiency of growth and development

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in *Kalanchoe blossfeldiana* (Table 1, Fig. 1). Minimum plantlets height (1.758 cm) was shown in the absence of exogenous BA and NAA. BA and NAA at 1.00 mg $l^{-1}$ in the MS media performed the best for increasing plant height (7.012 cm). Effect of NAA on induction of plant height was less than that of BA (the average of 2.25 vs. 3.70 cm) (Table 1). BA at 1.00 mg $l^{-1}$ (4.650 cm) and NAA at 0.50 mg $l^{-1}$ (2.438 cm) have been shown to have highest influence on plant height, singularly. In most cases, the combined treatments of BA and NAA showed a synergistic effect, with the increasing plant height greater than that of the singular treatments. After 5 weeks of culture, fully developed plantlets were produced from the explants (Fig. 1). Data analysis showed that the effect of BA and NAA were significant on the plant height ($p \leq 0.01$), but plant height was not significantly affected by kind of variety. The positive influence of BA and NAA was clear in enhancing the number of leaf and shoot. The highest shoot number (5.886), leaf number (8.980) and proliferation index (1.791) were obtained in medium containing 1 mg $l^{-1}$ BA + 0.5 mg $l^{-1}$ NAA. Also, apical buds cultured on MS media supplemented with 1 mg $l^{-1}$ BA + 0.5 mg $l^{-1}$ NAA including 5.39 shoots showed good growth of shoot (Table 1). The lowest shoot number (1.128) leaf number (2.015) and proliferation index (0.405) were obtained in medium without BA and NAA (control). BA had a significant effect on increasing leaf and shoot number (Table 1). Data presented in Table 1 shows that the least leaf and shoot number have been induced in media without BA. Minimum node number (1.283) was obtained in medium without BA and NAA (control). BA and NAA at 1.00 mg l-1 in the MS media performed the best for increasing node number (4.516) (Table 1, Fig. 1). Effect of BA on induction of node number was higher than that of NAA (the average of 2.800 vs. 1.800) (Table 1). BA at 1.00 mg $l^{-1}$ (3.516) and NAA at 0.50 mg $l^{-1}$ (1.983) have been shown to have highest influence on node number, singularly. In most cases, the combined treatments of BA and NAA showed a synergistic effect, with the increasing node number greater than that of the singular treatments. Data analysis showed that the effect of BA and NAA, singularly and in combination with each other were significant on the node number ($p \leq 0.01$ and $p \leq 0.05$, respectively), but node number was not significantly affected by kind of variety. The effects of different concentrations of BA and NAA were found significant on fresh and dry weight of plantlets. The

Table 1. Mean comparison of the effect of different concentrations of BA and NAA on some traits of *Kalanchoe blossfeldiana* cv. White

| Treatments (mg $l^{-1}$) | Plant height (cm) | Shoot number | Node number | Leaf number | Root length (cm) | Root number | Fresh weight (g) | Dry weight (g) | Proliferation index |
|---|---|---|---|---|---|---|---|---|---|
| NAA 0 + BA 0 | 1.758$^{g}$ | 1.128$^{h}$ | 1.283$^{ef}$ | 2.015$^{i}$ | 3.016$^{h}$ | 2.420$^{a}$ | 1.695$^{ghi}$ | 0.715$^{fgh}$ | 0.405$^{i}$ |
| NAA 0 + BA 0.5 | 2.657$^{efg}$ | 2.106$^{fgh}$ | 2.333$^{cdef}$ | 3.498$^{fgh}$ | 4.133$^{fgh}$ | 3.650$^{a}$ | 2.903$^{efg}$ | 1.260$^{de}$ | 0.696$^{fghi}$ |
| NAA 0 + BA 1 | 4.650$^{abc}$ | 3.720$^{bc}$ | 3.516$^{abc}$ | 6.115$^{bc}$ | 7.838$^{bc}$ | 6.860$^{a}$ | 4.803$^{ab}$ | 2.066$^{ab}$ | 1.238$^{bc}$ |
| NAA 0 + BA 2 | 3.700$^{cdef}$ | 2.551$^{def}$ | 2.833$^{cde}$ | 4.165$^{def}$ | 5.900$^{cdef}$ | 5.130$^{a}$ | 3.535$^{cde}$ | 1.495$^{cde}$ | 0.845$^{defg}$ |
| NAA 0.5 + BA 0 | 2.483$^{fg}$ | 1.545$^{gh}$ | 1.983$^{def}$ | 2.535$^{hi}$ | 3.766$^{fgh}$ | 3.330$^{a}$ | 1.741$^{fghi}$ | 0.863$^{gh}$ | 0.511$^{hi}$ |
| NAA 0.5 + BA 0.5 | 3.400$^{cdefg}$ | 2.608$^{def}$ | 2.483$^{cdef}$ | 4.318$^{def}$ | 5.416$^{defgh}$ | 4.570$^{a}$ | 2.865$^{efgh}$ | 1.198$^{efg}$ | 0.866$^{def}$ |
| NAA 0.5 + BA 1 | 5.950$^{ab}$ | 5.886$^{a}$ | 4.466$^{ab}$ | 8.980$^{a}$ | 10.160$^{ab}$ | 9.100$^{a}$ | 4.598$^{abc}$ | 1.995$^{abc}$ | 1.791$^{a}$ |
| NAA 0.5 + BA 2 | 3.466$^{cdef}$ | 2.885$^{cdef}$ | 2.700$^{cde}$ | 4.651$^{cdef}$ | 5.583$^{cdefg}$ | 4.830$^{a}$ | 3.230$^{de}$ | 1.320$^{de}$ | 0.958$^{cdef}$ |
| NAA 1 + BA 0 | 2.266$^{fg}$ | 1.496$^{gh}$ | 1.666$^{ef}$ | 2.476$^{hi}$ | 3.866$^{fgh}$ | 3.320$^{a}$ | 1.736$^{hi}$ | 0.960$^{gh}$ | 0.495$^{hi}$ |
| NAA 1 + BA 0.5 | 3.033$^{defg}$ | 2.663$^{def}$ | 2.666$^{cdef}$ | 4.388$^{def}$ | 4.716$^{efgh}$ | 4.100$^{a}$ | 3.053$^{de}$ | 1.350$^{de}$ | 0.883$^{def}$ |
| NAA 1 + BA 1 | 7.012$^{a}$ | 3.275$^{bcde}$ | 4.816$^{a}$ | 5.400$^{bcde}$ | 10.360$^{a}$ | 8.860$^{a}$ | 5.628$^{a}$ | 2.553$^{a}$ | 1.090$^{bcde}$ |
| NAA 1 + BA 2 | 4.316$^{cde}$ | 3.996$^{b}$ | 3.416$^{abcd}$ | 6.630$^{b}$ | 6.700$^{cde}$ | 6.020$^{a}$ | 3.463$^{cde}$ | 1.565$^{bcde}$ | 1.326$^{b}$ |
| NAA 2 + BA 0 | 2.000$^{g}$ | 1.551$^{fg}$ | 1.81$^{6f}$ | 2.573$^{ghi}$ | 3.316$^{gh}$ | 2.800$^{a}$ | 1.840$^{i}$ | 0.811$^{h}$ | 0.531$^{ghi}$ |
| NAA 2 + BA 0.5 | 3.660$^{cdef}$ | 2.718$^{def}$ | 2.616$^{cdef}$ | 4.458$^{def}$ | 6.083$^{cde}$ | 5.200$^{a}$ | 2.945$^{ef}$ | 1.216$^{efg}$ | 0.901$^{def}$ |
| NAA 2 + BA 1 | 4.483$^{bcd}$ | 3.386$^{bcd}$ | 3.282$^{bcd}$ | 5.623$^{bcd}$ | 7.150$^{cd}$ | 6.840$^{a}$ | 4.220$^{bcd}$ | 1.796$^{bcd}$ | 1.128$^{bcd}$ |
| NAA 2 + BA 2 | 3.383$^{cdefg}$ | 2.328$^{ef}$ | 2.416$^{cdef}$ | 3.821$^{efg}$ | 5.633$^{cdefg}$ | 4.840$^{a}$ | 2.793$^{efgh}$ | 1.200$^{efg}$ | 0.770$^{efgh}$ |

In each column, means with the similar letters are not significantly different at 5% level of probability using Duncan's test

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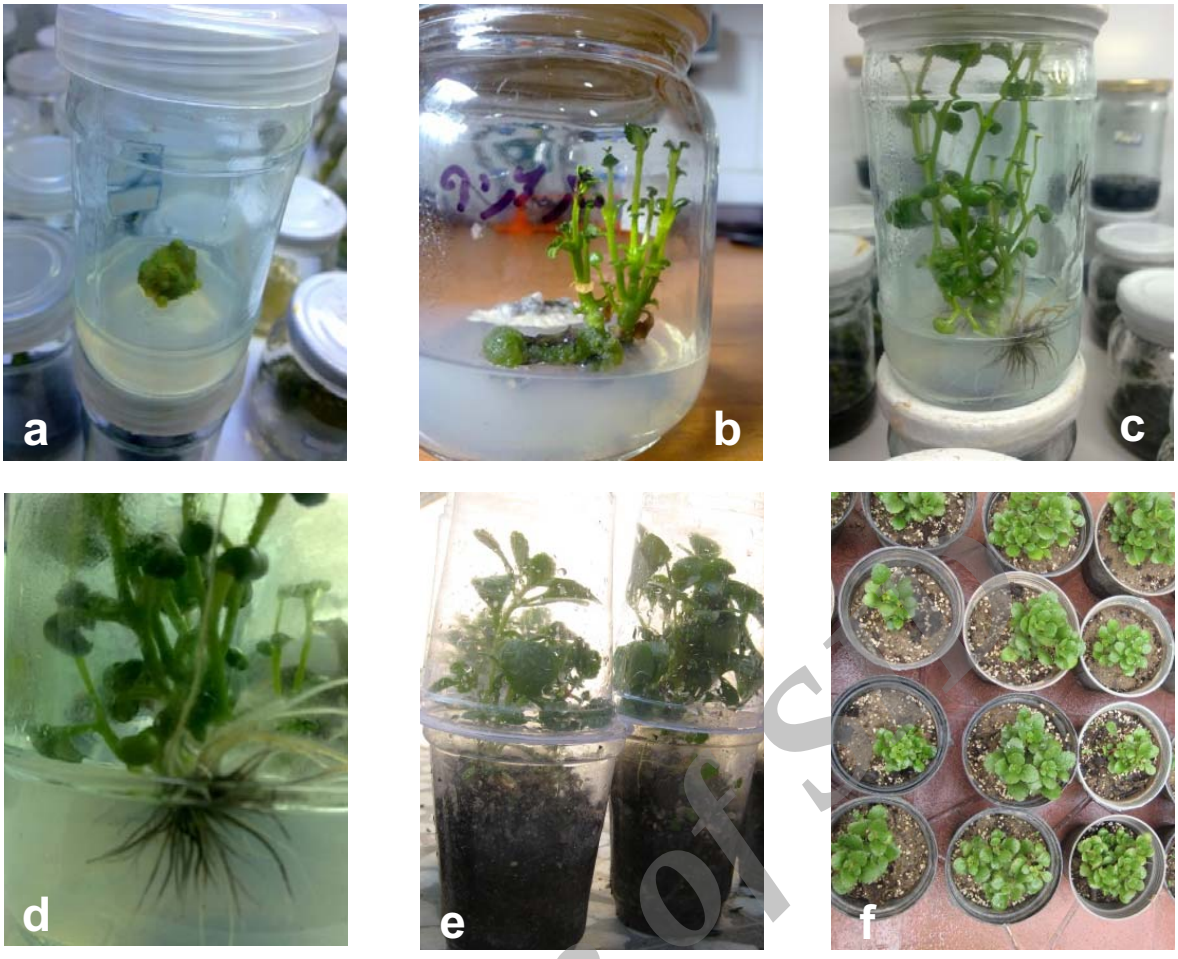


Fig. 1. Micropropagation process of *Kalanchoe blossfeldiana*. cv. White. a) Establishment of explants, b) Callus formation and production of shoots, c) Plantlets containing proliferated shoots, d) Plantlets containing roots, e) Hardening of plantlets, f) Hardened plants transferred to plastic pots containing a mixture of peat, perlite and sand (1:1:1).

highest average fresh (5.628 g) and dry (2.553 g) weight of plantlets was found with 1.00 mg $l^{-1}$ BA along with 1 mg $l^{-1}$ NAA (Table 1). Average fresh (1.695 g) and dry (0.715 g) weight of plantlets was minimum in absence of BA and NAA (control). It was appeared that less average weight of plantlets had found at all hormone concentrations of NAA without BA (Table 1).

Studies of Naz *et al.* (2009) on micropropagation of two species of Bryophyllum pinnatum and Bryophyllum daigremontianum from Crassulaceae family showed that thidiazuron (TDZ) has more potent as its lower concentrations (5 and 10 µM) for multiple shoots in B. daigremontianum. The regeneration frequency and number of shoots per explants were also enhanced on these concentrations. Contrary to our findings, these researchers showed that the concentrations of BAP (1, 2, and 3 µM) and combinations of BAP and NAA did not improve shoot regeneration. Only shoots were produced from leaf sections in lower concentrations of BAP (1 µM) while the higher concentration of BAP did not show the optimum response. The beneficial effect of BA on shoot regeneration and proliferation and induction of multiple shoots was reported in other species (Fuller and Fuller, 1995; Nhut, 2003; Fráguas *et al.*, 2004; Raj Poudel *et al.*, 2005). Some species may require a low concentration of auxins in combination with high levels of cytokinins to increase shoot proliferation (Van Staden, 2008). Study of Hashemabadi and Kaviani (2010) on micropropagation of *Aloe vera* L. using BA, IBA and NAA showed that the best proliferation of shoot per explants was shown on medium supplemented with 0.5 mg $l^{-1}$ BA + 0.5 mg $l^{-1}$ NAA. Naz *et al.* (2009) showed that 100% shoots frequency was observed in MS medium + 2 µM BAP in B. pinnatum

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and in MS medium + 2 µM BAP + 10 µM NAA in B. daigremontianum. This result is agreement with current study. In our work, combination of BA and NAA, both at 1.00 mg $l^{-1}$ promoted shoot proliferation. In both the Bryophyllum species different BAP concentrations did not affect the number of shoots per explant as in all the concentrations only one shoot per explant was produced. Our results do not confirm those of obtained by Khan *et al.* (2006). These workers showed that the maximum number of shoots, length of shoots, number of leaves, number of roots and number of plants were obtained on a hormone free MS based medium (control), suggesting that there is a little role of plant growth regulators in the *in vitro* development, multiplication and organogenesis of Kalanchoe tomentosa. In our work minimum of these traits were obtained in control plants. Some other studies on the micropropagation of Kalanchoe reported the use of different plant growth regulators like IAA, 2,4-D, NAA and TDZ for optimal in vitro proliferation (Dickens and Staden, 1990, Ioannou and Ioannou, 1992; Frello *et al*., 2002, Naz *et al*., 2009).

The medium containing 1 mg $l^{-1}$ BA + 1 mg $l^{-1}$ NAA resulted in the maximum root length (10.36 cm) and root number (8.860). Minimum root number (2.420) and root length (3.016 cm) was obtained in control medium (Table 1). Data analysis showed that the effect of BA and NAA were significant on root length ($p \leq 0.01$). There was no noticeable difference among different concentrations of NAA to response to root number and root length. Among different concentrations of BA, maximum root number (6.860) and root length (7.838 cm) were calculated in explants grown on medium containing 1.00 mg l-1. The number and length of root per explants were no increased with increasing the concentration of NAA and BA (Table 1).

Naz *et al*. (2009) showed that simple BAP failed to produce roots in Bryophyllum species (contrary to our finding), so combination of BAP with NAA proved to be excellent for root growth (consistent to our finding). In our studies, BA improved root formation and growth. Also, combination of BA and NAA proved to be excellent for root number and root length. Contrary to current study, Naz *et al*. (2009) revealed that there is difference between two cultivars for root production, because B. pinnatum produced 4.2 roots per explants and B. daigremontianum produced 7.2 roots per explants. Our findings demonstrated that the addition of BA and NAA in culture media was effective for increasing the number of root and root length. Current study showed the positive effect of NAA on root induction and root length. Some studies showed the positive effect of cytokinins on rooting (Gomes et al., 2010). Our studies demonstrated the positive effect of NAA in concentrations of 0.5 and 1 mg $l^{-1}$ on both root induction and root length.

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