Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(1):285-288

ISSN: 2248 –9215
CODEN (USA): EJEBAU

In vitro propagation of *Kalanchoe blossfeldiana* using BA and NAA

Mohaddeseh Kordi*, Behzad Kaviani and Davood Hashemabadi

Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran

---

ABSTRACT

Micropropagation is an efficient in vitro propagation method for Kalanchoe blossfeldiana. In the present research, the effect of plant growth regulators (PGRs) on root formation and adventitious shoot from apical buds explants of Kalanchoe blossfeldiana. Apical buds as explants were cultured on solid MS medium supplemented with BA and NAA, both with concentrations of 0, 0.5, 1 and 2 mg l^{-1}. The experimental design was R.C.B.D. MS medium supplemented with 1 mg l^{-1} BA + 1 mg l^{-1} NAA resulted in the highest plant height (6.22 cm), root number (9.34) and root length (10.36 cm). Maximum shoot number (5.39) was obtained in MS medium containing 1 mg l^{-1} BA + 0.5 mg l^{-1} NAA. About 85% of the micropropagated plantlets were established successfully in acclimatization medium. Regenerated plantlets were morphologically identical with mother plants.

Keywords: Apical buds, Crassulaceae, Ornamental plants, Phytohormones, Micropropagation.

INTRODUCTION

The ornamental species *Kalanchoe blossfeldiana*, belonging to Crassulaceae, is a pot plant. The economic value of ornamental plants has increased significantly worldwide and is increasing annually by 8-10% [9]. The techniques for *in vitro* propagation of ornamental plants and tissue culture laboratory equipment are being continuously improved to meet the demand of the floriculture breeding and industry [22]. Micropropagation through tissue culture permits the regeneration of large numbers of disease free plants from small pieces (explants) of stock plants in a relatively short period and, crucially, without seasonal restrictions [19]. In the field of ornamental plants, tissue culture has allowed mass propagation of superior genotypes and plant improvement, thus enabling the commercialization of healthy and uniform planting material [13, 24]. The success of the micropropagation method depends on several factors like genotype, media, plant growth regulators and type of explants, which should be observed during the process [14, 17]. Most important of these parameters are the plant growth regulators included in the culture media [4]. In tissue culture, cytokinins and auxins play a crucial role as promoters of cell division and act in the induction and development of meristematic centers leading to the formation of organs [18]. Some studies were carried out on micropropagation of *Kalanchoe blossfeldiana*. Studies of Xiu-Lina et al. [26] on micropropagation of *Kalanchoe blossfeldiana* showed plantlets regeneration from stems and leaves explants on MS medium containing BA and NAA. Xin-Zheng et al. [25] showed rooting of *Kalanchoe blossfeldiana* on MS medium supplemented with BA and IBA. In this paper, a protocol for multiplication of *Kalanchoe blossfeldiana* via organogenesis by using different concentration of BA and NAA will be detailed.

MATERIALS AND METHODS

Mother plants of *Kalanchoe blossfeldiana* were prepared from a commercial greenhouse in Amol city, Mazandaran, Iran. Micro-cuttings (apical buds) were isolated from the mother plants and used as explants. Apical buds were washed thoroughly under running tap water for 20 min and disinfected with a 15% 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 5 min in 70% ethanol followed by three times rinses with sterile distilled water (15 min each). Apical buds were cultivated on MS [11] 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. Five apical buds were cultivated in culture flasks. 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. The cultures were incubated in growth chamber whose environmental conditions were adjusted to 25±2°C and 75-80% relative humidity, under a photosynthetic photon density flux 50 µmol/m^2/s with a photoperiod of 14 h per day. Plant height, shoot number, root number and root length were measured 45 days after apical buds culture. 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 SAS statistical software and means were compared using LSD at 0.05 level of probability.

RESULTS AND DISCUSSION

In this study, the effect of different concentrations of BA and NAA on micropropagation of *Kalanchoe blossfeldiana*, an ornamental plant, was evaluated. Studied characteristics were plant height, shoot number, root number and root length. The results are summarized in Tables 1 and 2. Our data revealed that there are differences in the effect of the different concentrations of BA, NAA and interaction between these two growth regulators on these characters. Apical buds were excised and transferred on MS medium containing BA (0, 0.5, 1 and 2 mg l^{-1}) and NAA (0, 0.5, 1 and 2 mg l^{-1}). Subsequently, within the next 45 days, differences were observed. The medium containing 1 mg l^{-1} BA + 1 mg l^{-1} NAA resulted in the maximum plant height (6.22 cm), root length (10.36 cm) and root number (9.34). 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 2). Minimum plant height (1.980 cm), shoot number (1.221), root number (2.720) and root length (3.016 cm) was obtained in control medium (Table 2). Data analysis showed that the effect of BA and NAA were significant on the plant height, shoot number and root length ($p \leq 0.01$) (Table 1).

Our results indicated that there are differences in the effect of the different concentrations of BA and NAA for plant height, shoot number, root number and root length. Cytokinins are usually used on the micropropagation media to stimulate axillary shoot proliferation. Similar to our findings, many researchers showed that cytokinin BA induced multiple shoot formation and shoot length [2, 3, 12, 20]. Study of Xiu-Lina et al. [26] on micropropagation of *Kalanchoe blossfeldiana* showed that for shoot proliferation, BA and NAA resulted in the longest root, largest number of leaf and node and shoot number. Also, study of Hepaksoy and Aksoy [8] on micropropagation of *Ficus carica* L. revealed that the most shoot was obtained in medium supplemented with 5 mg l^{-1} BA along with 1 mg l^{-1} IBA. Some species may require a low concentration of auxin in combination with high levels of cytokinins to increase shoot proliferation [23]. Rout et al. [21] observed that the rate of growth in *Rosa* spp. was found to be very poor in a hormone-free medium. Contrary to our findings, studies of Osuna et al. [16] on micropropagation of *Lepidium virginicum* L. showed that the maximum shoot length was obtained in MS medium without hormones. Study of Ahmadi Hesar et al. [1] on micropropagation of *Matthiola incana* showed that multiple shoots containing roots can be obtained simultaneously on MS medium only supplemented with 0.5-2 mg l^{-1} KIN. Studies of Kaviani et al. [10] on micropropagation of *Matthiola incana* using NAA and KIN demonstrated the positive effect of plant growth regulators on shoot length and node number. Study of Hashemabadi and Kaviani [7] 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. The largest number of roots was obtained on medium supplemented with 0 mg l^{-1} IBA + 1 mg l^{-1} NAA (9.71). The longest (8.75 cm) root was achieved on medium supplemented with 1 mg l^{-1} IBA + 1 mg l^{-1} NAA. Our findings demonstrated that the addition of BA and NAA in culture media was effective for increasing the number of root and root length. Some studies showed the positive effect of auxins on rooting [6, 9, 15]. Current study showed the positive effect of NAA on root induction and root length. Some studies showed the positive effect of cytokinins on rooting [5]. Studies of Gomes et al. [5] on *Arbutus unedo* L. showed that shoots produced on higher cytokinin-containing medium are more amenable to root induction than shoots obtained with the lowest concentrations of BA. Study of Hashemabadi and Kaviani [7] on

micropropagation of *Aloe vera* L. using BA, IBA and NAA showed that the largest number of roots was obtained on medium supplemented with 0 mg l^{-1} IBA + 1 mg l^{-1} NAA. 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.

Table 1. Analysis of variance (ANOVA) for the effect of different concentrations of BA and NAA and varieties on some traits of *Kalanchoe blossfeldiana*.

Source of variations	df	Plant height	Shoot number	Root length	Root number
Variety (A)	1	0.396^{ns}	0.939^{ns}	0.436^{ns}	1357.19^{ns}
BA and NAA (B)	15	9.632^{**}	7.032^{**}	29.08^{**}	1363.01^{ns}
A × B	15	0.779^{ns}	1.051^{**}	2.549^{*}	1229.23^{ns}
CV (%)		21.10	17.40	20.06	29.10

**: Significant at α = 1%, *: Significant at α = 5%, ^{ns}=Not significant

Table 2. Mean comparison of the effect of different concentrations of BA and NAA on some traits of *Kalanchoe blossfeldiana*.

Treatments (mg l^{-1})	Plant height (cm)	Shoot number	Root length (cm)	Root number
NAA 0 + BA 0	1.988^{g}	1.221^{h}	3.016^{h}	2.720^{a}
NAA 0 + BA 0.5	2.766^{efg}	2.106^{fgh}	4.133^{fgh}	3.820^{a}
NAA 0 + BA 1	4.650^{abc}	3.720^{bc}	7.838^{bc}	6.860^{a}
NAA 0 + BA 2	3.700^{cdef}	2.551^{def}	5.900^{cdef}	5.130^{a}
NAA 0.5 + BA 0	2.483^{fg}	1.545^{gh}	3.766^{fgh}	3.330^{a}
NAA 0.5 + BA 0.5	3.400^{cdefg}	2.608^{def}	5.416^{defgh}	4.570^{a}
NAA 0.5 + BA 1	5.950^{ab}	5.386^{a}	10.160^{ab}	9.100^{a}
NAA 0.5 + BA 2	3.466^{cdef}	2.885^{cdef}	5.583^{cdefg}	4.830^{a}
NAA 1 + BA 0	2.266^{fg}	1.496^{gh}	3.866^{fgh}	3.320^{a}
NAA 1 + BA 0.5	3.033^{defg}	2.663^{def}	4.716^{efgh}	4.100^{a}
NAA 1 + BA 1	6.216^{a}	3.275^{bcde}	10.360^{a}	9.340^{a}
NAA 1 + BA 2	4.316^{cde}	3.996^{b}	6.700^{cde}	6.020^{a}
NAA 2 + BA 0	2.000^{g}	1.551^{fg}	3.316^{gh}	2.800^{a}
NAA 2 + BA 0.5	3.660^{cdef}	2.718^{def}	6.083^{cde}	5.200^{a}
NAA 2 + BA 1	4.483^{bcd}	3.386^{bcd}	7.150^{cd}	6.840^{a}
NAA 2 + BA 2	3.383^{cdefg}	2.328^{ef}	5.633^{cdefg}	4.840^{a}

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

REFERENCES

[1] A. Ahmadi Hesar, B. Kaviani, A.R. Tarang and S. Bohlooli Zanjani, *Plant Omics J*., **2011**. 4 (5), 236-238.
[2] C.B. Fráguas, M. Pasqual, L.F. Dutra and O. Cazzeta, *In Vitro Cell. Dev. Biol-Plant*, **2004**. 40, 471-474.
[3] M.P. Fuller and F.M. Fuller, *J. Biol. Edu*., **1995**. 20 (1), 53-59.
[4] F. Gomes and J.M. Canhoto, *In Vitro Cell. Dev. Biol*., **2003**. 39, 316-321.
[5] F. Gomes, M. Simões, M.L. Lopes and M. Canhoto, *New Biotech*., **2010**. 27 (6), 882-892.
[6] H.Y. Hammaudeh, M.A. Suwwan, H.A. Abu Quoud and R.A. Shibli, *Dirasat Agric. Sci*., **1998**. 25, 170-178.
[7] D. Hashemabadi and B. Kaviani, *Aus. J. Crop Sci*., **2010**. 4 (4), 216-222.
[8] S. Hepaksoy and U. Aksoy, *Biologia Plantarum*, **2006**. 50, 433-436.
[9] S.M. Jain SM and S.J. Ochatt, *Springer Protocols, Humana Press*, 2010.
[10] B. Kaviani, A. Ahmadi Hesar and A. Kharabian Masouleh, *Plant Omics J*., **2011**. 4 (7), 435-440.
[11] T. Murashige and F. Skoog, *Physiol. Plant*, **1962**. 15, 473-497.
[12] D.T. Nhut, *Plant Growth Regul*., **2003**. 40 (2), 179-184.
[13] D.T. Nhut, N.T. Don, N.H. Vu, N.Q. Thien, D.T.T. Thuy, N. Duy and J.A. Teixeira da Silva, *Global Science Books*, *UK*, **2006**. pp 325-335.
[14] D.T. Nhut, N.T. Hai and M.X. Phan, *Springer Protocols, Humana Press*, **2010**. pp 15-20.
[15] J. Nobre, A. Romano, U. Aksoy, L. Ferguson and S. Hepaksoy, *Acta Hortic*., **1998**. 480, 161-164.
[16] L. Osuna, M.E. Tapia-Perez, O. Figueroa, E. Jimenez-Ferrer, M.L.G. Ramírez, M.T. Gonzalez-Garza, P. Carranza-Rosales and D.E. Cruz-Vega, *In Vitro Cell. Dev. Biol*., **2006**. 42 (6), 590-600.
[17] P.K. Pati, S.P. Rath, M. Sharma, A. Sood and P.S. Ahuja, *Biotechnol. Adv*., **2005.** 94-114.
[18] A.J.M. Peeters, W. Gerards, G.W.M. Barendse and Wullems, *Plant Physiol*., **1991**. 97, 402-408.
[19] W. Preil, P. Florak, U. Wix and A. Back, *Acta Hortic*., **1988**. 226, 99-107.
[20] P. Raj Poudel, I. Kataoka and R. Mochioka, *Asian J. Plant Sci*., **2005**. 4 (5), 466-471.

[21] G.R. Rout, B.K. Debata and P. Das, *Proc. Natl. Acad. Sci. Ind*., **1990**. 60, 311-318.
[22] G.R. Rout, A. Mohapatra and S. Mohan Jain, *Biotech. Adv*., **2006**. 24 (6), 531-560.
[23] D. Van Staden, D., *Springer Press*, **2008**. pp 205-226.
[24] T. Winkelmann, T. Geier and W. Preil, *Plant Cell Tiss. Org. Cult*., **2006**. 86, 319-327.
[25] S. Xin-Zheng, L. Qingwei and L. Mingaing, *Chinese Agric. Sci*., **2006** (Abstract).
[26] L. Xiu-Lian, L. Zhong-Xoing, L. Huang-Shung, W. Jin-Shou, J. Huangy, H. Xiao-Xia and K. Xiang, *Chinese Agric. Sci. Bull*., **2005** (Abstract).