Micropropagation of *Matthiola incana* using BA and IBA

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Abstract

Matthiola incana (Brassicaceae) is an important ornamental plant, which is used as cut flower, pot plant, and landscape. Traditionally, it is propagated via seeds, but interest is given in vegetative propagation of parental lines as well as superior single plants. Micropropagation by organogenesis is an efficient *in vitro* propagation method for *Matthiola incana*. Starting from seeds, apical bud is produced and propagated in MS medium containing benzyladenine (BA) and indole-3-butyric acid (IBA). Seeds from mother plants were germinated on MS medium without growth regulators. Apical buds from *in vitro* germinated seedlings were subcultured on solid MS medium supplemented with BA and IBA, both with concentrations of 0, 0.5, 1 and 5 mg L^{-1}. Four-week-old *in vitro* plants obtained from apical buds showed successful shooting and rooting. MS medium supplemented with 5 mg L^{-1} BA + 1 mg L^{-1} IBA resulted in the highest shoot length (2.90 cm/plant). Largest number of node (5.72/plant) was obtained in MS medium containing 1 mg L^{-1} BA + 1 mg L^{-1} IBA. When the shoot tips were inoculated in the medium containing 0.5 mg L^{-1} IBA without BA, the best result was observed for root number (5.95/plant). Shoot tips cultivated in media containing 0.5 mg L^{-1} IBA without BA showed maximum root length (15.36 cm/plant). Also, the content of fresh weight and dry weight were obtained. About 85% of the micropropagated plantlets were established successfully in acclimatization medium. Regenerated plantlets were morphologically identical with mother plants. This protocol has proven useful for tissue culture propagation of *Matthiola incana*.

Keywords: Brassicacea; micropropagation; organogenesis; ornamental plants; plant growth regulators

Abbreviations:
BA: benzyladenine; IBA: indole-3-butyric acid; MS: Murashige and Skoog

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Introduction

The ornamental species *Matthiola incana*, belonging to Brassicaceae, is a pot plant, which is also used as cut flower. The Brassicaceae is a fairly large family with many economically important taxa while few studies have been done on tissue culture of this plant. Natural propagation of *Matthiola incana* takes place by seed. The economic value of ornamental plants has increased significantly worldwide and is increasing annually by 8-10% (Jain and Ochatt, 2010). The techniques for *in vitro* propagation of ornamental plants and tissue culture laboratory equipment are being continuously improved to meet the demands of the floriculture breeding

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and industry (Rout et al., 2006). Tissue culture has become a routine technique in agricultural and horticultural development which has revolutionized the ornamental industry and most popular application of this technique is micropropagation (Maira et al., 2010; Bhattacharya and Bhattacharyya, 2010). 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 (Preil et al., 1988). In general, the number of publications on different aspects of the culture of *Matthiola incana* is limited, with emphasis on micropropagation through somatic explants (Gautam et al., 1983). 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 (Winkelmann et al., 2006; Nhut et al., 2006). 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 (Pati et al., 2005; Nhut et al., 2010). The most important of these parameters are the plant growth regulators included in the culture media (Gomes and Canhoto, 2003). Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the developmental programs of plants (Mercier et al., 1997). Cytokinins are usually used in the micropropagation media to stimulate axillary shoot proliferation (Chawla, 2009; El-Agamy, 2009). However, the ideal concentrations differ from species to species and need to be established accurately to obtain the effective rates of multiplication. In general, three modes of *in vitro* plant regeneration have been in practice: organogenesis, embryogenesis and axillary proliferation. 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 (Peeters et al., 1991). Multiple shoot buds were differentiated from cotyledon explants of *Matthiola incana*, cultured in the medium containing BAP and NAA (Gautam et al., 1983). Plantlets were regenerated from protoplast culture of *Matthiola incana* in the medium supplemented with BAP, 2,4-D and NAA (Hosoki and Ando, 1989). Rooting is a crucial step to the success of micropropagation. Auxins enhance the germination, root induction and seedling growth of many species (Gautam et al., 1983; Isutsa, 2004; Kalimuthu et al., 2007; Jain and Ochatt, 2010; Hashemabadi and Kaviani, 2010; Eeckaut et al., 2010; Casas et al., 2010). Different organs of *Matthiola incana* exhibit differential morphogenic potential. Probably, the change in response depends on the exogenous and endogenous plant growth regulators (Gautam et al., 1983). Nowadays, studies generally analyze the effect that a plant growth regulator exercises on the explants after a short period of time, and not its influence on later development (Feito et al., 1994; Moncaleán et al., 2003). In this paper, a protocol for multiplication of *Matthiola incana* via organogenesis will be detailed using different concentrations of BA and IBA.

Materials and Methods

Seeds of *Matthiola incana* were prepared from Mohaghegh-e-Ardabili University, Iran. The seeds were washed thoroughly under running tap water for 20 min and disinfected with a 20% NaOCl aqueous solution and Tween-20 for 10 min then rinsed three times in sterile distilled water (10 min each). At the end, seeds were sterilized for 2 min in 70% ethanol followed by three times rinsing with sterile distilled water (15 min each). Five seeds were cultivated in culture flasks in MS (Murashige and Skoog, 1962) basal medium without growth regulators. Micro-cuttings (apical buds) were isolated from 4-week-old plants and cultivated in MS media supplemented with 0, 0.5, 1, and 5 mg L^{-1} BA, and 0, 0.5, 1, and 5 mg L^{-1} IBA. 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. Shoot length and node number were measured 30 days after apical buds culture. Some

Table 1
Mean comparison of the effect of different concentrations of IBA on some traits of *Matthiola incana*

Treatments (mg L^{-1})	Shoot length (cm)	Node number	Root length (cm)	Root number	Fresh weight (g)	Dry weight (g)
IBA 0	2.130^{b}	3.340^{b}	4.448^{a}	10.852^{a}	0.581^{a}	0.052^{a}
IBA 0.5	2.107^{b}	3.310^{b}	2.578^{ab}	6.778^{a}	0.409^{bc}	0.031^{b}
IBA 1	2.439^{a}	4.240^{a}	1.100^{b}	7.000^{a}	0.459^{b}	0.035^{b}
IBA 5	2.457^{a}	3.590^{b}	1.000^{b}	1.000^{b}	0.396^{bc}	0.035^{b}

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

Table 2
Mean comparison of the effect of different concentrations of BA on some traits of *Matthiola incana*

Treatments (mg L^{-1})	Shoot length (cm)	Node number	Root length (cm)	Root number	Fresh weight (g)	Dry weight (g)
BA 0	1.747^{c}	2.530^{c}	2.491^{b}	5.029^{b}	0.387^{c}	0.034^{a}
BA 0.5	2.537^{a}	4.250^{a}	5.936^{a}	5.360^{a}	0.492^{b}	0.039^{a}
BA 1	2.558^{a}	4.300^{a}	5.460^{a}	5.080^{a}	0.576^{a}	0.042^{a}
BA 5	2.297^{b}	3.500^{b}	2.140^{b}	2.143^{c}	0.389^{c}	0.039^{a}

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

other characteristics, such as root length, root number, fresh weight, and dry weight were recorded after 42 days. The experimental design was R.C.B.D. Each experiment was carried out in three replicates and each replicate included five specimens. Analysis of variance (ANOVA) was done using SPSS and MSTAT-C statistical software and means were compared using Duncan's test at 0.05 level of probability.

Results

In current study, the effect of different concentrations of BA and IBA on micropropagation of *Matthiola incana*, an ornamental plant, through organogenesis was evaluated. Studied characteristics were shoot length, node number, root number, root length, fresh weight, and dry weight. The results are summarized in Tables 1, 2, and 3. Our data revealed that there are differences in the effect of the different concentrations of BA, IBA and interaction between the two growth regulators on these characters. Apical buds were excised and transferred on MS medium containing BA (0, 0.5, 1, and 5 mg L^{-1}) and IBA (0, 0.5, 1, and 5 mg L^{-1}). Subsequently, within the next 3-4 weeks, differences were observed. The medium containing 5 mg L^{-1} BA + 1 mg L^{-1} IBA and 1 mg L^{-1} BA + 0.5 mg L^{-1} IBA, both resulted in the maximum shoot length (2.90 and 2.82 cm). Also, apical buds cultured on MS media supplemented with 1 mg L^{-1} BA + 1 mg L^{-1} IBA and 5 mg L^{-1} BA + 0.5 mg L^{-1} IBA including 2.808 and 2.676 cm shoot length, respectively, showed good growth of shoot (Table 3). Minimum shoot length (1.616 cm) was obtained in medium containing 1 mg L^{-1} IBA without BA (Table 3). Media without any plant growth regulators (controls) did not show suitable shoot length, too (Tables 2 and 3). Data analysis showed that the effect of BA, IBA and BA + IBA were significant on the length of shoot ($p \leq 0.01$) (Table 4). Our results indicated an overall significant positive correlation ($r=0.710$, $p \leq 0.01$) between shoot length and nodes number, as well as between shoot length and root number ($r=0.229$, $p \leq 0.05$), root length ($r=0.422$, $p<0.01$), dry weight ($r=0.122$, $p \leq 0.05$), and fresh weight ($r=0.258$, $p<0.01$) (Table 5). The largest number of node (5.72) was obtained in MS medium containing 1 mg L^{-1} BA + 1 mg L^{-1} IBA. Also, apical buds cultured in medium supplemented with 1 mg L^{-1} BA + 0.5 mg L^{-1} IBA showed good node number (4.92) (Table 3). Lowest node number (2.20) was obtained in medium containing 1 mg L^{-1} BA without IBA (Table 3). Data analysis showed that the effects of BA, IBA, and BA + IBA ($p \leq 0.01$) were significant on the node number (Table 4). Our results indicated a significant positive correlation between node number and root number ($r=0.411$, $p \leq 0.01$), root length ($r=0.387$, $p \leq 0.01$), and fresh weight

Mean comparison of the effect of different concentrations of BA and IBA on some traits of *Matthiola incana*.

Treatments (mg L^{-1})	Shoot length (cm)	Node number	Root length (cm)	Root number	Fresh weight (g)	Dry weight (g)
BA 0 + IBA 0	1.676^{h}	2.720^{fghi}	4.292^{b}	2.517^{b}	0.050^{abc}	0.485^{cde}
BA 0 + IBA 0.5	$2.176d^{ef}$	3.760^{cdef}	15.360^{a}	5.936^{a}	0.053^{ab}	0.661^{b}
BA 0 + IBA 1	2.412^{cde}	3.720^{cdef}	15.080^{a}	5.460^{a}	0.068^{a}	0.818^{a}
BA 0 + IBA 5	2.280^{de}	3.160^{fghi}	2.140^{b}	2.143^{b}	0.038^{bc}	0.355^{def}
BA 0.5 + IBA 0	1.920^{fgh}	2.640^{hij}	3.778^{b}	2.578^{b}	0.033^{bc}	0.426^{def}
BA 0.5 + IBA 0.5	2.476^{bcd}	4.320^{bc}	1.800^{b}	1.000^{b}	0.034^{bc}	0.412^{def}
BA 0.5 + IBA 1	2.112^{efg}	3.360^{efgh}	1.750^{b}	1.010^{b}	0.029^{bc}	0.369^{def}
BA 0.5 + IBA 5	1.920^{fgh}	3.320^{efgh}	1.580^{b}	1.120^{b}	0.027^{bc}	0.404^{def}
BA 1 + IBA 0	1.616^{h}	2.200^{j}	7.000^{b}	1.100^{b}	0.0282^{c}	0.332^{f}
BA 1 + IBA 0.5	2.820^{ab}	4.920^{b}	1.680^{b}	1.787^{b}	0.032^{bc}	0.451^{def}
BA 1 + IBA 1	2.808^{ab}	5.720^{a}	1.777^{b}	1.555^{b}	0.404^{bc}	0.597^{bc}
BA 1 + IBA 5	2.522^{bcd}	4.120^{cd}	1.889^{b}	1.234^{b}	0.039^{bc}	0.457^{def}
BA 5 + IBA 0	1.776^{gh}	2.560^{ij}	1.555^{b}	1.334^{b}	0.023^{c}	0.309^{f}
BA 5 + IBA 0.5	2.676^{abc}	4.000^{cde}	1.876^{b}	1.567^{b}	0.037^{bc}	0.444^{def}
BA 5 + IBA 1	2.900^{a}	4.400^{bc}	1.845^{b}	1.342^{b}	0.033^{bc}	0.494^{cd}
BA 5 + IBA 5	2.476^{bcd}	3.400^{defg}	1.565^{b}	1.989^{b}	0.0487^{abc}	0.336^{ef}

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

Table 4
Analysis of variance (ANOVA) for the effect of different concentrations of BA and IBA on some traits of *Matthiola incana*

Source of variations	df	Shoot length	Node number	Root length	Root number	Fresh weight	Dry weight
BA	3	3.572^{**}	16.843^{**}	1.009^{ns}	22.228^{ns}	0.695^{**}	0.00885^{*}
IBA	3	14.254^{**}	68.643^{**}	69.224^{**}	839.602^{**}	0.831^{**}	0.00135^{ns}
BA × IBA	9	1.327^{**}	7.45^{**}	1.000^{ns}	18.334^{ns}	0.228^{**}	0.0021^{ns}
Error		0.562	1.188	1.545	10.234	0.228	0.0425
CV (%)		24.628	22.596	26.563	21.903	19.377	10.943

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

(r=0.333, p≤0.01). There was no positive correlation between node number and dry weight (Table 5). When the shoot tips were inoculated in the medium containing 0.5 mg L^{-1} IBA without BA, the best result was observed for root number (5.936) (Table 3). Analysis of variance showed that the effect of IBA on the root number were significant (p≤0.01) (Table 4). The effect of BA and BA + IBA on the root number was not significant (Table 4). Current study indicated a significant positive correlation between root number and root length (r=0.691, p≤0.01) and fresh weight (r=0.625, p≤0.01). There was no positive correlation between root number and dry weight (Table 5). Maximum root lengths (15.36 and 15.08 cm) were obtained in shoot tips cultured on media supplemented with 0.5 and 1 mg L^{-1} IBA without BA, respectively (Table 3). Analysis of variance showed that the effect of IBA on the root length were significant (p≤0.01). The effect of BA and BA + IBA on the root length was not significant (Table 4). Our results indicated a significant positive correlation (r=0.381, p≤0.01) between root length and fresh weight. There was no positive correlation between root length and dry weight (Table 5). The highest fresh weight (0.818 g) was found when 1 mg L^{-1} IBA was used without BA (Table 3). The highest dry weight (0.068 g) was obtained when 1 mg L^{-1} IBA was used without BA (Table 3). Minimum fresh and dry weights (0.309 and 0.023 g, respectively) were recorded in media containing 5 mg L^{-1} BA without IBA. Data analysis showed that the effect of BA, IBA and BA + IBA were significant (p≤0.01) on the fresh weight (Table 4). Analysis of variance showed that the effect of BA on the dry weight was significant (p≤0.05). The effect of IBA and BA + IBA on the dry weight was not significant (Table 4). The study indicated a significant positive correlation (r=0.406, p≤0.01) between dry weight and fresh weight.

Table 5
Simple correlation of the effect of BA and IBA on some traits of *Matthiola incana*

	Shoot length	Node number	Root number	Root length	Fresh weight	Dry weight
Shoot length	1					
Node number	0.710**	1				
Root number	0.229*	0.411**	1			
Root length	0.422**	0.387**	0.691**	1		
Fresh weight	0.258**	0.333**	0.625**	0.381**	1	
Dry weight	0.122*	0.094	0.125	0.108	0.406**	1

Discussion

Our results indicated that there are differences in the effect of the different concentrations of BA and IBA for shoot length and node number. Cytokinins are usually used in 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 (Lin et al., 1997; Nhut, 2003; Fráguas et al., 2004; Raj Poudel et al., 2005). The study by Raj Poudel et al. (2005) on the effect of plant growth regulators on micropropagation of *Vitis ficifolia* showed that for shoot proliferation, BA at 5.0 µM concentration resulted in the longest shoots. Shoot numbers were significantly higher at higher concentrations (5.0 and 10.00 µM) of BA. Also, study of Hepaksoy and Aksoy (2006) on micropropagation of *Ficus carica* L. revealed that the longest shoot was obtained in the medium supplemented with 5 mg L^{-1} BA along with 1 mg L^{-1} IBA. This result is exactly in agreement with our findings. Increasing shoot length via higher concentrations of BA compared with IBA is due to decrease the apical dominant. Gumuscu et al. (2008) showed maximum shoot formation in *Neotchihatchewia isatida* with 1 and 2 mg L^{-1} BAP. In current study the highest rates of shoot production were obtained when shoot tips were cultured on the medium supplemented with 5 and 1 mg L^{-1} BA along with 1 mg L^{-1} IBA. In accordance with our finding, Hepaksoy and Aksoy (2006) showed that BA and IBA were unable to improve the multiplication rate. Best results were achieved on media containing 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 (Van Staden et al., 2008). Studies of Fuller and Fuller (1995) on the micropropagation of *Brassica* spp. showed that maximum shoot percentage (88.3%) was obtained in medium containing 2 mg L^{-1} IBA + 4 mg L^{-1} KIN. Rout et al. (1990) observed that the rate of growth in *Rosa* spp. was very poor in a hormone-free medium. Contrary to our findings, studies of Osuna et al. (2006) on micropropagation of *Lepidium virginicum* L. (belonging to Brassicaceae) showed that the maximum shoot length was obtained in MS medium without hormones. Study of Ahmadi Hesar et al. (2011) 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} kinetin. Studies of Kaviani et al. (2011) on micropropagation of *Matthiola incana* using NAA and KIN demonstrated the positive effect of plant growth regulators on shoot length and node number. These researchers showed that MS medium supplemented with 2 mg L^{-1} KIN without NAA resulted in the best shoot length (1.166 cm) and largest number of node (4.64). 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 explant was shown in the medium supplemented with 0.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA. The largest number of roots was obtained in the medium supplemented with 0 mg L^{-1} IBA + 1 mg L^{-1} NAA (9.71). The longest (8.75 cm) and thickest (4.3 cm) roots were achieved in the medium supplemented with 1 mg L^{-1} IBA + 1 mg L^{-1} NAA.

Our findings demonstrated that the addition of BA and IBA in culture media was effective for increasing the number of root and root length of *Matthiola incana*. Some studies showed the positive effect of auxins on rooting

(Gautam et al., 1983; Nobre et al., 1998; Hammaudeh et al., 1998; Lee-Epinosa et al., 2008; Jain and Ochatt, 2010). Rooting is a crucial step in successful micropropagation. Without effective root system, plant acclimatization will be difficult and the rate of plant propagation may be severely affected (Gonçalves et al., 1998). Current study showed the positive effect of IBA on root induction and root length. The largest number and the highest length of roots obtained in the medium containing 0.5 mg L^{-1} IBA without BA. Some studies showed the positive effect of cytokinins on rooting (Gomes et al., 2010). In agreement with our findings, root formation was inhibited in the medium culture of *Lilium longiflorum* Georgia containing BA (Han et al., 2004). Also, Fuller and Fuller (1995) observed maximum percentage of explants regeneration with root formation (65.0%) in *Brassica* spp. obtained in culture medium supplemented with 2 mg L^{-1} IBA without KIN. Studies of Gautam et al. (1983) on micropropagation of *Matthiola incana* by cotyledon explants revealed that a combination of auxin-cytokinin is antagonistic to the individual response of both and produced only a callus mass. Studies of Gomes et al. (2010) on *Arbutus unedo* L. showed that shoots produced in higher cytokinin-containing medium are more amenable to root induction than shoots obtained with the lowest concentrations of BA. A review of the literature clearly points out to a negative effect of cytokinins on shoot rooting (Van Staden et al., 2008), although a positive role has been occasionally referred to (Nemeth, 1979; Bennett et al., 1994). Studies of Isutsa (2004) on micropropagation of *Passiflora edulis* varieties showed that the shoots did not initiate roots in all IBA-augmented media but they initiated roots only in NAA-augmented medium. A study by Hashemabadi and Kaviani (2010) on micropropagation of *Aloe vera* L. using BA, IBA, and NAA showed the largest number of roots was obtained in the medium supplemented with 0 mg L^{-1} IBA + 1 mg L^{-1} NAA. The longest and thickest roots were achieved in the medium supplemented with 1 mg L^{-1} IBA + 1 mg L^{-1} NAA. Nobre et al. (1998) obtained the largest number of roots in the medium containing 2.5 µM IBA. Study of Raj Poudel et al. (2005) on micropropagation of *Vitis ficifolia* showed rooting frequency did not differ significantly with different concentrations of IBA, but root number increased significantly at higher concentrations (2.0 and 4.0 mg L^{-1}). Our studies demonstrated the positive effect of IBA at low concentrations on both root induction and root length. Our findings revealed that probably, the indigenous concentrations of IBA or other auxins are sufficient for root induction and growth.

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