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Matthiola incana Micropropagation Using Shoot Tips and Callus Induction Derived from Lamina Explants and Rooting Capacity from Callus

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Abstract

Tissue culture is an attractive alternative for plant propagation. Micropropagation is a technique to ensure a constant and uniform source of ornamental plants. *Matthiola incana* is an important ornamental species mainly cultivate by seed. *Matthiola incana* seeds were germinated on solid MS medium without plant growth regulators. Shoot proliferation and root formation are possible using kinetin (Kn) and naphthalene acetic acid (NAA). Shoot tips and leaf micro-cuttings derived from in vitro germinated seedlings were subcultured on solid MS medium containing Kn (0, 0.5, 1 and 2 mg l^{-1}) and NAA (0, 0.5, 1 and 2 mg l^{-1}) for shoot tips explants and Kn (0, 0.5 and 1 mg l^{-1}) and NAA (0, 0.5 and 1 mg l^{-1}) for leaf explants. Shoot tips media supplemented with 2 mg l^{-1} Kn without NAA and 2 mg l^{-1} NAA without Kn resulted in the best shoot length (1.20 cm) and root number (1.90), respectively. The callus was induced from most leaf media after four weeks of culture. MS mediums containing 0.5 mg l^{-1} Kn and 0.5 mg l^{-1}. The largest number (1.94) and the highest length (16.60 mm) of roots were obtained in MS medium supplemented with 1 mg l^{-1} Kn + 0.5 mg l^{-1} NAA. NAA prevented root formation originated from callus with concentration of 1 mg l^{-1} + 0.5 and 1 mg l^{-1} Kn.

Keywords: Brassicaceae, Organogenesis, Ornamental Plants, Phytohormones.

INTRODUCTION

The ornamental species *Matthiola incana*, belonging to Brassicaceae, is a pot plant. The Brassicaceae is a fairly large family with many economically important taxa, but from viewpoint of tissue culture, it has been little studied. 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 demand of the floriculture breeding 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). 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). The most frequently used growth regulators for micropropagation of ornamental plants by organogenesis, embryogenesis and axillary proliferation are naphthalenacetic acid (NAA), and benzyl adenine (BA) (Jain and Ochatt, 2010). Kn has been applied for micropropagation of many plants (Jain and Ochatt, 2010). In this paper, potential of shoot tips and leaf explants of *in vitro* grown *Matthiola incana* seedling to proliferation, and induction of callus and root by Kn and NAA has been discussed.

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 rinses with sterile distilled water (15 min each). Five seeds were cultivated in culture flasks on MS (Murashige and Skoog, 1962) basal medium without growth regulators. Micro-cuttings (shoot tips and leaves) were isolated from 4-week-old plants and cultivated on MS media supplemented with 0, 0.5, 1 and 2 mg l^{-1} Kn, and 0, 0.5, 1 and 2 mg l^{-1} NAA for shoot tips, also, 0, 0.5 and 1 mg l^{-1} Kn, and 0, 0.5 and 1 mg l^{-1} NAA for leaves. The media were adjusted to pH 5.7-5.8 and solidified with 7 g/L 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/m2/s with a photoperiod of 14 h per day. Some characters such as callus, fresh weight, number of root, and root length were calculated after 30 days. The experimental design was R.C.B.D. Each experiment was carried out in three replicates and each replicate includes five specimens. Data were subjected to ANOVA (analysis of variance) and significant differences between treatments means were determined by LSD test.

RESULTS AND DISCUSSION

The plant growth regulators are widely used for callus, rooting and shoot induction in tissue culture studies. Therefore, we studied the effect of Kn and NAA on shoot proliferation, callus production and rooting of *Matthiola incana*, an ornamental plant. The medium supplemented with 2 mg l^{-1} Kn without NAA resulted in the best shoot length (1.20 cm) (Table 1). Data analysis showed that the effect of Kn, NAA and Kn × NAA were significant on the length of shoot and ($p \leq 0.01$) (Table 2). When the shoot tips were inoculated in the medium containing 2 mg l^{-1} NAA without Kn, the best result was observed for root number (1.90) (Table 1). Analysis of variance showed that the effect of Kn was no significant on the root number, while the effect of NAA and Kn × NAA on the root number was significant ($p \leq 0.05$) (Table 2). Similar to our findings, many researchers showed that Kn induced multiple shoot formation (Sajina *et al.,* 1997b; Mathai *et al.,* 1997; Luo *et al.,* 2009). Some studies showed the positive effect of NAA on rooting (Gautam *et al.,* 1983; Hammaudeh *et al.,* 1998; Lee-Epinosa *et al.,* 2008). The results on leaf explants revealed that the largest number and highest length of root were obtained in MS basal medium containing 0.5 mg l^{-1}Kn + 1 mg l^{-1} NAA. Our data revealed that there are differences in the effect of the different concentrations of Kn and NAA on the root number and length. The most roots length (16.60 mm) and the most number of roots (1.94) were found when we used 0.5 mg l^{-1} Kn + 1 mg l^{-1} NAA (Table 3). This result was comparatively better than the growth of control. Data analysis showed that the effect of Kn and NAA was significant on the length and number of root ($p \leq 0.01$) (Table 4). Interaction effect of Kn and NAA was significant on the length and number of root ($p \leq 0.01$ and $p \leq 0.05$, respectively) (Table 4). The highest percent of callus induction (100%) was seen in explants grown in MS medium containing 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} Kn + 0.5 mg l^{-1} NAA (Table 3). Data analysis showed that the effect of Kn and NAA were significant on the callus formation ($p \leq 0.01$) (Table 2). The effect of Kn + mg l^{-1} NAA was no significant on the callus formation (Table 4). The most fresh weight between explants was obtained in explants grown in MS medium supplemented with 0.5 mg l^{-1} NAA (0.833 g) and 0.5 mg l^{-1} Kn + 1 NAA (0.817 g) (Table 3). Data analysis showed that the effect of Kn was significant on the fresh weight ($p \leq 0.01$) (Table 4). No the effect of NAA and Kn + NAA were significant on the fresh weight (Table 4).

In case of ornamental plants, leaf especially obtained from *in vitro* grown plantlets has more extensively been applied. We used from leaf explants taken from *in vitro* germinated seeds of *Matthila incana*. Many researchers applied leaves of ornamental plants as explants (Ibrahim and Debergh, 2000; Pati *et al.,* 2004; Tyagi *et al*., 2010; Godo *et al*., 2010; Eeckant *et al*., 2010; Radice, 2010). Organogenesis takes place either directly or after callus formation. Studies on many ornamental plants showed both kinds of organogenesis (Jain and Ochatt, 2010). There are many reports on organogenesis via callus formation (Pati *et al.,* 2010; Jain and Ochatt, 2010). Studies of Maira *et al.,* (2010) on *Anthurium andreanum* Lind cv Rubrun revealed that the four-week-old in plants obtained from micro-cuttings, showed callus proliferation at the stem base. The development of plantlets was observed from callus tissue. *In vitro* leaf explants in *Rosa damascena* and some other ornamental plants were used for direct organogenesis (Leffering and Kok, 1990; Ibrahim and Debergh, 2001; Dubios and de Vries, 1995). Nencheva (2010) showed direct organogenesis from pedicel explants of Chrysanthemum. Cytokinins and auxins are usually known to promote the formation of callus and root in many excited and *in vitro* cultured organs (Jain and Ochatt, 2010). Proper type and concentration of these hormones are different for each species. We observed that callus was formed on the explants in many treatments. NAA did not stimulate much callus induction and root formation when it was applied alone (Table 3). Similar to our findings, many researchers showed that cytokinins and auxins induced callus induction and root formation in ornamental plants (Fuller and Fuller, 1995; Sangavai and Chellapandi, 2008; Hashemabadi and Kaviani, 2010; Dorion *et al.,* 2010; Pati *et al*., 2010; Ochatt *et al*., 2010; Jain and Ochatt, 2010). Callus induction and root formation was performed for most Rhododendron genotypes by indole-3-acetic acid (IAA), NAA, indole-3-butyric acid (IBA) and 2,4-Dichlorophenoxy acetic acid (2,4-D) (Eeckaut *et al.,* 2010). Rout *et al.,* (1990) observed that the addition of benzylaminopurine (BAP) (2.0-3.0 mg l^{-1}) as the only growth regulator in the culture medium resulted in feeble callusing at the cut ends of the explants and the shoot elongation was considerably slow.

Rooting is an important process in micropropagation. Without an effective root system, plant acclimatization will be difficult and the rate of plant propagation may be severely affected (Gomes *et al.,* 2010). The ideal concentrations of cytokinins and auxins differ from species to species and need to be established accurately to achieve the effective rates of multiplication (Gomes *et al.,* 2010). The most types of cytokinins and auxins applied for root formation on callus or organs are BA, Kn and IAA, and NAA, IBA and 2,4-D, respectively. Some studies showed the positive effect of cytokinins on rooting (Gomes *et al.*, 2010). A review of the literature clearly points out to a negative effect of cytokinins on shoot rooting (Van Staden, 2008), although a positive role has been occasionally referred (Nemeth, 1979; Bennett *et al.*, 1994). Studies of Godo *et al.,* (2010) and Wong and Bhalla (2010) on *Lysionotus pauciflorus* Maxim. and Scaevola, respectively, showed that the regenerated shoots rooted easily on medium without any plant growth regulators. Current study showed the positive effect of Kn and NAA on root formation. Contrary to our findings, root formation was inhibited in the medium culture of *Lilium longiflorum* Georgia containing BA (Han *et al.*, 2004). Nayak *et al.,* (2010) showed that the lowest rooting of *Bambusa arundinacea* was observed in medium without Kn. Fuller and Fuller (1995) demonstrated that the least and most percentage of explants regeneration with root percent (5.0% and 65.0%) in *Brassica* spp. obtained in culture medium without IBA and Kn, and 2 mg l^{-1} IBA without Kn, respectively. The studies of Gautam *et al.,* (1983) on *in vitro* regeneration of plantlets from somatic explants of *Matthiola incana* showed only a few shoots developed on explants reared on MS medium supplemented with 0.1 mg l^{-1} Kn. Also, NAA (1 and 4 mg l^{-1}) induced profuse rooting in explants. Nhut *et al.,* (2010) demonstrated adventitious shoots of *Begonia tuberous* can be rooted on MS medium supplemented with 0.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA. Root was induced on nodal segments of *Vanda teres* on medium containing 2 mg l^{-1} Kn + 0.5 mg l^{-1} NAA (Alam *et al*., 2010). Tyagi *et al.,* (2010) showed root induction at the cut ends of shoots obtained from leaf explants of *Crataeva adansonii* on MS basal medium devoid of growth regulators. Shoot cuttings induce roots on MS medium with 1 mg l^{-1} NAA in 4-5 weeks, and in *Dianthus caryophyllus* L. with NAA and IBA (Casas *et al*., 2010). IAA (0.5-1 mg l^{-1}) helped rooting in *Pelargonium* × *hortorum* (Dorion *et al.,* 2010). Studies of Ruffoni *et al.,* (2010) on *Myrtus communis* showed that rooting was better in medium containing IAA than control, BA and BA + IAA. Ochatt *et al.,* (2010) demonstrated that for rooting of *Lathyrus odoratus* L. micro-shoots, they are explanted onto medium with 0.5-1 mg l^{-1} NAA for 3 weeks.

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Tables

Table 1. Effect of different concentrations of Kn and NAA on the shoot length and root number of *Matthiola inca.*

Regulators (mg l⁻¹)	Traits	
	Shoot length (mm)	Root No.
0 Kn	8.46a	0.85a
0.5 Kn	6.58b	0.42a
1 Kn	7.37ab	0.75a
2 Kn	8.58a	0.81a
0 NAA	9.26a	0.50b
0.5 NAA	7.25b	0.50b
1 NAA	5.76c	0.76ab
2 NAA	8.72a	1.05a
0 Kn + 0 NAA	6.95c	0.36cd
0 Kn + 0.5 NAA	7.65b	0.25d
0 Kn + 1 NAA	9.45a	1.25ab
0 Kn + 2 NAA	9.48a	1.90a
0.5 Kn + 0 NAA	9.20a	0.56cd
0.5 Kn + 0.5 NAA	7.50c	0.38cd
0.5 Kn + 1 NAA	3.65h	0.45d
0.5 Kn + 2 NAA	6.50d	0.75bc
1 Kn + 0 NAA	8.92a	0.70cd
1 Kn + 0.5 NAA	5.85e	1.60ab
1 Kn + 1 NAA	4.75g	0.40d
1 Kn + 2 NAA	10.00a	0.75bc
2 Kn + 0 NAA	12.00a	0.45cd
2 Kn + 0.5 NAA	8.55b	0.20d
2 Kn + 1 NAA	5.28f	1.80a
2 Kn + 2 NAA	8.92a	0.85bc

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

Table 2. Analysis of variance (ANOVA) for the effect of different concentrations of Kn and NAA on the shoot length and root number of *Matthiola incana*

Source of variations	df	M.S.	
		Shoot length	Root No
Kn	3	0.174**	0.770ns
NAA	3	0.477**	1.120*
Kn × NAA	9	0.175**	2.470**
Error	64	0.03782	0.402
c.v.		25.18	9.8

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

Table 3. Effect of different concentrations of Kn and NAA on the root length and number, callugenesis percent and fresh weight of *Matthiola incana.*

Plant growth Regulators (mg l-1)	Traits			
	Root length (mm)	Root No.	Callugenesis (%)	Fresh weight (g)
0 Kn	7.00a	1.28a	74.12a	0.80a
0.5 Kn	8.12a	1.00a	50.17b	0.69a
1 Kn	1.38b	0.17b	29.17c	0.49b
0 NAA	2.54b	0.22b	14.88c	0.69a
0.5 NAA	5.68a	0.73a	85.00a	0.58a
1 NAA	7.80a	1.33a	57.00b	0.59a
0 Kn + 0 NAA	7.33d	0.81e	45.02e	0.76b
0.5 Kn + 0 NAA	1.08g	0.26h	7.11g	0.52e
1 Kn + 0 NAA	1.05g	0.30f	8.12g	0.47f
0 Kn + 0.5 NAA	2.07f	1.15c	100.00a	0.85a
0.5 Kn + 0.5 NAA	8.45c	1.10d	100.00a	0.66c
1 Kn + 0.5 NAA	9.07b	0.21i	55.71d	0.52d
0 Kn + 1 NAA	6.67e	1.30b	80.23b	0.67c
0.5 Kn + 1 NAA	16.60a	1.94a	55.93c	0.79a
1 Kn + 1 NAA	1.07g	0.32g	33.79f	0.39f

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

Table 4. Analysis of variance (ANOVA) for the effect of different concentrations of Kn and NAA on the root length and number, callugenesis percent and fresh weight of *Matthiola incana.*

Source of variations	df	M.S.			
		Fresh weight	Callus induction	Root No.	Root length
Kn	2	160.56**	3.22**	4601.59**	0.17**
NAA	2	55.88**	1.40**	11139.37**	0.03ns
Kn × NAA	4	63.68**	0.70*	510.44ns	0.03ns
Error	15	7.68	0.20	258.44	0.01
Total	23				
c.v.		50.40	59.04	30.04	22.46

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

