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Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*)

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Abstract

We present a simple and reliable strategy for micro-propagation of *Matthiola incana,* an ornamental plant, in the presence of the single growth regulator, kinetin, which enables the production of stock plants. *In vitro* single nodes of *Matthiola incana* were cultured in MS basal medium with different concentrations of kinetin to produce shoots and roots. Multiple shoots containing roots simultaneously obtained on MS medium only with supplementation of 0.5-2 mg/L kinetin. The best shoot length (11.72 mm) and the most number of nodes (4.64) were obtained when we used 2 mg/L of kinetin. The largest number (3.40) and the longest length of roots (54.0 mm) were achieved using 1 mg/L kinetin. Data analysis showed that the effect of kinetin was significant on the length of shoot and root, and the number of node and root.

Keywords: Cytokinin, *in vitro*, *Matthiola incana*, root induction, shoot multiplication.
Abbreviations: KIN-kinetin; MS-Murashige and Skoog.

Introduction

Matthiola incana (Brassicaceae) is an ornamental plant. The Brassicaceae is a fairly large family with many economically important taxa. Ornamental plants are produced mainly for their aesthetic value. The economic value of ornamental plants has significantly been increased worldwide and to be continued 8-10% annually (Jain and Ochatt, 2010). Traditionally, *Matthiola incana* is propagated via seed. 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). Plant growth regulators are the most important factors for successful plant regeneration. In tissue culture, cytokinins 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, mainly shoots (Peeters et al., 1991). Kinetin (KIN) has been applied for micropropagation of many ornamental plants (Jain and Ochatt, 2010). *In vitro* shoot proliferation and multiplication are largely based on media formulations containing cytokinins as a major plant growth regulators (Mamidala and Nanna, 2009; Hoque, 2010). Some reports indicated that the presence of cytokinin in the culture medium helped in the multiplication of shoots (Kumar et al., 2001). Rooting can be initiated by transferring the regenerated shoots to a medium containing only auxins. The best explants for micropropagation of most plants, without any intervening callus phase, are shoot tips and axillary buds (Pati et al., 2005; Hashemabadi and Kaviani, 2010). In general, the number of publications on different aspects of the culture of *Matthiola incana* with emphasis on somatic explants micropropagation is limited; (Gautam et al., 1983). This paper deals with the effects of KIN on shoot and root regeneration of *Matthiola incana.*

Results & discussion

The plant growth regulators are widely used for callus, shoot induction and rooting in tissue culture studies. Therefore, we studied the effect of KIN on regeneration of *Matthiola incana*, an important ornamental plant, and the results are presented in Table 1. The results revealed that the length of shoot and the number of node raised with increasing concentration of KIN. Our data revealed that there are differences in the effect of the different concentrations of KIN. The best shoot length (11.72 mm) and the most number of nodes (4.64) were obtained when we used 2 mg/L of KIN (Table 1 and Fig. 1e). This was comparatively higher rate compared with control. Data analysis showed that the effect of KIN was significant on the length of shoot and the number of node ($p \leq 0.05$ and $p \leq 0.01$, respectively)(Table 2). Cytokinins are usually known to make promotion the formation of buds in many *in vitro* cultured organs. Similar to our findings, many researchers showed that cytokinins induced multiple shoot formation and shoot length (Van Staden, 2008; Chawla, 2009; Jafari Najaf-Abadi and Hamidoghli, 2009; Tornero, 2009; Gomes et al., 2010; Hashemabadi and Kaviani, 2010). Studies of Fuller and Fuller, (1995) showed that the most and least percentage of

Table 1. Effect of different concentrations of kinetin on the shoot length, number of node, root length and number of root of *Matthiola incana*.

Cytokinin concentration (mg/L)	Traits			
	Shoot length (mm)	Node No.	Root No.	Root length (mm)
0	7.08 [b]	2.20 [c]	0.46 [b]	6.8 [b]
0.5	9.16 [ab]	3.61 [b]	2.80 [a]	36.0 [ab]
1	8.92 [ab]	3.64 [b]	3.40 [a]	54.0 [a]
2	11.72 [a]	4.64 [a]	2.20 [ab]	40.0 [a]

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

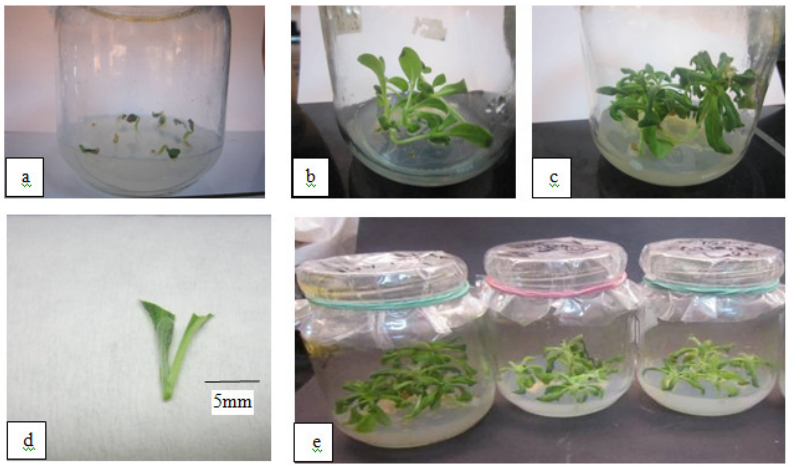

Fig 1. (a-e) Process of culture, growth and proliferation of *Matthiola incana* through *in vitro* culture of seeds. (a) Culture and germination of seeds on MS basal medium without plant growth hormones. (b) Development of plantlets from seeds cultured on MS basal medium without plant growth hormones after 4 weeks. (c) Development of plantlets from seeds cultured on MS basal medium without plant growth hormones after 5 weeks. (d) A single node for proliferation. (e) Shoot and root induction from single node segments after 4 weeks of culture on MS basal medium containing 0.5, 1 and 2 mg/L KIN (right to left).

Table 2. Analysis of variance (ANOVA) for the effect of different concentration of kinetin on the shoot length, number of node, root length and number of root of *Matthiola incana*.

Source of variations	df	M.S.			
		Shoot length	Node No.	Root No.	Root length
Kinetin concentration	3	18.20*	5.15**	8.04*	19.66*
Error	12	2.39	0.26	0.21	0.60
Total	19				
c.v.		16.78	15.20	21.09	22.76

**: Significant at α = 1% , *: Significant at α = 5%

explant regeneration of *Brassica* spp. (88.3% and 8.3%) obtained in medium containing 2 mg/L IBA + 4 mg/L KIN and, without growth regulators, respectively. Tatari Vernosefadrani et al. (2009) used different growth regulators on micropropagation of *Gerbera jamesonii* and showed that the most proliferation and plantlets length obtained in medium containing 2 mg/L KIN. Studies on *Bambusa arundinacea* showed that the highest multiplication can be obtained in medium without KIN (Nayak et al., 2010). However, Rout et al. (1990) observed that the rate of growth in *Rosa* spp. to be very poor in a hormone-free medium. The ideal concentrations of cytokinins differ from species to species and need to be estimated accurately to achieve the effective rates of multiplication (Gomes et al., 2010). We observed that no callus was formed on explants in controls (without plant regulators). Rout et al. (1990) observed that addition of BAP (2.0-3.0 mg/L) as growth regulator in the culture medium resulted in weak callusing at the cut ends of the explants and the shoot elongation was considerably slow. In the current study, the largest number and the longest length of roots (3.40 and 54.0 mm, respectively) were obtained using 1 mg/L KIN (Table 1 and Fig. 1e). Data analysis showed that the effect of KIN was significant on the number and length of root ($p \leq 0.05$) (Table 2). Contrary to our findings, root formation was inhibited in the medium culture of *Lilium longiflorum* Georgia containing BA (Han et al., 2004). Moreover, the lowest rooting of *Bambusa arundinacea* was observed in medium without KIN (Nayak et al., 2010). Fuller and Fuller, (1995) demonstrated that the least and most percentage of explants regeneration (5.0% and 65.0%) in *Brassica* spp. obtained in culture medium without IBA and KIN, and 2 mg/L IBA without KIN, 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 et al., 2008), although a positive role has

been occasionally referred (Bennett et al., 1994). Krishnamurthy et al. (2001) showed that the longest root was obtained with application of 0.5 mg/L BAP in *Polianthes tuberosa* L. The studies of Gautam et al. (1983) on *in vitro* regeneration of *Matthiola incana* showed that only a few shoots developed on explants on MS medium supplemented with 0.1 mg/L KIN. Also, NAA (1 and 4 mg/L) induced profused rooting in explants. In conclusion, type and concentration of growth regulators and species (genotype) are the most important factors in plant micropropagation. Fewer studies have shown the positive effect of a single growth regulator on micropropagation of different plant. Current study showed the positive effect of a single growth regulator, KIN, on micropropagation of *Matthiola incana*.

Materials & 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 placed on the culture flasks containing MS basal medium (Murashige and Skoog, 1962) without growth regulators (Fig. 1a). Seeds were germinated aseptically on MS medium (Fig. 1a, b and c). Micro-cuttings (single nodes) (Fig. 1d) were isolated from 5-week-old plants (Fig. 1c) and after removing the extra leaves, they were placed on MS medium supplemented with 0.5, 1 and 2 mg/L KIN (Fig. 1e). The media were adjusted to pH 5.7-5.8 and solidified with 7 g/L Agar-agar. The pH was adjusted before autoclaving at 121°C, 1 atm. for 20 min. The cultures were incubated in growth chamber that environmental conditions were adjusted to 25±2°C and 75-80% relative humidity, under a photosynthetic photon density flux of 50 µmol/m^2/s with a photoperiod of 14 h light. Some characters such as shoot length, number of node, number of root, and root length were calculated after 30 days. The experimental design was RCBD. Each experiment was carried out in five replicates and each replicate contained five specimens (totally; 25 specimens for each treatment). Data were subjected to ANOVA (analysis of variance) and significant differences between treatments means were determined by LSD test.

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