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Effect of Spermidin and Cytokinin on *in vitro* Induction Flowering & Micropropagation of Rosa sp. ('Dolsevita') 2014-2015

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ABSTRACT: In vitro flowering of rose, auxins and cytokinins can play a very important role in rapid multiplication of roses with desirable traits and production of healthy plants. The aim of this study was to investigate the performance of some plant growth hormones on the growth characteristics of rosa sp.cv. 'Dolcevita' in two plantation periods. Dolcevita were cultured on Murashige and skoog medium (MS medium) supplemented with different concentrations and combinations of Naphthylacetic acid (NAA) (0.1 ppm), Spermidine (Spd) (2 mM) and benzyladenine (BA) and Kinetin (KN) (0.5, 1.5 and 2.5 ppm). The experiments in an in-vitro condition were carried in a completely randomized design with 3 replications. The results indicated that at first plantation period, number of shoots was significantly higher in 2.5ppm KN+0.1ppm NAA compared with other treatments. Total leave were significantly higher in 0.5ppm BA+0.1ppm NAA than other treatments. The 0.5ppm KN + 1.5 or 2.5 ppm BA + 0.1ppm NAA treatment had lowest of yellow leave content among treatments. At second plantation period, number of shoots was higher in 1.5ppm KN + 2.5ppm BA + 0.1ppm NAA than other treatments. The 1.5ppm KN + 2.5ppm BA + 2mM spd + 0.1ppm NAA treatment had highest and lowest of total and yellow leave content among treatments, respectively. The highest of number of root and root length in 2.5ppm KN + 2mM spd + 0.1ppm were observed. Floral bud was produced only in 1.5ppm KN + 2mM spd + 0.1ppm NAA at second plantation period.

Key words: Rose, spermidine, cytokinins, in vitro flowering, flower

INTRODUCTION

Roses are one of the world's most popular flowers (Castilon *et al.,* 2006). These plants are a group of important garden species of the genus Rosa, within the family Rosaceae Juss (Wu *et al.,* 2005). Rose is commonly propagated by asexual methods such as sucker, hardwood cutting, budding and grafting (Hudson *et al.,* 2002). However, these methods are very slow and generally associated with different problems (long production time and limitation of stock plant) (Skirvin *et al.,* 1990). Tissue culture methods were developed as a potential tool for mass and rapid propagation in some plant (Khan & Shaw, 1988). Auxin and cytokinins are two plant growth regulators that are associated with plant growth (Fishel, 2009). Auxins regulate many physiological processes which include cell division and elongation, apical dominance in shoot, root initiation, vascular tissue differentiation, fruit drop or retention, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting and vegetative growth, fruit ripening, flowering, and suppress the growth of side buds and stimulate root growth (Alan, 2003; Taiz and Zeiger, 2006). The most biologically active and the most practical auxins are Naphthalene Acetic Acid (NAA) and Indole-3-Butyric Acid (IBA) (Belendez, 2008). The most biologically active and the most practical auxins are Naphthalene Acetic Acid (NAA) (Belendez, 2008). Plant hormones both growth of pre-existing roots and adventitious root formation. In horticulture, NAA is usually used to stimulate root initiation when rooting cuttings of plants (Belendez, 2008). Kinetin (KN) is phyto-hormones involved in different developmental processes. KN can stimulate cell division, leave expansion, and chlorophyll synthesis (Zhao *et al.,* 2011). Cytokinins such as benzyladenine (BA), kinetin (KIN) are important plant hormones that regulate various processes of plant growth anddevelopment including cell division and differentiation, enhancement of leaf expansion, retention of chlorophyll, promote light induced formation of chlorophyll, lateral bud development, and regulation of sink/source relationships and nutrient mobilization (Taiz and Zeiger, 2006; Mazher *et al.,* 2011).

Mor *et al.* (1983) reported that BA was as effective like kinetin in delaying senescence of detached carnation petals. Spermidine (Spd) involves in regulatory processes such as promotion of growth, DNA replication, cell division and differentiation (Groppa et al., 2001). BA delayed carnation senescence (Cook *et al.,* 1985). There are many reports on in vitro propagation of roses. Also, the application of auxins and cytokinins to advance flowering in vitro is well documented in many plant species including roses. Nonetheless, information regarding the effects of auxins and cytokinins on in-vitro flowering of rosa sp. cv. 'Dolcevita'. is scarce. Therefore, the present study was conducted to evaluate the effect of some plant hormones (NAA, Spd, KN and BA) on growth properties of rose sp. cv. 'Dolcevita'.

MATERIALS AND METHODS

The experiments in an in-vitro condition were carried in a completely randomized design with some auxin, cytokinins and their combinations with 3 replications. The auxin consisting naphthylacetic acid (NAA) in combination with some such as kinetin (KN) and benzyladenine (BA) and also spermidine (Spd) were maintained in a growth room at 25 ± 1ºC with 16 height daily in two plantation period. The concentrations of applied hormones were 0.1 ppm for NAA, 2 mM for Spd, 0.5, 1.5 and 2.5 ppm for KN and BA. Also, a control treatment (without plant hormone) was investigated. Overall, total 18 hormone treatments were used.

Treatments to NAA, Spd, KN and BA that the combination was as follows:

1) No treatment as control
2) 0.1ppm NAA
3) 1.5ppm KN + 0.1ppm NAA
4) 2.5ppm KN + 0.1ppm NAA
5) 0.5ppm BA + 0.1ppm NAA
6) 1.5ppm BA+ 0.1ppm NAA
7) 0.5ppm KN + 1.5ppm BA + 0.1ppm NAA
8) 0.5ppm KN + 2.5ppm BA + 0.1ppm NAA
9) 1.5ppm KN + 1.5ppm BA + 0.1ppm NAA
10) 1.5ppm KN + 2.5ppm BA + 0.1ppm NAA
11) 1.5ppm KN + 2mM spd + 0.1ppm NAA
12) 2.5ppm KN + 2mM spd + 0.1ppm NAA
13) 0.5ppm BA + 2mM spd + 0.1ppm NAA
14) 1.5ppm BA + 2mM spd + 0.1ppm NAA
15) 0.5ppm KN + 1.5ppm BA + 2mM spd + 0.1ppm NAA
16) 0.5ppm KN + 2.5ppm BA + 2mM spd + 0.1ppm NAA
17) 1.5ppm KN + 1.5ppm BA + 2mM spd + 0.1ppm NAA
18) 1.5ppm KN + 2.5ppm BA + 2mM spd + 0.1ppm NAA

The shoots of rose (Dolcevita cv) were collected. The shoots were cut into 3-4 cm sections. These were placed in flax of detergent water. Then shoots were rinsed with tap water and dipped into 70% alcohol for one minute and rinsed with HClO4 and distilled water. Then shoots were rinsed with distilled water. The shoots were cut into 1 cm sections containing one or two buds and explanted on half strength MS proliferation medium. MS medium was included 0.7 % agar, 150 mg/L myo-inositol, 100 mg/L ascorbic acid, and 10 mg/L arginine. The pH of the medium was adjusted to 5.6 or 5.7 with HCl and NaOH before autoclaving. The medium was dispensed into culture jar and autoclaved at 121 oC at 1.2 atm for 20 minutes. The experimental medium for shoot regeneration consists of full strength MS macro and micro mineral elements and different concentrations of auxins and cytokinins. The jars placed in a culture room maintained at 23±2o C under white fluorescent light with 16 hours photoperiod. At the first week of October 2014, the following data were recorded: number of shoots, total leave, and yellow leave were determined.

Statistical analysis

The impacts of auxins and cytokinins on the morphogenesis of rose Dolcevita was carried out by one-way analysis of variance (ANOVA). Means were compared by Duncan test ($p<0.05$). Statistical procedures were performed using the software package SPSS 19 for Windows.

RESULTS AND DISCUSSION

A. Number of shoot

Results indicated that significant variation ($p<0.05$) in number of shoots at both first and second plantation periods (Table 1 and 2). The results showed that at first plantation period, number of shoots was greater in 2.5ppm KN+0.1ppm NAA treatment compared with other treatments (Table 1). At second plantation period, number of shoots was higher in 1.5ppm KN + 2.5ppm BA + 0.1ppm NAA, than other treatments, respectively (Table 2). Many research observed variable effects of some plant hormones on shoot regeneration in different varieties of rose.

Asadi *et al.* (2009) reported that the shoot number per explants increased as the BAP concentration increased but decreased as the NAA concentration increased. They found that highest number of shoots was produced in control of NAA and 3 ppm BAP. Also, Vijaya *et al.* (1991) reported that NAA was more effective in the production of multiple shoots. Khan *et al.* (2006) indicated that maximum bud sprouting, bud spread and shoots length in damask rose cuttings were recorded at 50 ppm NAA. Based on the results obtained by Jabbarzadeh and Khosh-Khui (2005), BA (2.5-3 ppm) in combination with a low rate of IBA was the most suitable treatment for in vitro multiplication of rose. However, Carelli and Echeverrigaray (2002) found that BA promotes a higher number of shoots compared with KIN. They reported the number of shoots increased with increasing concentration of BA. Based on results of these researchers, the association of 3 ppm of BA and 0.5 ppm of NAA was adopted in the following experiments. The combination of 1 ppm BA and 0.5 ppm NAA positively affected the multiplication of the Baronesse cultivar compared with 1 ppm BA, leading to an increase in the number of shoots. Other researchers have obtained different results for other roses with the combination of BA and NAA. Results of Kanchanapoom *et al*. (2010) revealed that 13.3 mM BA in combination with 9.3 mM kinetin gave the highest number of shoots. This may suggest that bud formation in this cultivar required cytokinins.

Table 1: **Effect of NAA, KN, spd and BA in MS medium on number of shoot, total leave and yellow leave of Dolcevita in first plantation period**.

Treatments	Shoot number	Total leave number	Yellow leave number
Control	1.25ab	12.00 c-g	3.75 c
0.1_{ppm} NAA	1.25ab	9.50 d-h	2.50 def
1.5_{ppm} KN + 0.1_{ppm} NAA	1.25ab	11.75 c-g	2.00 f
2.5_{ppm} KN + 0.1_{ppm} NAA	1.50 a	16.00 ab	0.75 g
0.5_{ppm} BA + 0.1_{ppm} NAA	1.25 ab	16.75 a	3.50 c
1.5_{ppm} BA+ 0.1_{ppm} NAA	1.25 ab	14.25 abc	0.75 g
0.5_{ppm} KN + 1.5_{ppm} BA + 0.1_{ppm} NAA	1.00 ab	12.50 b-e	0.00 h
0.5_{ppm} KN + 2.5_{ppm} BA + 0.1_{ppm} NAA	1.25ab	15.00 abc	0.00 h
1.5_{ppm} KN + 1.5_{ppm} BA + 0.1_{ppm} NAA	1.00ab	12.25 c-f	0.25 g
1.5_{ppm} KN + 2.5_{ppm} BA + 0.1_{ppm} NAA	1.00ab	13.50 abc	2.25 ef
1.5_{ppm} KN + 2_{mM}spd + 0.1_{ppm} NAA	0.75 b	6.00 h	1.75 f
2.5_{ppm} KN + 2_{mM}spd + 0.1_{ppm} NAA	1.00ab	8.50 gh	3.50 c
0.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.00ab	13.75 abc	5.75 b
1.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.00ab	9.25 e-h	3.00 cde
0.5_{ppm} KN + 1.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.00ab	11.25 c-g	0.75 g
0.5_{ppm} KN + 2.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	0.75 b	7.00 h	2.50 def
1.5_{ppm} KN + 1.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.00ab	8.75 fgh	3.25 cd
1.5_{ppm} KN + 2.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.00ab	13.00 bcd	8.25 a

Means within a column followed by the same letter are not significantly different at $p<0.05$ by Duncan test.

Table 2: **Effect of NAA, KN, spd and BA in MS medium on number of shoot, total leave and yellow leave of Dolcevita in second plantation period.**

Treatments	Shoot number	Total leave number	Yellow leave number
Control	0.89 j	11.98 ij	7.89gh
0.1_{ppm} NAA	1.11 hij	10.22 j	6.55 h
1.5_{ppm} KN + 0.1_{ppm} NAA	1.22 hij	11.44 ij	6.11 h
2.5_{ppm} KN + 0.1_{ppm} NAA	1.55 fgh	11.00 ij	6.89 h
0.5_{ppm} BA + 0.1_{ppm} NAA	1.78 efg	19.22 fgh	14.22bc
1.5_{ppm} BA+ 0.1_{ppm} NAA	1.89 ef	24.33 def	6.33 h
0.5_{ppm} KN + 1.5_{ppm} BA + 0.1_{ppm} NAA	2.66 bc	21.33 efg	11.66 c-f
0.5_{ppm} KN + 2.5_{ppm} BA + 0.1_{ppm} NAA	2.47 bcd	30.84 bc	13.55bcd
1.5_{ppm} KN + 1.5_{ppm} BA + 0.1_{ppm} NAA	2.77 b	27.22 cd	15.66 b
1.5_{ppm} KN + 2.5_{ppm} BA + 0.1_{ppm} NAA	3.33 a	25.89 cde	10.66ef
1.5_{ppm} KN + 2_{mM}spd + 0.1_{ppm} NAA	1.44 f-i	16.00 ghi	9.78fg
2.5_{ppm} KN + 2_{mM}spd + 0.1_{ppm} NAA	1.00 ij	13.77 hij	6.77 h
0.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.89 ef	27.33 cd	9.55fg
1.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.33 g-j	33.11 b	11.32def
0.5_{ppm} KN + 1.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	2.15 de	33.00 b	15.66 b
0.5_{ppm} KN + 2.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.33 g-j	16.00 ghi	12.66cde
1.5_{ppm} KN + 1.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	2.33 cd	35.33 ab	30.66 a
1.5_{ppm} KN + 2.5_{ppm} BA + 2_{mM}spd + 0.1_{ppm} NAA	1.82 ef	39.66 a	3.33 i

Means within a column followed by the same letter are not significantly different at $p<0.05$ by Duncan test.

B. Number of total leave

The height number total leaves were in applications of 0.5ppm BA+0.1ppm NAA treatment (Table 1). However, at second plantation period number of total leave was highest in 1.5ppm KN + 2.5ppm BA + 2mM spd + 0.1ppm NAA (Table 2). In both periods, the control produced the lower number of leaves than the most treatments. Khan et al. (2006) showed that the mean number of leaves per rose cuttings increased with increase in growth regulator concentration. Increase in leaf number may be due to their important impact on inducing strong rooting system by hormones thus enabling the cuttings to absorb more nutrients thereby producing more leaves (Stancato *et al.*, 2003).

C. Number of yellow leave

At first plantation period, number of yellow leave was lowest in the 0.5ppm KN + 1.5 or 2.5 ppm BA + 0.1ppm NAA treatment (Table 1). However, number of yellow leave was lowest in 1.5ppm KN + 2.5ppm BA + 2mM spd + 0.1ppm NAA at first plantation period (Table 2). Roy *et al.* (2004) reported that the number of roots and longest roots of rose explants was highest in 1 ppm Benzyl amino purine (BAP) and 0.5 ppm NAA. Also, Asadi *et al.* (2009) stated that rose cultivated on MS medium in vitro showed a highest number of shoots with 3 ppm (BAP) without NAA. They found that highest number of shoots was produced in control of NAA and 3 ppm BAP.

D. Number of root

Results showed that the highest of number of root in 2.5ppm KN + 2mM spd + 0.1ppm NAA was observed (Fig. 1). Many researchers have found variable impacts of cytokinin and auxin on shoot and root regeneration in different varieties of rose (Khosh-khui and Sink, 2008). Jabbarzadeh and Khosh-Khui (2005) stated that using different concentrations of abscisic acid (ABA) in combination with various concentrations of IAA, IBA and NAA, did not produce any roots of damask rose. Based on the results obtained of these researchers, the best regulator for rooting of shoots is 2.5 ppm 2,4-dichlorophenoxyacetic acid for two weeks in MS medium.

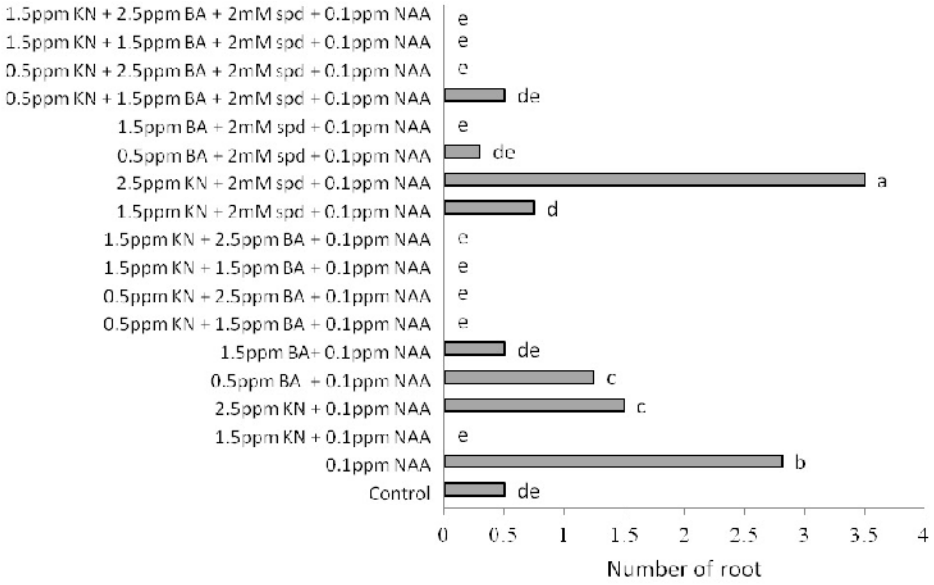

Fig. 1. Effect of NAA, KN, spd and BA in MS medium on number of root of Dolcevita in second plantation period

E. Root length

According to Fig. 2 the highest of root length in 2.5ppm KN + 2mM spd + 0.1ppm NAA was observed. In horticulture, auxins, especially NAA, are usually used to stimulate root initiation when rooting cuttings of plants. Nonetheless, high concentrations of NAA prevent root elongation and instead enhance adventitious root formation (Belendez, 2008). Shoot cuttings of many plant species, when dipped or coated with low concentration of auxins such as NAA, develop roots more quickly and in higher numbers (Khan *et al.*, 2006). Many studies have reported that exogenous application of auxins results in increased initiation of lateral roots and that lateral root development is highly dependent on auxin (Chhun *et al.*, 2003).

F. Floral bud

Floral bud was produced only in 1.5ppm KN + 2mM spd + 0.1ppm NAA at second plantation period (Fig. 3). These results are agreed with the findings of Kaur-Sawhney *et.al* (1988) who showed that using different concentrations of spermidine produce floral bud of tobacco. BA as a cytokinin has been used for most experiments on flowering in vitro of roses and other plants (Lin *et al.*, 2004; Taylor *et al.*, 2005; Vu *et al.*, 2006).

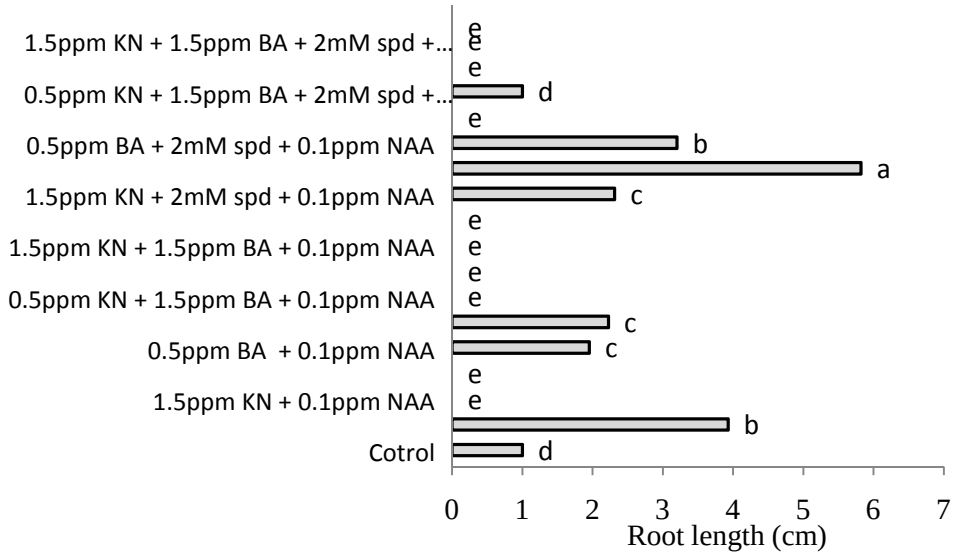

Fig. 2. Effect of NAA, KN, spd and BA in MS medium on root length of Dolcevita at second plantation period

Demeulemeester and DeProft (1999) demonstrated that the age of mother plants influences flower induction of chicory. However, Vu *et al.* (2006) revealed that the highest percentage of flowering of rose (hybrid tea) was obtained on MS medium complement with 3 ppm BA, 0.1 ppm NAA and 30 g l^{-1} sucrose. In another study, Wang *et al.* (2002) observed that the most efficient floral bud induction was procured on media complement with 0.5 ppm thidiazuron (TDZ) and 0.1 ppm NAA for cultivar Orange Parade. Vu *et al.* (2006) found cytokinin (BA, TDZ) and zeatin) increases the flowering percent and helps the normal development of floral buds. Wang *et al.* (1997) concluded that MS medium with BA (2 mg l-1) was the most effective on floral bud formation for *Dendrobium candidum*. The buds are smaller than normal size, however the flowers that formed in the Dolcevita was larger. Flowers was complete, and containing 5 petals and 5 sepals, average length of petals (1.7 cm), and the average length of the sepals (1.9 cm). Also, there was observed an average width of 1.18 and 0.26 cm, petals and sepals respectively. Vase life of flower formation in the second period has been approximately 100 days.

Fig. 3. The floral bud cultured on MS medium supplemented with 1.5ppm KN + 2mM spd + 0.1ppm NAA flowered in vitro after two subcultures.

CONCLUSIONS

Based on the results obtained in this study, 1.5ppm KN + 2mM spd + 0.1ppm NAA was the most suitable treatment for in vitro multiplication of Dolcevita rose. Floral bud was produced only in this treatment at second plantation period.

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