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EFFECT OF CYTOKININS ON *IN VITRO* SHOOT MULTIPLICATION OF ROSE

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ABSTRACT

Nodal explant of rose cvs. 'Bianca' and 'El Torro' were cultured in *in vitro* to determine the effectiveness of equimolar (8.87 µM) three different types of cytokinins on *in vitro* shoot multiplication. The MS medium supplemented with BA showed the best response for shoot multiplication and gained highest fresh weight per explant in both the cultivars. Although highest elongation of shoot as well as maximum number of leaves and highest percent of dry matter was obtained from MS medium supplemented with 2iP, but it failed to achieve good multiplication of shoot in both the cultivars. Among the three investigated cytokinins, kinetin had no effect on shoot multiplication in both the cultivars. Poor shoot multiplication with big leaves was obtained from cytokinin free MS medium. Regarding the rose cultivars, 'Bianca' showed higher response than 'El Torro'.

Key words: Cytokinin, *in vitro*, multiplication, rose

INTRODUCTION

Rose is one of the major cut flower in the world. Germany is the most important country in Europe for business of this attractive cut flower and it took the first place in the commerce of cut flower in 2014 (Anonymous, 2015). The cultivation area of roses in greenhouse in Germany was 361 ha in 2012, which is 28% of the greenhouse area in use for cultivation of floriculture crops (Anonymous, 2013). The majority of ornamental roses are heterozygous and do not breed true to type. Therefore, rose is propagated asexually by cutting, budding and grafting. But these methods are time-consuming and laborious with very low percentage of success and also have risk of spreading viral and bacterial diseases (Schneider, 2005). In contrast, *in vitro* has been considered as an efficient tool for mass production of true to type and pathogen free rose round the year. Furthermore, these plants are more compact, branch better and sometimes produce more flowers (Patie*t al.*, 2006). Success of *in vitro* propagation varies considerably with type of explant, culture media, genotype and type and concentration of plant growth regulators (Schneider, 2005). Cytokinin is one of the most important plant growth regulators that promote cell division and differentiation and their inclusions in the culture media is important for bud break and shoot multiplication (McGaw and Burch, 1995). Skirvin and Chu (1979) had published first report on micropropagation of rose, then a number of studies have been reported on shoot multiplication (Ibrahim and Debergh, 2001; Senapati and Rout, 2008; Kanchanapoom*et al.*, 2010) and cultivars (Marcelis-van Acker and Scholten, 1995; Carelli and Echeverrigaray, 2002; Kim *et al.*, 2003; Misra and Chakrabarty, 2009). However, single protocol is not much specifically for all cultivars of roses due to their poor shoot proliferation tendency. Therefore, the aim of the present study was to investigate the effect of cytokinins on shoot multiplication in two economically important rose cultivars.

MATERIALS AND METHODS

Plant material and explant preparation: Healthy and vigorous flowering shoots with the length of 20-25 cm were collected from the Greenhouse Laboratory Center, Dürnst of the TechnischeUniversitätMünchen in Freising, Germany. About 5-7 cm proximal and distal portion of each shoot was discarded and only the axillary buds from middle portion of stem were taken. After removing of leaflets and thorns, the shoots were cut into pieces of 3-4 cm length and each nodal segment bearing a quiescent axillary bud with a fragment of petiole. The explants were initially washed thoroughly running tap water for 10 min. The nodal segments were then surface sterilized with 70% (v/v) ethanol for 1 min and 1% (v/v) sodium hypochloride with few drops of Tween 20 for 15 min. Thereafter, the explants were rinsed in three times with sterile deionized water and distilled water, each time it required 5 minutes to remove all the traces of sodium hypochloride. Finally, the cut ends of the nodal

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segments were trimmed and about 1.5-2 cm long single-bud stem were used as explant.

Culture establishment: Modified Murashige and Skoog (MS) (Davies, 1980) containing 40 g L^{-1} of sucrose and 7 g L^{-1} agar were used in the present study. The pH of the medium was adjusted to 5.8 using 0.1 (N) NaOH and/or 0.1 (N) HCl prior to the addition of agar. The glass test tubes (150 × 25 mm) having 15 ml of media were autoclaved at 121ºC and 1.2 kg cm^{-2} of pressure for 15 min. After disinfection, the nodal segments were placed on the modified MS medium and afterward the culture tubes were incubated at 24 ± 1ºC and 70% relative humidity under 16 h photoperiod of 60 µmol m^{-2} s^{-1} light intensity maintained by cool white fluorescent tubes (OSRAM L 36W/32-930).

Treatments: Multiplication of shoots was observed in equimolar (8.87 µM) of three different types of cytokinins*viz.,* 6-Benzylaminopurine (BA) (Sigma-Aldrich, USA), 2-isopentenyladenine (2iP) (Sigma-Aldrich, USA) andKinetin (Duchefa, Biochemie BV, the Netherlands). Data on shoot multiplication and development were recorded after 5 weeks of culture initiation.

Percent dry weight of micro-shoots per explant: Dry weight of micro-shoots was determined after drying the samples at 65°C during 72 hours. Percentage of dry weight of micro-shoots per plantlet were calculated using the following formula.

% of dry weight = (Dry weight/Fresh weight) × 100

Statistical analyses: The experiment was arranged in a Complete Randomized Design (CRD). The experiment was replicated thrice and each treatment consisted of 15 culture tubes. Data were subjected to one-way analysis of variance (ANOVA) using the StatgraphicsPlus Version 2.1 statistical program (STSC, Inc., 1987). Treatment means were compared using the Fisher's Least Significant Difference (LSD) at $P \leq 0.05$ where the F value was significant.

RESULTS AND DISCUSSION

Number of micro-shoots per explant: Variation in the number of multiplied micro-shoots per explant was observed in the MS medium containing different types of cytokinins (Figure 1 and Table 1). The highest number of micro-shoots per explant was obtained in the MS medium supplemented with BA, in which the average number of micro-shoot was 3.78 in 'Bianca' and 3.31 in 'El Torro'. However, 2iP and kinetin had no significant effect on multiplication of micro-shoot and only 1 or rarely 2 micro-shoots were formed per explant in both the cultivars (Figure 1 and Table 1). The result revealed that inclusion of BA favoured multiplication of micro-shoot compared to 2iP and kinetin in both the cultivars. Similar response of BA also reported in various cultivars of roses by Hasegawa (1980), Marcelis-van Acker and Scholten (1995), Carelli and Echeverrigaray (2002), Kim *et al.* (2003) and Misra and Chakrabarty (2009). In contrast, some researchers observed maximum multiplication of micro-shoot from the MS medium supplemented with kinetin in the range of 1-2 mg L^{-1} than BA (Ibrahim and Debergh, 2001; Kanchanapoom*et al.*, 2010). Genotypical differences may be responsible for this contradictory result.

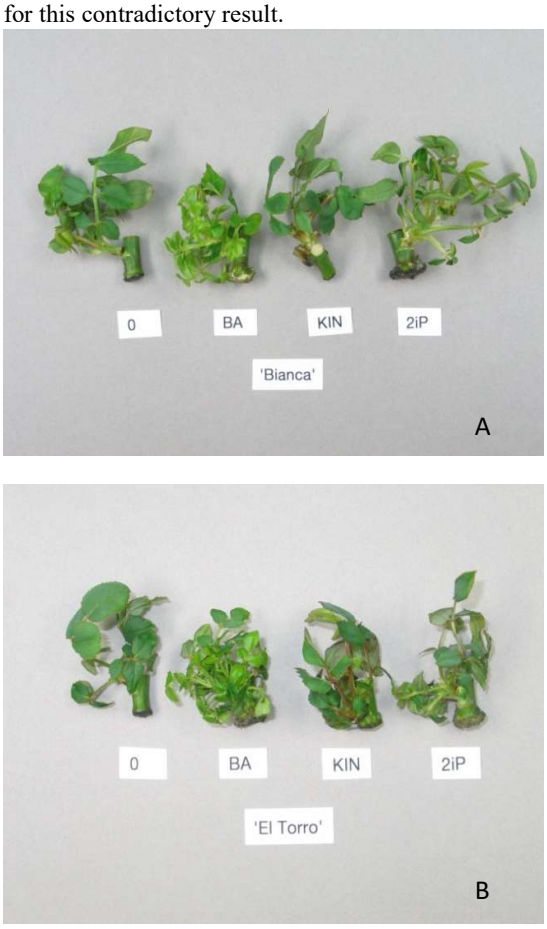

Figure 1. Development of plantlets of 'Bianca' (A) and 'El Torro'(B) in the multiplication phase as affected by the different types of cytokinins in the MS media.

Length of micro-shoot: The MS medium containing different types of cytokinins showed significant variation in the average length of new micro-shoot (Table 1). Comparing equimolar concentration (8.87 µM) of the cytokinins, the tallest micro-shoots was derived from the MS medium containing 2iP whereas, little elongation of micro-shoots was occurred in the medium presence of BA in both the cultivars (Table 1). The minimum elongation of micro-shoot in the MS medium supplemented with BA might be due to their higher multiplication that caused a reduction in the length of micro-shoot. The results are in agreement with those obtained in other rose cultivars by Skirvin and Chu (1979), Bressan*et al.* (1982) and Horn (1992). But Marcelis-van Acker and Scholten(1995) observedthat BA

Table 1. Influence of different types of cytokinins on various shoot growing parameters of 'Bianca' and 'El Torro'.

Type of cytokinins	Number of micro-shoot per explant	Length of micro-shoot (cm)	Number of leaves per micro-shoot
Bianca			
Control	1.04 c	1.04 b	4.96 b
BA	3.78 a	1.09 b	5.24 b
2iP	1.49 b	1.31 a	6.68 a
kinetin	1.27 b	0.94 c	4.32 c
Lsd (0.05)	0.24	0.08	0.29
El Torro			
Control	1.00 c	1.02 b	5.84 b
BA	3.31 a	0.84 c	6.00 b
2iP	1.33 b	1.16 a	7.60 a
kinetin	1.16 bc	0.76 d	5.00 c
Lsd (0.05)	0.18	0.06	0.44

In each column, means followed by the same letters are not significantly different according to Fisher's least significant difference test ($P \leq 0.05$).

Table 2. Influence of different types of cytokinins on various shoot growing parameters of 'Bianca' and 'El Torro'.

Type of cytokinins	Length of leaf (cm)	Fresh weight of micro-shoots per explant (cg)	% of dry weight of micro-shoots per explant (cg)
Bianca			
Control	5.51 a	35.67 c	12.25 b
BA	4.07 c	49.32 a	10.93 c
2iP	5.02 b	46.84 b	13.37 a
kinetin	5.12 b	31.12 d	10.03 d
Lsd (0.05)	0.18	1.63	0.46
El Torro			
Control	4.36 a	28.71 b	16.04 b
BA	3.19 d	37.83 a	15.83 b
2iP	3.43 c	28.24 b	18.57 a
kinetin	3.96 b	25.44 c	12.67 c
Lsd (0.05)	0.14	1.47	0.85

In each column, means followed by the same letters are not significantly different according to Fisher's least significant difference test ($P \leq 0.05$).

stimulated the length of micro-shoot in rose cv. 'Sweet Promise'. In the present study, it was also observed that kinetin were unable to elongate micro-shoot in both the cultivars and even the length of micro-shoots were shorter than the micro-shoots obtained from the MS medium without cytokinin (Table 1). This result is in contrary with the findings of Ibrahim and Debergh (2001) where highest elongation of micro-shoot was obtained from the MS medium with 1-2 mg L^{-1} of kinetin than BA, this may be due to the genotypic differences of rose cultivars.

Number of leaves per micro-shoot and length of leaf : Types of cytokinin significantly influenced the average number of leaves produced in the micro-shoot as well as average length of leaf (Table 1 and 2). The maximum number of leaves (in 'Bianca' 6.68 and in 'El Torro' 7.60) was formed in the MS medium containing 2-iP followed by BA, control and kinetin, respectively. Like other parameters, kinetin was also found ineffective in developing new leaves in both the cultivars. Regarding length of leaf, the presence of cytokinin in the medium significantly reduced the length of leaf in both the cultivars (Table 2). Hence, the largest leaf was developed by the free cytokinin MS medium. When the three cytokinins were evaluated then it was found that kinetin enhanced the length of leaf than that of 2-iP and BA. This result supported the findings of Jacob*et al.* (1969) who concluded that kinetin is important for leaf growth in hybrid tea rose cv 'Super Star'. But in another experiment Marcelis-van Acker and Scholten (1995) reported that kinetin had no effect on length and number of leaves of 'Montrea' cultivar of rose.

Fresh and percentage of dry weight of micro-shoots per explant: The fresh and percentage of dry weight of micro-shoots per explant of both the cultivars were significantly affected by the types of cytokinins (Table 2). Compare to 'El Torro', 'Bianca' produced higher fresh weight of micro-shoots per explant in all the treatments. It might be due to their higher response to cytokinin in multiplication phase. However, both cultivars gained maximum fresh weight in the MS medium presence of BA, while lowest fresh weight was achieved by the MS medium supplemented with kinetin (Table 2). Maximum multiplication of micro-shoot and moderate growth in the MS medium containing BA secured the highest fresh weight per explant in 'Bianca' (49.32 g) and 'El Torro' (37.83 g). This result is in accordance with the findings of Marcelis-van Acker and Scholten (1995) who compared equimolar (0.44µM) concentration of BA, kinetin and 2-iP and found highest fresh weight in BA containing MS media in rose cv. 'Sweet Promise'. But regarding the percentage of dry weight of micro-shoots per explant, MS media supplemented with 2-iP secured highest percentage of dry weight compared to BA and kinetin in both the cultivars (Table 2). Even though BA containing MS media produced highest fresh weight of micro-shoots per explant, but gave intermediary result in respect of percent of dry weight of micro-shoots per explant. In the present study, the explants grown on MS media containing BA showed some extent of hyperhydricity symptom but no hyperhydricity symptom was observed in 2-iP and kinetin (data not shown). The highest fresh weight of micro-shoots per explant in correlation with lower dry weight (%) of micro-shoots per explant might be due to the hyperhydricity characteristics and BA is known as inducing hyperhydricity (Sandal *et al.*, 2001).

CONCLUSION

From the above results, it is revealed that cytokinin is useful for establishing and multiplication of rose. Kinetin had virtually no effect on shoot multiplication even it inhibited the elongation of micro-shoot and leaves, and developing new leaves. Although 2-iP enhanced better elongation of micro-shoot but failed in multiplication of shoots which do not ensure rapid propagation of rose. On the other hand, the MS medium containing BA found more effective in multiplication of micro-shoot and also showed moderate vigorous growth. Based on these results, BA can be adopted for *in vitro* multiplication of shoot in rose.

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