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Influence of Explant Nodal Positions on the *In Vitro* Shoot Regeneration of Rose

Shreef Mahmood[1*] and Bernhard Hausera[2]
[1] Department of Horticulture, Faculty of Agriculture Hajee Mohammad Danesh Science and Technology University Dinajpur 5200, Bangladesh.
[1] Greenhouse Laboratory Center Technische Universität München Dürnast 3, D-85354 Freising, Germany.
[2] Hochschule Weihenstephan-Triesdorf Fakultät Gartenbau und Leben smittel Technologie Am Staudengarten 8 D-85354 Freising, Germany.

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*Corresponding author's email: shreefmahmood@yahoo.com

Abstract

The influence of explant nodal positions on the *in vitro* shoot growth and proliferation were studied in the two rose cvs. 'Bianca' and 'El Torro'. Third, fourth and fifth nodal explants were cultured on the modified MS medium supplemented with 1.0 and 5.0 mg/l BA. In both the cultivars, higher rate of proliferation ('Bianca' 3.75; 'El Torro' 2.65) were obtained from the explants distal to the apex than those of the proximal position with 5.0 mg/l BA. But proliferating shoots derived from the fifth nodal explant with 1.0 mg/l attained highest shoot length ('Bianca' 1.43 cm; 'El Torro' 1.19 cm) and produced higher number of leaves ('Bianca' 5.45; 'El Torro' 6.30) and fresh weight ('Bianca' 659.38 mg; 'El Torro' 255.95 mg) per explant than the third and fourth nodal explants. The fifth nodal explant with 1.0 mg/l BA was found the best treatment for the shoot regeneration of rose cvs. 'Bianca' and 'El Torro'.

Keywords: Node, Proliferation, *Rosa Hybrid* L., 6-benzylaminopurine,

INTRODUCTION

Rose is one of the most important florist crop grown all over the world. In Germany, the production area of roses in greenhouses is 124.2 hectares, which is 32.14% of the greenhouse area in use for cultivation of cut flowers (Anonymous, 2013). Commercial propagation of roses are usually done by cutting, although they can also be propagated by budding and grafting, which are difficult, undesirable and tedious processes (Horn, 1992). *In vitro* culture on the other hand, is an alternative method for propagation of a large number of pathogen-free plants in a short time with high uniformity. In Germany, it is the second most micropropagated species within the woody plants, with approximately 330,000 plantlets per year (Anonymous, 2009). In rose, the most commonly used explant is a nodal stem segment, where in the axillary bud is made to proliferate to form multiple shoots. The success of micropropagation of roses is involved with several factors: the composition of the medium used (Davies, 1980; Podwyszynska and Olszewski, 1995; Asadi *et al.,* 2009), cultural environment (Bressan *et al.,* 1982; Horn, 1992; Rout *et al.*, 1999; Carelli and Echeverrigaray, 2002) and genotype (Marcelis-van Acker and Scholton, 1995; Kim *et al.*, 2003; Misra and Chakrabarty, 2009). There are some other factors like the explant's position on the mother plant which have much less studied, but can be determinant in the success of micropropagation of roses. Hence, the present experiment was initiated with the aim to investigate the effects of explants nodal position on the shoot regeneration of rose cultivars.

MATERIALS AND METHODS

About 15-20 cm long young healthy flowering shoots of rose (*Rosa hybrid* L.) cvs. 'Bianca' and 'El Torro' were collected from the Greenhouse Laboratory Center, Technische Universität München, Freising, Germany. The top and basal axillary buds were discarded and only the axillary buds of the third, fourth and fifth nodal portions of the stem were taken. After removing leaves and thorns, the shoots were neatly cut into nodal pieces (3-4 cm long) each bearing one axillary bud with a fragment of petiole. The mean diameter of the third, fourth and fifth nodal explants were 4.85, 5.29 and 5.47 cm, respectively for 'Bianca' and 4.45, 4.81 and 5.06 cm, in that order for 'El Torro'. The explants were disinfected by immersing for 1 min. in 70% ethanol, afterwards for 15 min. in 1% sodium hypocholoride solution with some drops of Tween 20. The nodes were rinsed 3 times with sterile deionized distilled water and approximately 0.5 cm was trimmed from both the ends of each nodal segment to remove damaged tissues and used as the explant sources. About 1.5-1.8 cm long explants was planted vertically into 150 × 25 mm culture-tube containing 15 ml of the modified MS medium.

The modified MS medium (Davies, 1980) having 40 g/l of sucrose and 7 g/l agar was used in the proliferation phase. The pH of the medium was adjusted to 5.8 before agar was added. The medium was supplemented with 1.0 and 5.0 mg/l (equivalent to 4.43 and 22.17 µM) of 6-benzylaminopurine (BA). The test tubes containing the media were autoclaved at 1.2 kg cm^{-2} pressure and 121ºC temperature for 15 min. The instruments like scalpels, forceps, needles etc. were also pre-sterilized by autoclaving and subsequent sterilization was done by flaming and cooling method inside the laminar air-flow cabinet. The cabinet was usually started half an hour before using and wiped with 70% ethyl alcohol to reduce the chances of contamination. Hands were also sterilized by wiping with the mixture of 0.26% glycerine and 70% ethyl alcohol solution. The neck of the test tubes with the glass cap were flamed before opening and closing.

The culture tubes containing the explants were transferred to the growth room where the temperature was maintained at 24 ± 1ºC and 70% relative humidity under 16 h photoperiod. Artificial light was provided by parallel white fluorescent tubes installed above the culture. Photosynthetic photon flux density was 60 µmol m^{-2} s^{-1} at the plant level. The data had been taken after 5 weeks from the culture initiation when the new shoots are optimum to transfer into another madia for rooting although the new shoots started to grow after 25 days from the culture initiation. The

experiments were arranged in completely randomized design (CRD). In the experiments, treatments consisted of three explant nodal positions (third, fourth and fifth) and three different concentration of BA (control, 1.0 mg/l and 5.0 mg/l). These treatments were applied to the cvs. 'Bianca' and 'El Torro'. In all cases, each treatment combinations consisted of 30 culture tubes. A general ANOVA was conducted for all the variables using Statgraphics Plus Version 2.1 statistical program (STSC, 1987). The means were compared using Fisher's Least Significant Difference (LSD). All analyses were regarded as significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Number of shoot per explant

The rates of shoot proliferation in the both cultivars were significant due to the explant nodal positions cultured in the differents of BA (Table 1).

But no significant difference was observed among the explant nodal positions when those were grown in BA lacking MS medium. A gradual increase in number of shoots were observed with an increasing position of nodal explant in both the cultivars in different concentrations of BA (Table 1). However, the fifth nodal explant produced the highest number of shoots in different concentrations of BA followed by the fourth and the third nodal explants, respectively (Table 1). Higher level of endogenous auxins presence in the nodal explant closest to the apex zone may inhibit the proliferation of shoot in *in vitro* conditions. A similar behavior was reported by Ara et al. (1997), where the second nodal explant was found to be best for multiple shoot regeneration than shoot tip grown in MS media supplemented with 1.0 mg/l BA. But a contradictory report of Hisa and Korban (1996) indicated that the higher shoot proliferation was possible from shoot tips than the lateral buds. In another report, Salehi and Khosh-Khui (1997) concluded that due to the nutritional factors particularly carbohydrate availability in the explant, the explants with larger in diameter and length were best for the shoot multiplication and development compared to the explants with lower diameter and length, which is in support of the present findings. However, Horn (1992) found that the size of the explant did not affect the proliferation rate of roses. It was observed that different nodal explants of both the cultivars produced about only one shoot from the control treatment (Table 1). The supplementation of BA in the culture media resulted in increasing number of shoots per explant for all the nodal explants and both 'Bianca' and 'El Torro' yielded the maximum number of shoots which were 3.70 and 2.35, respectively with 5.0 mg/l BA. The *in vitro* shoot proliferation is mainly based on medium containing cytoknins as the major plant growth regulator in stimulating shoot proliferation in roses (Vijaya *et al*., 1991).

Table 1. Effect of explant nodal positions on the number and length of shoots per explant of 'Bianca' and 'El Torro'

Explant nodal position	No. of shoots per explant			Length of main shoot (cm)		
	Control	1.0 mg/l BA	5.0 mg/l BA	Control	1.0 mg/l BA	5.0 mg/l BA
Bianca						
3rd	1.05 a	2.25 c	3.15 b	1.01 c	1.20 b	0.81 c
4th	1.20 a	2.55 b	3.40 b	1.22 b	1.37 a	0.96 b
5th	1.20 a	2.85 a	3.75 a	1.30 a	1.43 a	1.02 a
Lsd (0.05)	0.21	0.28	0.26	0.06	0.09	0.05
El Torro						
3rd	1.00	1.05 b	2.25 b	0.67 b	0.73 c	0.62 b
4th	1.00	1.40 a	2.30 b	0.94 a	0.92 b	0.59 b
5th	1.00	1.55 a	2.65 a	0.97 a	1.19 a	0.75 a
Lsd (0.05)	00	0.30	0.32	0.04	0.08	0.07

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

Table 2. Effect of explant nodal positions on the number and length of leaves of 'Bianca' and 'El Torro'.

Explant nodal position	No. of shoots per explant			Length of main shoot (cm)		
	Control	1.0 mg/l BA	5.0 mg/l BA	Control	1.0 mg/l BA	5.0 mg/l BA
Bianca						
3rd	4.35 a	4.75 b	4.25 b	5.59 b	3.82 b	2.95 b
4th	4.25 a	5.25 a	4.65 a	6.05 a	4.31 a	3.19 a
5th	4.50 a	5.45 a	4.70 a	6.16 a	4.41 a	3.24 a
Lsd (0.05)	0.30	0.38	0.33	0.19	0.15	0.13
El Torro						
3rd	4.05 b	4.95 c	4.75 b	3.96 c	2.97 c	2.48 b
4th	3.90 b	5.55 b	5.35 a	4.43 b	3.32 b	2.61 a
5th	4.60 a	6.30 a	5.75 a	4.56 a	3.56 a	2.68 a
Lsd (0.05)	0.35	0.44	0.46	0.11	0.10	0.08

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

Length of the shoot

The explant nodal positions significantly influenced the length of the shoot grown in the different concentrations of BA (Table 1). It was observed that the nodal explant furthest from the apex grew quickly and developed in the tallest shoots than the nodal explant closest to the apex. In both cultivars, the fifth nodal positions produced the tallest shoot followed by the fourth and the third nodal positions, respectively (Table 1). Possibly, the fifth nodal position with the large diameter had better nutrient translocation and, therefore, produced the tallest shoot. This result is in full agreement with the findings of Bressan *et al*. (1982), Kim *et al.* (2003) and Razavizadeh and Ehsanpour (2008) where they reported that lateral buds from midsection of a stem grew vigorously than those closest to the shoot-tips. It was also observed that the growth of the buds near the apex was slow while the fifth nodal explant developed very quickly. A similar finding was also observed by Ma *et al*. (1996) where the buds nearest to the apex exhibited the slowest rate of development and the best growth of explants was obtained from the fourth and the advanced nodal positions. However, inclusion of BA in the medium promoted the elongation of the shoot in both the cultivars but regenerated shoots failed to elongate when the explants were grown in the MS medium containing the higher concentration of BA (Table 1). BA is a strong cytoknin which depresses the length of the shoot by an increased number of axillary buds (Hameed *et al*., 2006; Waseem *et al.*, 2009) as all the nutrients are utilized for the formation of lateral shoots (Yakimova *et al.*, 2000). In both the cultivars, the highest lengths of the shoot was measured in 1.0 mg/l BA followed by the 5.0 mg/l and the BA lacking medium.

Number and length of leaves

The number and the length of the leaves varied significantly among the explant's nodal positions, except in 'Bianca' no significant variation was found in terms of number of leaves when those were grown on the medium without BA (Table 2).

The present results showed that the number of leaves increased with the advancement of nodal positions and simultaneously the length of the leaves also increased. The fifth nodal explant had significantly higher number of big leaves than the fourth nodal explant where the third nodal position, in turn, had the minimum number of smallest leaves in different concentrations of BA (Table 2). A possible explanation could be that the higher storage of carbohydrate in the fifth nodal explant induces for developing of new larger leaves in the plantlet. The results is in accordance with the findings of Hisa and Korban (1996) who observed that the explants derived from the distal nodes produced the higher number of leaves. However, the higher number of leaves in the furthest nodal explant from the apex increasing the chance of quick development of microshoots in the

Table 3. Effect of explant nodal positions on the fresh and percentage of dry weight of shoots per explant of 'Bianca' and 'El Torro'.

Explant nodal position	No. of shoots per explant			Length of main shoot (cm)		
	Control	1.0 mg/l BA	5.0 mg/l BA	Control	1.0 mg/l BA	5.0 mg/l BA
Bianca						
3rd	4.35 a	4.75 b	4.25 b	5.59 b	3.82 b	2.95 b
4th	4.25 a	5.25 a	4.65 a	6.05 a	4.31 a	3.19 a
5th	4.50 a	5.45 a	4.70 a	6.16 a	4.41 a	3.24 a
Lsd (0.05)	0.30	0.38	0.33	0.19	0.15	0.13
El Torro						
3rd	4.05 b	4.95 c	4.75 b	3.96 c	2.97 c	2.48 b
4th	3.90 b	5.55 b	5.35 a	4.43 b	3.32 b	2.61 a
5th	4.60 a	6.30 a	5.75 a	4.56 a	3.56 a	2.68 a
Lsd (0.05)	0.35	0.44	0.46	0.11	0.10	0.08

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

rose cultivars. In relation to the use of different levels of BA, the highest number of leaves was achieved from 1.0 mg/l BA followed by higher concentration of BA (5.0 mg/l) and also BA lacking medium. This result indicates that the inclusion of BA in the medium was synergistic for producing new leaves but the higher concentration (5.0 mg/l) had the adverse effect on the number of leaves in both the cultivars. An increasing trend in the length of leaf was observed with the advancement of nodal positions. However, the presence of BA in the medium suppressed the length of leaf in both the cultivars (Table 2). Davies (1980) also found a similar result where higher concentration of BA induced the smaller leaves and also reduced the number of leaves per explant.

Fresh and percent of dry weights per explant

The explant nodal positions significantly influenced the fresh and percent of dry weights per explant due to different concentrations of BA for cvs. 'Bianca' and 'El Torro' (Table 3).

Both the fresh and the percent of dry weight per explant increased with the advanced nodal position which indicated higher development of explant in the increasing positions of node (Table 3). Horn *et al.* (1988) reported that the fourth and the fifth nodal positions were superior regarding the growth and the shoot weight, which supports the present findings. The fifth nodal position of both the cultivars cultured in 1.0 mg/l BA secured the highest fresh weight per explant. It might be due to the cumulative effect of the tallest shoot and the maximum number of leaves produced by the same treatment (fifth nodal explant with 1.0 mg/l BA). The poor performance of nodes near the apex was possibly due to their less diameter and herbaceous nature (Ma *et al.,* 1996). However, fresh weight declined when the concentrations of BA were further increased. In relation to the percent of dry weight, better accumulation of dry matter per explant was observed from the control treatment whereas the supplementation of BA in the medium reduced the dry weight in both the cultivars (Table 3). The decrease in dry weight of explant with the increasing the BA concentration was caused, in part, probably by the occurrence of explant with hyperhydricity symptoms (data not shown), which consequently reduced the dry weight of explant. This is not surprising as higher concentrations of cytokinin are known to induce hyperhydricity in *in vitro* raised culture, which reduce the percentage of dry matter in explant. In the present study, 'Bianca' was more responsive in presence of BA in the medium than 'El Torro', which could be attributed to their genetic constituents. However, the fifth nodal explant with 1.0 mg/l BA performed better in the *in vitro* shoot regeneration of rose cvs. 'Bianca' and 'El Torro'.

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