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EFFECT OF GROWTH HORMONES ON SHOOT PROLIFERATION OF ROSE CULTIVARS

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Abstract

Roses are commonly propagated asexually but the conventional methods are tedious and time consuming with low percentage of success. Tissue culture technique has been proved as a potential tool for quick and mass propagation of several plant species. The research was conducted to explore the optimum levels of Kinetin, NAA, IAA and BA supplemented in MS medium for micropropagation of rose cvs. Queen Elizabeth and Angel Face. Maximum shoot initiation percentage (80%) was achieved with BAP 3.0 mgL^{-1} + Kinetin 3.0 mgL^{-1}. Shoot length was maximum (3.0 cm) on MS medium with IAA (3.0 mgL^{-1}) and BAP 0.3 mgL^{-1} BAP while on MS medium added with BAP 3.0 mgL^{-1} + Kinetin 3.0 mgL^{-1} shoots attained the length of 1.5 cm. It was also observed that young shoot tips as an experiment performed better than mature shoot tips. Overall performance of Queen Elizabeth was better than Angel Face cultivar.

Introduction

Rose (*Rosa hybrida* L.) is the most popular of the flowers because of its beauty and fragrance that is why it is rightly called the queen of flowers. The genus *Rosa contains* more than 1400 cultivars and 150 species (Gualt and synge, 1971), which are grown for rootstocks, curiosity value and striking floral display. Apart from its ornamental value, it is also used for the production of essential oil and vitamin C (Bose & Yadav, 1989, Marchant *et al.*, 1996). These characters make it an important cash crop.

Conventionally, it is propagated asexually through cuttings, budding or grafting scion cultivars on specific rootstocks in the particular seasons. These methods are laborious and time taking with very low percentage of success. It has also been observed that plants raised from these methods are infected with different diseases that affect flower production and quality, and ultimately their market value is decreased (Norton & Boe, 1998). In general cuttings of hybrid roses are difficult to root.

Tissue culture methods have been developed as a potential tool for rapid and mass propagation in number of plant species. The central concept of tissue culture is totipotency i.e., every living cell has the genetic information needed to develop into complete organism (Khan & Shaw, 1988). Thus, new avenues became an important alternative of conventional propagation procedures for the plants, especially those propagated vegetatively. Micropropagation offers not only quick propagation of plants, but also eliminates diseases and provides scope for development of new cultivars (Debergh & Read, 1990).

There are many reports on *in vitro* propagation of roses but the frequency of establishment was low and highly variable (Hasegawa, 1980; Khosh-Khui & Sink, 1982). Hence the present project was initiated to standardise the micropropagation system of some hybrid roses.

Materials and Methods

Shoot tips (2.0 cm long) were taken from young and mature branches of rose ***cvs.*** Queen Elizabeth and Angel Face. The excised young and mature shoot tips (0.5 cm long) were washed with distilled water. Both explants were sterilized with 70 % alcohol having 2-3 drops of Tween 20 per 100 ml of water. The explants were then immersed in sodium hypochlorite solution (0.25%) for 5 minutes followed by 3-4 rinses with autoclaved distilled water. Shoot tips were cultured individually in test tubes containing 10 ml of the MS media. Rose explants excrete phenolic compounds, which caused browning of media and mortality of explants. Therefore, activated charcoal (0.5 mgL^{-1}) was added to MS media to control browning.

MS medium (Murashige & Skoog, 1962) was used for the regeneration of shoots from the explants along with different growth regulators IAA, NAA, BAP and Kinetin (0, 0.3, 3 and 10 mgL^{-1}). After 8 weeks, the micro shoots were subcultured for rooting on MS medium supplemented with IAA alone and combination with BAP (0.3 mgL^{-1}). pH of medium was adjusted at 5.7-5.8 before autoclaving and media was autoclaved at 121^{o}C with a pressure of 1.5 kgm^{-2} for 20 minutes. Uniform culture conditions were maintained as 16-hour photoperiod and (25 ± 2) ^{o}C growth temperature.

Data were collected on shoot initiation, number of days to initiate shoot, number of shoot per plant, shoot length and percentage of rooted plants. The experiments were laid out in completely randomized design (CRD). Each treatment was replicated thrice and ten test tubes were used per replication. The data collected was analyzed and the means were compared by Least Significance Difference Test (Steel & Torrie, 1984).

Results and Discussion

The data presented in the Table 1 shows that IAA in combination with BAP and Kinetin presented variable results for shoot initiation. IAA (3.0 mgL^{-1}) in combination with BAP (3.0 mgL^{-1}) and Kinetin (3.0 mgL^{-1}) initiated 71.5% and 72.3% shoots, respectively, in both cultivars. Similarly higher shooting percentage was observed in mature shoots on same media combinations. The higher dose (10 mgL^{-1}) of both cytokinins (BAP and Kinetin) reduced the shoot initiation. As the response of varieties is concerned for shooting percentage, Queen Elizabeth initiated more shoots than *Angel Face* as shown in Table 1. Young shoot tip explants gave slightly higher number of shoots than mature shoot tips.

Different levels of IAA and NAA in combination with BAP showed that BAP (3.0 mgL^{-1}) initiated maximum shoots (76%). The lowest concentration 0.3 mgL^{-1} of IAA with combination of BAP initiated 74% shoots while the higher amount of IAA (10 mgL^{-1}) with combination of BAP initiated the lowest (31%) number of shoots. Similarly the higher concentrations of NAA with combination of BAP had the lowest (35%) shoots induction. Young shoot tips responded better for shoot proliferation than the mature shoot tip explants. It was observed that 62% shoots initiated from young shoot tip and 57% in mature shoot tip explants. These studies confirmed the findings of Hasegawa (1979) who reported that 3.0 mgL^{-1} BAP was important for shoot growth while Kinetin was also useful for initiation and growth of shoots. These results are in contrary with the studies of Jacobs *et al.,* (1969) who concluded that 4.0 mgL^{-1} Kinetin was important for leaf, growth in hybrid Tea rose cv. 'Super Star'. This variation might be attributed due to the varietal difference.

Table 1. Effect of auxin and cytokinin on shoot initiation percentage in rose *cvs.* Queen Elizabeth and Angel Face.

Treatments (mgL^{-1})	Young shoot		Means	Mature shoot		Means
	Queen Eizabeth	Angel Face		Queen Eizabeth	Angel Face	
IAA+BAP	43.3 i	42.1 i	42.7 d	42.8 d	42.7 d	42.7 d
3+0	50.5 h	57.8 g	54.2 c	53.0 h	55.3 gh	54.2 c
3+0. 3	71.5b cd	65.3 de	68.4 b	71.0 c	65.8 de	68.41 b
3+3	40.0 ijk	38.5 ijk	39.2 ef	42.0 i	36.5 jkl	39.2 ef
3+10						
IAA+Kinetin	42.5 i	40.8 ij	41.7 de	43.0 i	40.3 ij	41.7 de
3+0	67.3d ef	65.3 ef	66.3 b	62.0 ef	70.6 c	66.3 b
3+0. 3	72.3 abc	71.8 abcd	72.1 a	73.0 bc	71.2 c	72.1 a
3+3	35.0 kl	29.6 m	32.3 h	32.8 lm	31.8 lm	32.3 h
3+10						
BAP+IAA	77.0 ab	75.0 ab	76.0 a	80.0 a	70.2 cd	75.1 a
3+0	73.0a bc	64.8 ef	68.9 cd	74.8 bc	73.2 bc	74.0 a
3+0. 3	72.5 abc	39.1 ijk	55.8 g	72.5 c	64.8 e	68.6 b
3+3	37.0 jk	73.3 abc	55.2 g	41.5 i	34.7 kl	38.1 f
3+10						
BAP+NAA	76.3 ab	63.5 bc	69.9 b	77.5 ab	72.2 c	74.8 a
3+0	68.5 b	63.0 bc	65.7 de	72.0 c	59.5 fg	65.7 b
3+0. 3	37.0 jk	36.5 jkl	50.2 h	41.0 ij	32.5 lm	36.7 fg
3+3	36.0 jkl	32.0 lm	34.0 gh	38.5 ijk	29.5 m	33.7 gh
3+10	58.6	53.6		55.8	52.7	
CVS. Means						

Data presented in Table 2 indicates that IAA (3.0 mgL^{-1}) and BAP (3.0 mgL^{-1}) alone initiated shoots earlier. The increased concentration of BAP and IAA in combination took additional days to initiate shoots. The cultivar Queen Elizabeth took less time to initiate shoot than Angle Face both in young and mature shoot tip explants. Higher doses (10 mgL^{-1}) of Kinetin and NAA in combination with IAA and BAP took an additional day to initiate shoots. MS media supplemented with NAA (0.3, 3 and 10 mgL^{-1}) took maximum (8.5) days to initiate shoots in both cultivars. Mature shoot tip explant of Angel Face took additional time (9.0 days) than young shoot tip on the same media treatments. Similarly, IAA in combination with higher-level Kinetin (10 mgL^{-1}) took 8.7 days to initiate shoots with young shoot tip and 9.2 days for Angel Face with mature shoot tip explant. It was observed that there was no clear difference among the varieties for time to initiate shoots. However, mature shoot tips took an additional day to initiate shoots than young shoot tip explants. These results are not in alliance with Ara *et al.* (1997) who tested four types of explants; young and old shoot tips, and 1st and 2nd nodes from the apex, and reported that all types of explants took about 7-8 days to initiate shoots.

Table 2. Effect of auxin and cytokinin on days to initiate shoots in rose *cvs*. Queen Elizabeth and Angel Face.

Treatments (mgL^{-1})	Young shoot		Means	Mature shoot		Means
	Queen Elizabeth	Angel Face		Queen Elizabeth	Angel Face	
IAA+BAP						
3+0	7.5 bc	8.0 abc	7.7 bc	7.5 bc	8.0 ab	7.7 cde
3+0. 3	8.0 abc	8.0 abc	8.0 abc	7.5 cc	8.5 ab	8.0 bcd
3+3	8.0 abc	7.5 bc	7.7 bc	7.5 bc	8.5 ab	8.0 cde
3+10	7.5 bc	8.5 ab	8.0 abc	7.5 bc	8.5 ab	8.0 bcd
IAA+Kinetin						
3+0	7.0 c	8.5 ab	7.7 cde	7.5 bc	8.0 abc	7.7 cde
3+0. 3	8.0 abc	9.0 a	8.5 ab	7.5 bc	9.5 a	8.5 ab
3+3	7.5 bc	8.0 abc	7.7 bc	7.5 bc	8.0 abc	7.7 cde
3+10	8.7 a	8.0 abc	8.3 abc	7.5 bc	9.2 a	8.3 abc
BAP+IAA						
3+0	7.5 bc	7.5 bc	7.5 bc	7.5 bc	8.0 abc	7.7 dc
3+0. 3	9.0 a	8.0 abc	8.5 ab	8.0 ab	9.0 a	8.5 ab
3+3	7.5 bc	8.0 abc	7.7 bc	7.5 bc	8.0 abc	7.7 cde
3+10	9.0 a	8.5 ab	8.7 a	8.0 abc	9.5 a	8.7 a
BAP+NAA						
3+0	7.0 c	7.5 bc	7.2 bc	7.0 c	7.5 bc	7.2 e
3+0. 3	8.5 ab	8.5 ab	8.5 ab	8.0 abc	9.0 a	8.5 ab
3+3	8.5 ab	8.5 ab	8.5 ab	8.0 abc	9.0 a	8.5 ab
3+10	8.5 ab	8.5 ab	8.5 ab	8.0 abc	9.0 a	8.5 ab
CVS. Means	7.4	8.6		7.5	8.5	

MS medium supplemented with IAA (3.0 mgL^{-1}) induced maximum (3.0) shoots per explant in mature shoot tip of cv. Angel Face. Similarly, MS medium supplemented with BAP (3.0 mgL^{-1}) produced 2.0 shoots per explant as evident in Table 3. When auxin and cytokinin were added in combination, the results were unexpected and it could be observed that the presence of IAA and BAP in the media has no positive effect (Table 3). Higher concentrations of auxin and cytokinin had adverse effects on number of shoots per explant in both young and mature shoot tip of both cultivars. The data presented in Table 3 shows that BAP (3.0 mgL^{-1}) alone or with 0.3 and 3.0 mgL^{-1} IAA produced 2.0 shoots per plant in young shoot tip of both cultivars while in mature shoot tip produced 3.0 shoot per explant in cv. Queen Elizabeth. The BAP with higher concentration of IAA and NAA yielded single shoot per explant. The second group of media where BAP was used with IAA and NAA for shoot proliferation showed more shoots with Angel Face than Queen Elizabeth. Young shoot tip explants produced 1.6 shoots per explant than mature shoot tip explant (1.2 per explant). These results are supported by Hasegawa (1980) who reported that cultured shoot tips proliferated into multiple shoots on a basal medium supplemented with 3.0 mgL^{-1} IAA + 3.0 mgL^{-1} BAP. Our findings are also supported by Skirvin & Chu (1979) who reported that shoot tips of rose cv. 'Forever Yours' raised on MS medium supplemented with BAP 3.0 mgL^{-1} + NAA 0.1mgL^{-1} gave rise to multiple shoots.

Table 3. Effect of auxin and cytokinin on number of shoot per explant in rose *cvs*. Queen Elizabeth and Angel Face.

Treatments (mgL^{-1})	Young shoots		Means	Mature shoot		Means
	Queen Elizabeth	Angel Face		Queen Elizabeth	Angel Face	
IAA+BAP						
3+0	2.5 ab	2.0 bc	2.2 ab	1.5 cd	3.0 a	2.2 a
3+0. 3	2.5 ab	1.5 cd	2.0 bc	1.5 cd	2.5 ab	2.0 bc
3+3	2.5 ab	2.0 bc	2.2 ab	3.0 a	1.5 cd	2.2 ab
3+10	1.5 cd	1.5 cd	1.5 cd	2.0 bc	1.0 d	1.5 bcd
IAA+Kinetin						
3+0	2.0 bc	1.5 cd	1.7 bc	1.5 cd	2.0 bc	1.7 abc
3+0. 3	1.0 d	1.5 cd	1.2 cd	1.5 cd	1.0 d	1.2 cd
3+3	1.5 cd	1.5 cd	1.5 cd	2.0 bc	1.0 d	1.5 bcd
3+10	1.5 cd	1.5 cd	1.5 cd	2.0 bc	2.0 bc	2.0 ab
BAP+IAA						
3+0	2.0 bc	2.0 bc	2.0 bc	2.0 bc	1.2 cd	1.6 cd
3+0. 3	1.7 bc	1.5 cd	1.6 cd	2.0 bc	1.0 d	1.5 bcd
3+3	2.0 bc	1.0 d	1.5 cd	2.0 bc	1.0 d	1.5 bcd
3+10	1.0 d	1.0 d	1.0 d	1.0 d	2.0 bc	1.0 d
BAP+NAA						
3+0	2.0 bc	1.5 cd	1.7 bc	2.0 bc	1.5 cd	1.7 ab
3+0. 3	1.5 cd	1.0 d	1.2 d	1.5 cd	1.0 cd	1.2 bcd
3+3	1.0 d	1.0 d	1.0 d	1.0 d	1.0 d	1.0 d
3+10	1.3 cd	1.0 d	1.2 cd	1.0 d	1.3 cd	1.2 cd
CVS. Means	1.8	1.8		1.7	1.4	

It can be observed from Table 4 that growth regulators had least effect on the shoot length. Maximum shoot length (3.0 cm) was observed on higher concentration of IAA and BAP with shoot tip of Queen Elizabeth. It was observed that MS medium supplemented with IAA and BAP showed increased shoot length than on MS medium supplemented with BAP, NAA or IAA in combination with Kinetin (Table 4). These results confirm the findings of Hasegawa (1980) and Proft *et al*., (1985). Shoots obtained were subcultured on MS medium supplemented with 0.3 mgL^{-1} IAA alone or in combination with BAP (0.3 mgL^{-1}). Both media failed to induce roots from shoots. It needs further standardization of media. It can be concluded from results that auxin and cytokinins combination were the best for shoot proliferation. Young shoot tip perform better than mature shoot tips while overall performance of Queeen Elizabeth was good.

Table 4. Effect of auxin and cytokinin on shoot length (cm) in rose *cvs*. Queen Elizabeth and Angel Face.

Treatments (mgL^{-1})	Young shoot		Means	Mature shoot		Means
	Queen Elizabeth	Angel Face		Queen Elizabeth	Angel Face	
IAA+BAP						
3+0	2.5 ab	2.0 bcd	2.2 ab	1.5 cde	3.0 a	2.2 ab
3+0. 3	2.5 ab	1.5 cde	2.0 bcd	1.5 cde	2.5 ab	2.0 bcd
3+3	2.5 ab	2.0 bcd	2.2 ab	3.0 a	1.5 cde	2.2 ab
3+10	1.5 cde	1.5 cde	1.5 bcd	2.0 bcd	1.5 cde	1.7 abc
IAA+Kinetin						
3+0	2.0 bcd	1.5 cde	1.7 bcd	1.5 cde	2.0 bcd	1.7 abc
3+0. 3	1.0 de	1.5 cde	1.2 e	1.5 cde	1.0 de	1.2 cd
3+3	1.5 de	1.5 cde	1.5 cde	2.0 bcd	1.0 de	1.5 bcd
3+10	1.5 de	1.5 cde	1.5 cde	2.0 bcd	2.5 ab	2.0 ab
BAP+IAA						
3+0	2.0 bcd	2.0 bcd	2.0 bcd	2.0 bcd	1.2 de	1.6 bcd
3+0. 3	1.7 cde	1.5 cde	1.6 de	2.0 bcd	1.0 de	1.5 bcd
3+3	2.0 bcd	1.0 de	1.5 cde	2.0 bcd	1.0 de	1.5 bcd
3+10	1.0 de	1.0 de	1.0 de	1.0 de	2.0 bcd	1.5 bcd
BAP+NAA						
3+0	2.0 bcd	1.5 cde	1.7 bcd	2.0 bcd	1.5 cde	1.7 abc
3+0. 3	1.5 cde	1.0 de	1.2 de	1.5 cde	1.0 de	1.2 cd
3+3	1.0 de	1.0 de	1.0 de	1.0 de	1.0 de	1.0 d
3+10	1.33 e	1.0 de	1.16 e	1.0 de	1.3 e	1.2 cd
CVS. Means	1.8	1.8		1.6	1.7	

References

Ara, K.A., M.M. Hossain, M.A. Quasem, M. Ali and J.U. Ahmed. 1997. Micropropagation of rose: *Rosa sp. cv.* Peace. *Plant Tissue Cult*., 7(2): 135-142.

Bose, T.K. and L.P. Yadav. 1989. *Commercial Flowers*. First Ed. Naya Prokas, Calcutta, India. pp. 15-20.

Debergh, P.C. and P.E. Read. 1991. Micropropagation. pp. 1-13. In: *Micropropagation: Technology and Application.* (Ed.): P.C. Debergh and R.H. Zimmerman. Kluwer Acad. Publishers, London.

Gault, S.M. and P.M. Synge. 1971. *The Dictionary of Roses in Color*. Ebury Press and Michael Joseph, Hague. pp. 11.

Hasegawa, P.M. 1979. *In vitro* propagation of Rose. *HortScience*, 14(5): 610-612.

Hasegawa, P.M. 1980. Factors affecting shoot and root initiation from cultured Rose shoot tips. *J. Amer. Soc. Hort. Sci.*, 105(2): 216-220.

Jacobs, G., P. Allan and C.H. Bornman. 1969. Tissue culture studies on Rose: Use of shoot tip explants. i). Auxin and Cytokinin effects. ii). Cytokinin: Gibberllin effects. *Agroplantae* 1:179-187 (*Hort. Absts*., 41(4): 8488; 1971).

Khan, I.A. and J.J. Shaw. 1988. *Biotechnology in Agriculture*. Punjab. Agric. Res. Coordination Board Faisalabad, Pakistan. pp. 2.

Khosh-Khui, M. and K.C. Sink. 1982. Micropropagation of new and old world rose species. *HortScience*, 57(3): 315-319.

Murashige, T and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*., 15: 473-479.

Marchant, R., M.R. Davey, J.A. Lucas and B. Power. 1996. Somatic embryogenesis and plant regeneration in Floribunda rose (*Rosa hybrida* L.) *cvs*. Trumpeter and Gland Tidings. *Plant Sci*., 120: 95-105.

Norton, M.E. and A.A. Boe. 1982. *In vitro* propagation of ornamental Rosaceous plants. *HortScience*, 17: 190-191.

Proft, M.P., G.V. Broek and R.V. Dijck. 1985. Implication of the container- atmosphere during micropropagation of plants. *Antwerpen Belgium* 50(1): 129-132 (*Hort. Absts*., 55(10): 7908; 1985).

Skirvin, R.M. and M.C. Chu. 1979. Propagation of rose with tissue culture. *Illinois Research*, 21(2): 3 (*Hort. Absts*., 49 (12): 9538; 1979).

Steel, R.G.D. and J.H. Torrie. 1984. *Principles and Procedures of Statistics*. McGraw Hill Book Co. Inc., New York. pp. 336-354.

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