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IN VITRO PROPAGATION OF SOME ROSE CULTIVARS

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Abstract

Micropropagation of three rose cultivars West Paul, Masquered and Charles Malareen was achieved *in vitro*. A single shoot was induced from nodal segment explants when cultured on Murashige and Skoog's (1962) basal medium containing basic salt, vitamins, 30g /l sucrose and 9g/l bactoagar, supplemented with 0.5 mg/l to 5.0 mg/l BA in all the rose cultivars. For further multiplication, nodal segment excised from in vitro formed shoots were transferred to fresh medium of same concentrations of BA. The maximum number of shoots proliferation (80%) and highest length (2-3cm) and highest number of shoots per explant (7-9) were achieved on medium containing 5.0 mg/l BA in Rose species West Paul within 4-5 weeks. It was concluded that 5.0 mg /l BA was optimal concentration for the induction of a large number of shoots.

Microcuttings obtained from *in vitro* proliferated shoots were cultured on half strengths MS medium supplemented with IBA 0.5 to 4.0 mg/l alone or in combination to NAA of same concentrations. Maximum rooting (60-70-%) with 3-8 roots per shoot was obtained at 0.5 mg/l of each IBA and NAA in West Paul and Masquered rose cultivars and at 2.0 mg/l IBA alone in all rose cultivars after 4-6 weeks of culture period.

Keywords: Rose, Tissue Culture. Regeneration, Micro propagation.

Introduction

Rose is probably the most popular garden plant and is considered as the queen of the flowers. The flowers may be single or double in white, red, pink, orange and yellow colour. In field, the roses are commonly propagated asexually by budding and grafting. It has also been observed that plants thus raised are infected with different types of diseases, which affect flower production.

Tissue culture has recently opened new avenues of research and has become an important alternative of conventional propagation procedure for the plants especially those propagated vegetatively. The traditional multiplication of roses is very low, the potential of which may greatly be enhanced by micropropagation.

A great success has been achieved in multiplication of many economically important plants. The significant features of vegetative propagation have been reported on shoot tip and nodal segment multiplication of *R.hybrida* scion cultivars (Skirvin and Chu, 1979; Davies,1980). Improved Blaze (Hasegawa, 1980), new and old world rose (Khosk-khui and Sink, 1982), *Rosa hybrida* (Dubios *et al,* 1988)Madame Goorgen Delbased cv (Gosper *et al,* 1989); *Rosamini* (Scotti-Campos and Pais, 1990); *Rosa* cv (H-T) *Dr.Verhage* (Voyatzi *et al,* 1988), *Hybrid tea, floribunda,* and miniature rose (Alekhno and Vysotskii, 1986) and *Rosa hybrida* cv (Shagufta *et al,* 1993) budded roses (Brave *et al,* 1984) Rose sp. Judy Fisher (Rehman *et al,* 1996) Rose plant (Dobres and Williams, 1998) Rosa sp. Soraya (Kintzios *et al,* 1999) rose plant (Firoozabady and Robenson 1999) Iceberg and Yellow page (Soomro *et al,* 2001). However, there are no reports on in vitro propagation of West Paul, Masquered, Charles Malareen rose cultivars. In the present study, the experiments were conducted to establish micropropagation of nodal segment explants with respect to different concentrations of growth regulators.

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Materials and Methods

Theree rose cultivars, viz, West Paul, Masquered and Charles Malareen were collected from nursery of National Agricultural Research Center, Islamabad during the year 1995. The 5-8cm long twigs were taken from new growth flushes. The twigs were washed thoroughly under running tap water, and transferred immediately to tap water until surface sterilization. Before sterilization all the leaves were removed and then surface sterilized in 0.1% Mercuric Chloride for 3-4 minutes. The sterilized material was rinsed 4 -5 times with autoclaved distilled water to remove the traces of sterilant. Stem segments with single node were prepared aseptically and these excised explants were cultured on Murashige and Skoog's (1962) basal medium (MS) containing salts, vitamins, 3% sucrose and 0.9% bactoagar supplemented with different concentration of Benzyle adinine (BA) ranging from 0.5-5.0 mg/l. The pH of medium was adjusted to 5.8 before autoclaving. The medium was then autoclaved at 120 ^{0} C at 15 pound/sq. inch for 20 minutes (Meynell and Meynell, 1975).

The MS medium was used at full strength throughout the experimental work. One explant was inoculated in 100 ml flask containing 50 ml of appropriate medium in vertical position. The cultured flasks were incubated in growth room under 2000 Lux light intensity provided by a series of warm white fluorescent lamps at 25 ^{0} C ± 2 temperature and 18/6 hrs light / dark regime (photoperiod) controlled by automatic timer in growth room.

Data was taken for shoot multiplication on initial cultures after 4 weeks. The sub culturing of the shoots were made on fortnightly basis.

Results and Discussion

The micropropagation of explants was markedly influenced by the nature and combination of growth regulators. The explants resumed new growth either by proliferating callus or by proliferating shoots within three to four weeks in culture media. The present investigations reveal that nodal segments excised *in vitro* from three rose cultivars, *West Paul, Masquered* and *Charles Malareen* were cultured on modified Murashige and Skoog's (1962) basal medium supplemented with a series of different concentrations, 0.5 mg/l to 5.0 mg/l BA to induce the shoots. Almost all the concentrations of BA induced single shoot primordial in all rose cultivars from nodal segment explants withing 25 days of inoculation. Similarly, Borkowska *et al* (1988); Rehman *et al* (1992,1996) and Soomro *et al* (2001) reported the shoot initiation in medium containing BA. The best multiplication rate of shoots in this investigation was achieved in West Paul (65%), masquered (45%) and Charles Melareen (40 %) at concentration of 5.0 mg/l BA after 4 weeks.

The rate of multiplication of shoots for each cultivar varied in different subcultures. The concentrations of BA had significant effect on both the number of shoot multiplication and length of shoots (Table 1). The highest number of shoots per explant 4 – 9 was recorded over a period of 4 – 6 weeks on MS medium containing 5mg/l BA in *West Paul* than 3 – 7 in *Charles Malareen* and 3 – 6, *Masquered*, Hasegawa (1979) reported that multiplication of Improved Blaze was found over a range of 10mg/l BA. Similar finding were also reported by Vijaya *et al* (1991) in *Andhra red;* Alekhno *et al* (1986) in Hybrid tea, *Floribunda* and Miniature rose; Rehman *et al* (1996) in *Judy fisher,* Soomro *et al* (2001) in *Iceberg* and

Yellow page at 5mg/l BA. However, Kintzios *et al* (1999) reported formation of plantlets on medium containing 5.2 mg/l BA and 5.7 mg/l Indole - 3 - butylic acid (IAA) in combination in rose cultivar Soraya. Dobres and Williams (1998) reported shoot initiation by using cytokinin, auxin and gibbrellin from node segment in rose plant. The multiplication rate can be improved by subculturing the shoots produced *in vitro* on fresh medium with same concentration of B A.

An increase in shoot length (2–3cm) was also achieved by the addition of 5mg/l BA to the medium while other concentrations have been found to grow no more than 1 to 2.5 cm in length in all rose species. The variable response of explants at different concentrations of BA seems highly dependent on cultures used. Douglous *et al,* (1988), Short and Robert (1991) Rehman *et al,* (1996), Soomro *et al* (2001). BA was also found most superior cytokynin for the production of shoots in various other rose species Chu *et al* (1993), Scotti and Pais (1990) Roa *et al* (1991) Vijaya and Satyanarayana (1991).

The results of the present study showed that the rate of highest shoot proliferation in West Paul could be attributed to high concentration of BA which is 5 times more than the previous reports Shagufta *et al* (1993) in *Rosa hybrida;* Short *et al* (1981) in *Rosa arvensis* and *Rosa coopri* and Bjarnason *et al* (1985) in *Rosa tropica.*

The shoots regenerated in vitro of all three cultivars were excised aseptically and sub cultured on half strength MS medium supplemented with 0.5 to 4mg/l each of Nepthelene acetic acid (NAA) and IBA in combination or IBA alone with same concentrations. The maximum rooting (50-65%) with 3 - 8 roots per shoot with an increase in root length 2-3cm were obtained at 0.5 mg/l of each IBA and NAA in West paul and Masquered cultivars respectively after 4-6 weeks of culture period. All other concentrations showed less or no roots in these cultivars (Tab :II). The similar results were reported by Khush khui and Sink (1982); Hasegave (1979). However, when IBA was supplied in the medium the shoot explants produced maximum number of roots (60-70%) with 3-6 roots per shoot (Tab:III) at concentration 2.0mg/l IBA in all 3 cultivars after 3-4 weeks of culture period. The maximum number of roots 3-8 and increase in root length (3-3.2) was obtained at concentration 2mg/l IBA only in *West paul* after 4-6 weeks of culture period. Similarly Reman *et al* (1992,1996) in *Tajmahal* and in *Judy Fisher*, Soomre *et al* (2001) in *Iceberg* and *Yellow page*, reported formation of roots in IBA containing medium. However, Rehman *et al* (1992,1996) also reported better rooting in anther popular variety of rose, *Tajmahal* and in *Judy Fisher* by using IAA in culture medium.

Among auxins, IBA was found to be a superior hormone in root formation in various plant species. It was also observed that IBA at low concentration gave best rooting response while higher concentration inhibited the root formation. The roots produced were large, healthy and maximum in number.

The induction of maximum number of roots may be due to the fact that cambial activity involved in root initiation was stimulated by growth regulators (Digby and Wanegrman, 1965) in pea, while increase in root length may be due to the effect of IBA on metabolic translocation. Carbohydrate metabolism may be involved in the effect of auxin (Mc David *et al,* 1972).

Table 1: Effect of different concentrations of BAP on shoot induction and proliferation in West Paul. Masquered and Charles Malareen,Segments after 6 weeks.

Species	No.of explants	Hormonal conc: mg/l (BA)	% explants responded	NO of Shoots formed	Height of Shoots (cm)	Culture period (weeks)
West Paul	10	0.5	30	2-3	0.5-1.0	4 - 6
	10	1.0	30	2-3	0.7-1.0	4 - 6
	10	2.0	50	2-5	1.0-1.5	4 – 6
	10	3.0	55	2-5	1.0-2.0	4 – 6
	10	4.0	65	3-6	1.0-2.5	4 – 6
	10	5.0	80	4 -9	2 –3	4 – 6
Masquered	10	0.5	20	1-2	0.7-1.0	4 – 6
	10	1.0	20	1-2	0.5-1.0	4 – 6
	10	2.0	35	2-4	0.4-1.0	4 – 6
	10	3.0	40	2-4	0.5-1.5	4 – 6
	10	4.0	50	2-5	1-1.5	4 - 6
	10	5.0	60	4-6	1-2.5	4 – 6
Charles Malareen	10	0.5	25	2-3	0.5-1.0	4 – 6
	10	1.0	30	2-3	0.8-1.0	4 – 6
	10	2.0	40	2-4	1-1.5	4 – 6
	10	3.0	35	2-4	1-1.0	4 – 6
	10	4.0	50	2-6	1-2.0	4 – 6
	10	5.0	65	3-7	1-2.0	4 – 6

Table 2: Effect of IBA concentrations on root formation in West Pual. Masquered and Charles Malareen, shoots after 6 weeks.

Species	Hormonal conc: mg/l (NAA+ IBA)	% of Shoots rooted	No: of Roots /Shoots	Root Length (cm)	Culture Period (weeks)
West Paul	0.5+0.5	65	3-8	3-3.2	4-6
	1.0+1.0	44	3-7	2-2.7	4-6
	1.5+1.5	35	3-4	2-2.1	4-6
	2.0+2.0	20	1-2	0.5-2.1	4-6
	2.5+2.5	07	1-0	0.5-1	4-6
	3.0+3.0	00	00	00	4-6
	3.5+3.5	00	00	00	
	4.0+4.0	00	00	00	
Masquered	0.5+0.5	50	3-7	2-2.3	4-6
	1.0+1.0	38	3-5	1.5-2.1	4-6
	1.5+1.5	25	2-4	2-2.1	4-6
	2.0+2.0	17	1-2	1-1.7	4-6
	2.5+2.5	10	1-2	0.5-0.8	4-6
	3.0+3.0	02	00	00	4-6
	3.5+3.5	00	00	00	
	4.0+4.0	00	00	00	
Charles Malareen	0.5+0.5	08	1-3	1-0.9	4-6
	1.0+1.0	10	1-3	1-0.5	4-6
	1,5+1.5	05	1-2	1-0.5	4-6
	2.0+2.0	00	00	00	4-6
	2.5+2.5	00	00	00	4-6
	3.0+3.0	00	00	00	4-6
	3.5+3.5	00	00	00	
	4.5+4.5	00	00	00	

Table 3: Effect of IAB concentrations on root formation in West Pual. Masquered and Charles Malareen , shoots after 4 weeks .

Species	Hormonal conc: mg/l (IBA)	% of Shoots rooted	No:of Roots /Shoots	Root Length in cm	Culture Period
West Paul	0.5	20	1-3	1-1.5	4-6
	1.0	37	2-4	1-1.7	4-6
	1.5	55	3-5	2-2.5	4-6
	2.0	70	3-6	3-3.2	4-6
	2.5	25	2-3	1-2.0	4-6
	3.0	5	2-3	1-1.5	4-6
	3.5	00	00	00	
	4.0	00	00	00	
Masquered	0.5	25	1-3	0.5-1	4-6
	1.0	40	2-4	0.5-1.5	4-6
	1.5	58	2-5	1-2.5	4-6
	2.0	60	3-5	2-2.8	4-6
	2.5	30	2-3	1-1.9	4-6
	3.0	15	2-3	1-1.5	4-6
	3.5	00	00	00	
	4.0	00	00	00	
Charles Malareen	0.5	35	2-3	1-1.3	4-6
	1.0	36	2-3	1-1.5	4-6
	1.5	45	2-3	1-1.9	4-6
	2.0	52	2-4	1.7-2.5	4-6
	2.5	30	1-3	1.3-2	4-6
	3.0	12	1-2	1-1.2	4-6
	3.5	2	1-2	0 -.5	4-6
	4.0	00	00	00	4-6

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