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REGENERATION OF ROSES VIA NODAL SEGMENTS IN VITRO

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Abstract

Nodal segments excised from rose caltivers, Iceberg & Yellow Page, when cultured on MS medium supplemented with BAP at 2 mg/L to 5 mg/L induced shoots. The mini-cuttings taken from proliferated shoots in vitor were tested for there ability to produce adventitious roots. Rooting was achieved by sub-culturing the mini-cutting on ½ MS medium supplement with 0.5 mg/L to 2 mg/L each of NAA and IBA. It was also observed that auxin was most affective growth regulator in stimulating roots.

Key words: Indole butyric acid, 6-benzyle denine, nephthelene acetic acid regeneration- rose

Introduction

Rose a symbol of perfection and elegance also called as "the queen of flowers" is perennial plant and it is hot house flower of many colour. The application of tissue culture techniques to the regeneration and commercial propagation of rose cultivars is more recently developed and allowed rapid multiplication of new varieties. As lot of work has been done on roses in the world, but very little attention has been given to Pakistan to propagate roses through tissue culture techniques. Most of the tissue culture studies on rose cultivars have been directed mainly towards micro-propagation and production of virus free plants through callus cultures, shoot tips, nodal segments, lateral and axillary buds, leaf segments and petioles Scotti & pais, 1990; Damiano et al., 1992; Roa et al., 1991 Rehman et al., 1992; Arnold et al., 1992; Vijaya, 1991; curier & Damiana, 1985; Kunitake et al., 1993; and Chu et al., (1993) used liquid medium for rapid propagation of shoot culture in miniature rose , Leffering et al., 1990 obtained regeneration of Rosa hybrida via leaf segment, Villalobose,1986 obtained virus free plants from meristem culture of carnation , Mercier & Roggamans, 1985 used traits with leaf petiole and shoots tips. The present investigation described the ability of shoots proliferated in vitro to produce adventitious roots in rose cultivars, Iceberg & yellow page.

Material and Methods

The rose cultivars, Icebery & yellow page were obtained from nursery of national agricultural research center, Islamabad. The nodal segments after removal of leaves were immediately transferred to tap water until sterilization. Before sterilization the nodal segments re-cut to 1-1.5 cm pieces in length and were surface sterilized by immersion in 0.1% HgCl for 3-4 mins. The sterilized material rinsed 4-5 times with autoclaved distilled water to remove the traces of sterilant. The excised explants were cultured on Murashige & Skoog (1962) basal medium supplemented with different concentration of BAP along with 3% sucrose & 0.9% bactoager. MS medium was used at full and ½ strenth throughout the experimental work. The pH of the medium was adjusted to 5.8 with 1 N-N AOH or HCL before autoclaving. The medium was autoclaved at 120 C at 1.5 kg cm-1 pressure (Meynell & meynell 1975) for 20 mins. Hunderd ml capacity flasks each containing 50 ml of medium was used for shoot formation. The explants were cultured in an upright position. After inoculation, the cultured flasks were kept

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under 2000 lux light intensity provided by fluorescent lamps at 26+2 C^0 temperature and 18/6 L/D regime photo-period controlled by automatic timer in growth room. For obtaining the shoots, stem nodal segments were excised in 1-1.5 cm in length and cultured on MS medium with different concentrations of BAP. Root, formation was achieved by transferring the mini-cuttings of proliferate shoots in vitro to 1/2 strength MS medium supplemented with auxins NAA and IBA in 20x150 mm test tubes and incubated in growth room. The whole process was carried out under aseptic conditions.

Table 1: Effect of BAP on shoot induction in ICE Berg and yellow page nodal segments

No of explements cultured	Hormonal conc mg./L	Age of explants I.B Y.P	Culture Period (weeks)	Remarks
10	2.0 BAP	20 + 10	4	Single shoot formed
10	3.0 BAP	30 +25	4	Single shoot formed
10	4.0 BAP	35 +35	4	Multiple shoots formed
10	5.0 BAP	60 +40	4	Multiple shoots formed

Table 11. Effect of different concentrations of NAA and IBA on root formation in Ice Berg and yellow page mini cuttings taken form induction medium.

Species	Hormonal conc. Mg/L MAA	IBA	Culture period(weeks)	Remarks
Ice Berg	0.5	0.5	3	Single root formed from one point Large roots fromed from one point No rrot No root
	1.0	1.0	4	
	1.5	1.5	4	
	2.0	2.0	4	
Yellow page	0.5	0.5	4	Single root formed Many roots formed from different points No roots No roots
	1.0	1.0	4	
	1.5	1.5	4	
	2.0	2.0	4	

Results and Discussion

The results of this study showed that explants taken form rose cultivars, lceberg and Yellow Page were cultured in medium with several combinations of BAP greatly influenced the response of shoot induction (Table l). the optimum number of shoot formation 60% in lceberg and 40% in Yellow page was observed in 5 mg/L BAP after 3-4 weeks (Figure I and II). Chu et al. (1993) In miniature rose, Vijaya and Satyanara yana (1991). In Andhrared Scott and pais 1990 in cv. Rosa mini, also found shoot regeneration MS medium containing BAP In general It was observed that induction and multiplication of roses reported at high BAP concentrations. Rao et al. (1991) and Voyatzi et al. (1988) found shoot formation and multiplication at 4-8 mg/L BAP.

In the present studies it was also investigated the ability of shoots produced in vitro to induce the adventitious roots in both rose cultivars, when subcultured on

½ MS medium supplemented with 0.5 mg/L to 2 mg/L each of NAA and IBA. Only 0.5 mg/L NAA and 1 mg/L IBA successfully induced the adventitious roots in Iceberg and yellow page respectively after 3-4 weeks of culture and attaining a length of 1-2 cm (Figure III & IV) while other concentrations failed to induce the roots in both cultivars (Table II). An increase in length (2 cm) was obtained in concentrations 1 mg/L IBA in Iceberg. The similar results were reported by Curier et al. (1985) in rose cultivars, Burger et al. (1990) in Rosa hybrida, Chaudhary (1990) in Priyadarshimi. In the present studies it was also noted that auxins in small concentrations play an important role to induce the roots in in vitro proliferated shoots.

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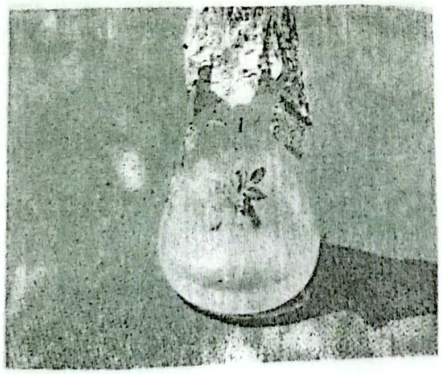


Fig. 1. Multiple shoot formed from Nodal Segment of Iceberg cultured on Full MS medium supplimented with 5.0 mg /L BAP after 4 weelss.

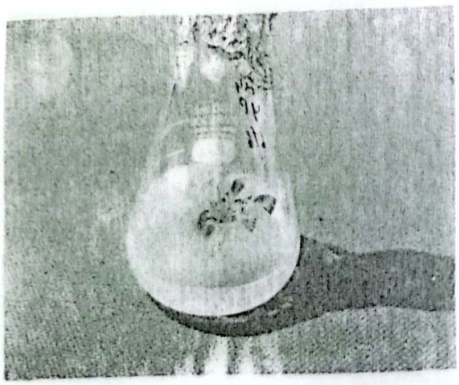


Fig. 2. Multiple shoot formed from Nodal Segments of yellow page cultured on Full MS medium supplemented with 5.0 mg/L BAP after 4 weeks.

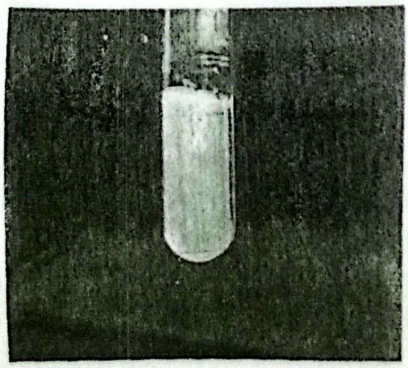


Fig.3. Root formation of shoot on ½ MS medium containing 1.0 mg/L NAA and IBA each with a culture period of 4 weeks.

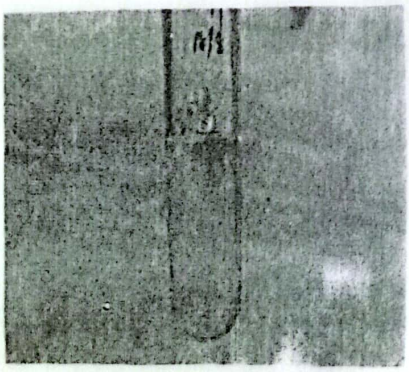


Fig.4. Multiple root formation of shoot on ½ MS medium containing 1.0 mg/L NAA and IBA each after 4 weeks of inoculation.