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Medium Engineering for Micropropagation of Rose (*Rosa hybrida* L.) cv. First Red

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ABSTRACT: In present study, the axillary buds were used to regenerate whole plant under *in vitro* conditions. Out of 23 media compositions supplemented with different combinations of plant growth regulators, only 10 medium concentrations responded and MS basal medium with BAP 4mg/l and GA3 3mg/l was best with 70% bud break after 5 weeks of initial culturing. Releases of phenols were checked through addition of ascorbic acid @ 200 mg/l in combination with PVP (100mg/l). Finally MS medium supplemented with TDZ (0.5mg/l) in addition to antioxidants, totally controlled yellowing & senescence. On an average 9 shoots were obtained on this medium with average shoot length of 2.37 cm after every four weeks. 22°C was the most congenial temperature for shoot multiplication. Stem tissue of about 2cm gave average eight shoots while only an average of 2 shoots were obtained when the size was 0.5cm. Additionally, it was observed that rate of bud break was much higher when explants were collected after 40 days of pinching than other wise. Significant differences were observed in the response of bud break from plants collected from middle of the side branch than the ones collected from lower & upper part of the branch. A maximum of 51% of the *in vitro* shoots regenerated roots on full strength MS medium supplemented with 1mg/l IBA 60% plantlets survived on cocopeat during hardening process.

Key words: Axiilary buds, *in vitro* propagation, Murashige and Skoog medium, shooting, rooting.

INTRODUCTION

Rose (*Rosa Hybrida* L.) belonging to family Rosaceae is one of the most important ornamental plant, which is used in cut flower industry. Rose blooms in a wide spectrum of colours, its demand as a cut flower is mainly due to use in decoration of commercial and domestic units. Rose water is used as perfume, and in medicines and confectionary (Narayananswami and Biswas, 1957).

Roses are cultivated by budding and grafting which is labour intensive, time consuming and above all, rate of propagation of rose through plant tissue culture will be an alternative of huge significance in floriculture industry. Most of the cultivars of rose are hybrids & their demand is ever increasing. The present investigations have been carried out on *in vitro* propagation of rose (*Rosa hybrida* L.) cv. First Red which is commercially viable due to its stem length, bright red colour and number of petals, which sometimes reaches fifty. It occupies first place in the International Auction centre, Alsameer, Netherlands. The protocol developed for *in vitro* propagation of cv. First Red will be helpful in meeting demands of floriculture industry for this cultivar. Propagation of rose varieties with increased rooting potential, improved rhizogenesis, faster varietal renewal and modification of flower colour are several commercial reasons behind the tissue culture of rose species all over the world.

Keeping in view the importance of roses, the present investigations were undertaken to develop an *in vitro* propagation protocol for rose cv. First Red, which is a highly prized variety due to its stem length, red colour and number of petals. In addition *in vitro* protocols can be utilized in modification of resistance to diseases, insects & pests.

MATERIALS AND METHOD

The source plant material of rose has been collected from the field of department of Floriculture and Landscaping, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan. Explants have been prepared by excising buds along with a slice of stem.

The thoroughly washed explants were treated with 0.2% bavistin for about 10 minutes and washed thoroughly with water, then treated with sodium hypochlorite for 20-30 minutes and subsequently washed with water for a total duration of 5 minutes. Then the sterilized explants were inoculated on MS medium both liquid and solid supplemented with various concentrations and combinations of different growth regulators i.e. BAP (0.25mg- 4mg/l), IAA (0.1mg- 2mg/l), GA3 (0.5-3mg/l) and TDZ was tried only one single concentration i.e. 0.5 mg/l as given in the Table 1.

Table 1: Establishment of cultures of rose cv. First Red on MS medium supplemented with different combinations of BAP, GA3, Kn, IAA, NAA.

Medium (MS)	MS basal/MS basal + combinations of growth regulators	Antioxidants		Percentage of bud break after 1 week of culturing	Percentage proliferation of explants after 4 weeks
		Ascorbic acid	PVP		
Liquid medium					
M1	MS basal	-		Explants turned brown within two hours.	-
M2	BAP 0.5 mg/l	-			-
M3	BAP 0.5 mg/l	200 mg/l	100 mg/l		-
M4	BAP 1.0 mg/l	200 mg/l	100 mg/l		-
M5	BAP 2.0 mg/l + IAA 0.2 mg/l	200 mg/l	100 mg/l		-
M6	BAP2.0 mg/l + GA3 0.5 mg/l + IAA 0.2 mg/l	200 mg/l	100 mg/l		-
M7	BAP 2.0 mg/l + GA3 0.5 mg/l + Kn 0.5 mg/l	200 mg/l	100 mg/l		-
M8	BAP 3.0 mg/l + Kn 0.5 mg/l	200 mg/l	100 mg/l		-
M9	BAP 3.0 mg/l + GA3 0.5 mg/l + Kn 0.5 mg/l	200 mg/l	100 mg/l		-
Solid medium					
M10	MS Basal	-	-	-	-
M11	BAP 0.5 mg/l	-	-	-	-
M12	BAP 0.5 mg/l	200 mg/l	100 mg/l	-	-
M13	BAP 1.0 mg/l	200 mg/l	100 mg/l	-	-
M14	BAP 2.0 mg/l + IAA 0.2 mg/l	200 mg/l	100 mg/l	5.1	-
M15	BAP 2.0 mg/l + GA3 0.5 mg/l + IAA 0.2 mg/l	200 mg/l	100 mg/l	16.6	60.0
M16	BAP 2.0 mg/l + GA3 0.5 mg/l + Kn 0.5 mg/l	200 mg/l	100 mg/l	17.0	60.0
M17	BAP 3.0 mg/l + Kn 0.5 mg/l	200 mg/l	100 mg/l	25.0	80.0
M18	BAP 3.0 mg/l + GA3 0.5 mg/l + NAA 0.25 mg/l	200 mg/l	100 mg/l	60.0	83.0
M19	BAP 3 mg/l + GA3 1 mg/l + Kn 0.5 mg/l	200 mg/l	100 mg/l	66.6	80.0
M20	BAP 4 mg/l + GA2 2.0 mg/l	200 mg/l	100 mg/l	67.0	85.0
M21	BAP 4 mg/l + GA3 3.0 mg/l	200 mg/l	100 mg/l	70.0	95.0
M22	BAP 4 mg/l + NAA 0.5 mg/l	200 mg/l	100 mg/l	60.0	83.0
M23	BAP 4 mg/l + Kn 3.0 mg/l	200 mg/l	100 mg/l	60.0	80.0
S.E.M.				1.55	2.23
C.D.0.05				4.41	6.36

Role of antioxidants, temperature, explant factors and iron source in the medium was studied upon success of the cultures. Well-developed shoots of about 3-5 cm length from lavishly multiplying shoot cultures were transferred singly to culture tubes having 15 ml of MS basal medium with 30% sucrose, 1mg/l IBA, pH adjusted at 5.8, autoclaved at 121°C for 15 min. After about four weeks on rooting medium and with fully developed root systems the plantlets were subjected to acclimatization. The plantlets were hardened separately on cocopeat as well as on a mixture of sand: soil: FYM (1:1:1) and maintained at 25 ± 10°C, 16/8 hr. photoperiod in 15 cm diameter pots. Finally the plants were transferred to nursery of floriculture plants.

Table 2: Effect of alternative medium composition on the rate of shoot multiplication.

Medium (MS basal)	Composition (GR)	Ascorbic acid	PVP	Remarks
MS + Fe EDDHA 96 mg/l	BAP 4 mg/l + GA3 3 mg/l	200 mg/l	100 mg/l	Died within 10 days
MS + $AgNO_3$ 15 mg/l	BAP 4 mg/l + GA3 3 mg/l	200 mg/l	100 mg/l	Died within 5 days
MS + $AgNO_3$ 25 mg/l	BAP 4 mg/l + GA3 3 mg/l	200 mg/l	100 mg/l	Died within 5-7 days
MS containing Ammonium Nitrate (8.25 g/l)	BAP 4 mg/l + GA3 3 mg/l	200 mg/l	100 mg/l	Died within 10 days
MS	TDZ 0.5 mg/l	200 mg/l	100 mg/l	100 % survival of subcultured shoots

RESULTS ANDDISCUSSION

In vitro propagation of rose, an axillary bud is successfully used as explants. After the preparation of explants for micro propagation, the most demanding task for successful establishment of cultures is the surface sterilization of explants. 95% of the uncontaminated buds were obtained after treating with 0.2% solution of bavistin and 10% solution of sodium hypochlorite for 30 minutes. Rose being a woody plant material needs more time duration and higher concentration of sterilants like other woody plant materials e.g. apple (Modgil *et al*. 1999), 'colt' Cherry (Sharma *et al*. 1992). MS Medium was found suitable for initial culture establishment and shoot multiplication; therefore no other basal medium was tried. BAP was superior for bud break and shoot multiplication then Kn. BAP in combination with low concentrations of NAA gave better results than BAP & Kn together due to the synergistic effect of NAA along with BAP on continuous cell division (Syamal & Singh, 1996; Ganga *et al*., 1998; Aryan and Rani, 2016)) with the increase in concentration of BAP led to an increase in percentage establishment of initial cultures of shoot multiplication. Addition of GA3 (3mg/l) to MS Medium containing BAP (0.5- 4.0mg/l) led to increase in the rate of culture establishment &*in vitro* shoot multiplication. Role of BAP, GA3, NAA in rose micro propagation at bud break and shoot multiplication phases has been studied by Sahoo & Debata (1997), Chaudhary and Prasad (1990), Rout *et al*. (1989), Singh & Syamal (1999). Contrarily, Marcellis –Van & Scholten (1995) reported that BAP at low concentration also works for micro propagation of rose. Shoot multiplication is the most crucial stage at which most of the failures occurs in *invitro* propagation of rose plants. Under present investigation 23 media combinations including liquid & solid based on MS medium, were tried. No liquid medium with any of the growth regulator combinations responded. Cultures died within few hours of culturing due to release of phenols in the medium as has been reported by Mahajan (1997) in strawberry similar results were obtained on MS solidified medium and axillary buds died within 2 days of initial culturing due to browning and did not sprout at all. Therefore, antioxidants PVP and ascorbic acid were added to MS medium then browning was checked. However shoot multiplication could not be contained on the same medium after 2nd subculture as all the shoots turned yellow. Addition of Ag NO_3 and Fe EDDHA in the medium did not help to control senescence of sub cultured shoots. Different workers have tried to control this constraint in *in vitro* propagation of rose Salm*et al.* (1996) in one there on *Rosahybrida* cv. Money way concluded that Fe EDDHA was a more photostable iron source than Fe-EDTA in higher availability of iron controlling senescence of *in vitro* shoots. Chakrabarty *et al*. (2000) reported that addition of $AgNO_3$ in the medium resulted in the reduction of leaf senescence as well as enhance the growth of axillary shoots. In present studies 0.5mg/l TDZ in MS along with PVP (100mg/l) & ascorbic acid (200mg/l) worked for *in vitro* shoot multiplication during 3rd or 4th subculture. Both bud sprouting and *in vitro* shoot multiplication showed 100 per cent survival and further growth was recorded. These data are supported by Barna and Wakhlu (1995), Kumar *et al.* (2001), Singh & Syamal (2001) in rose. The present results demonstrate that TDZ induces more vigorous growth of rose cv. First Red and at the same time also checks senescence.

Fig. 1. (A) *In vitro* axillary shoots after 15 days of culturing of axillary buds. (**B)** Multiplication of shoots originated from different axillary buds after 4 weeks. (**C)** *In vitro* root formation after 15 days of transfer on rooting medium. **(D)** Plants covered with poly bags.

Additionally, it has been observed that day of pinching have a remarkable effect on *in vitro* shoot proliferation. When the explants were collected after 10-60 days pinching, proliferation was better than collected earlier or later. Nodal segments taken from the middle proliferated earlier than from the apical or basal parts of the shoot twig as substantiated by Bressan *et al*. (1982). The best temperature for rose cv. First Red micro propagation has been observed to be 22^{o}C. Kumar *et al*. (2001) has found that 22°C- 25°C is the optimum range for *in vitro* propagation of *Rosa damascena.* Root formation is very important for vegetative propagation of plants. The success of any micro propagation protocol lies on the quality of rooting of micro shoots. In the present studies, different strength of medium with different concentrations of IBA was tried.

At the outset only IBA was tried since it has been reported conducive for induction in woody plants. Full strength MS with 1 mg IBA was the best treatment for inducing 51 per cent rooting. Different workers successfully used different auxins alone or in combination for inducing roots in rose (Barve*et al.*, 1984; Chakrabarty *et al.*, 2000; Currir *et al.* 1985). *In vitro* rooted plants were acclimatized and transplanted to cocopeat with survival rate of 51 per cent while none survived on FYM : sand : soil (1:1:1). There are several reports where micropropagated plants showed good survival rate on different potting mixture like unsterilized soil, perlite, peatmoss, sand : soil : FYM (1:1:1), soilrite, vermiculite and leaf mould.

CONCLUSION

A fully reproducible protocol for *in vitro* propagation of rose (*Rosa hybrida* L.)cv. First Red has been developed using axillary buds as explants. Rose is an important ornamental plant which needs to be multiplied at a fast rate to fulfill the demands of floriculture industry. In the present investigations various obstacles were met at different stages of micro propagation. These were one by one overcome by following/ modifying different cultural conditions and practices.

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