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Original Research Article

Effect of Growth Regulators on *in vitro* Shoot Multiplication and Plant Regeneration of *Rosa hybriad* L. from Nodal Explants

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A B S T R A C T

The present experiment was done to examine the effect of various growth regulators on *in vitro* establishment, multiplication and rooting of *Rosa hybrida* L. and all related experiments were conducted at Center for Plant Biotechnology, Hisar, using nodal segment as explants. The excised explants were washed thoroughly in liquid detergent followed by 3-4 washings with distilled water. The explants were then treated with 0.2% Bavistin and finally then 0.1% $HgCl_2$ followed by 4-5 washings with double distilled sterilized water. Aseptic explants were inoculated in Gem bottles containing sterilized MS media supplemented with various concentrations of plant growth regulators alone and in combinations. MS medium supplemented with 2mg/l BAP was found to be best medium for regeneration among the various concentrations used as it provided the maximum number of shoots per explant. Media having 2.0 mg/l BAP + 0.25 mg/l IAA showed maximum numbers of shoots (6.6) in minimum number of days. Half strength MS media supplemented 1.0 mg/l NAA+ IBA (1.0 mg/l) was found best for rooting and responded with least time (11 days).

Keywords

Rose, Micropropagation, Plant growth regulators, Shoot regeneration

Introduction

Rose "Queen of Flowers" is an important horticultural and most popular ornamental plant in the World (Ozel *et al.,* 2006). Rose occupies the first position among various cut flowers popular in the import-export trade in the world. They are woody perennials belongs to family Rosaceae grown as ornamental and for their essential oils and have gained importance word wide for their use in the pharmaceutical and food industries (Halawani *et al.,* 2014). The present day Hybrid Teas (Rosa hybrid L.) are the most popular rose types, bearing large highly centered flowers, originally developed from crossing between Hybrid perpetual and Tea roses. Roses can be propagated by seeds, cuttings, budding, layering and grafting. Seed propagation often results in variation while other methods of rose propagation are slow and time consuming and along with this dependence on season and slow multiplication rates are some of other major limiting factors in conventional propagation

(Pati *et al.,* 2006). So, there is a need to introduce efficient methods to fasten the propagation methods of roses. Micropropagation is one of the most important techniques of tissue culture that offers cost-effective implication in commercial floriculture (Soomro *et al.,* 2003). Tissue culture system in roses has been tried to established by Ibrahim and Debergh (2001) through leaf explants. Nodal segments as explants has been found more responsive in *in vitro* regeneration of roses (Shyama *et al.,* 2017). However, many workers demonstrated the *in vitro* propagation of roses in past years (Hameed *et al.,* 2006, Drefahl *et al.,* 2007, Previati *et al.,* 2008). Tissue culture technique produces roses with higher quality under a virus indexing programme, attending in this way the market demand. Tissue culture system in roses has been established by many scientists. In vitro cultures are now being used as tools for the study of various basic problems in plants sciences and now a day it is possible to progapagate all plants of economic importance through tissue, organs, embryos, single cells or protoplast culture on nutrient media under aseptic condition. Recently, *in vitro* flower induction in roses was demonstrated by many workers (Vu *et al.,* 2006). Keeping this in view, the main aim of the present study was to improve the establish protocols for micropropagation of disease free and high quality plants of roses. The experiment was done to investigate the effect of BAP, IAA on explants establishment and shoot multiplication and NAA and IBA on rooting of micro shoots.

Materials and Methods

The present investigation was carried out in the Center for Plant Biotechnology, Hisar, Dept. of Science & Technology, Haryana, CCS Haryana Agricultural University, Hisar Campus in the year 2010-2011.

Plant material

Nodal segment is used as explants and were excised from the field grown rose plants of CPB, Hisar. Single cultivar of rose was used for this experiment. From the field grown rose plants, young and tender current season growth vegetative shoots of 10-15 cm length were collected during morning hours and brought to the laboratory in glass jars containing tap water. The different explants of appropriate size were excised with the help of scalpel and scissor. The excised explants were washed thoroughly in liquid detergent Tween 80 for 2 min. followed by 3-4 washings with distilled water and then explants were treated with different duration of 0.2% Bavistin and 0.1% $HgCl_2$ followed by 4-5 washings with sterilized double distilled water in the laminar air flow.

Culture medium and conditions

For shoot proliferation and multiplication aseptic plant material were cultured in Gem bottles containing sterilized MS media (Murashige and Skoog's (1962)) supplemented with different concentrations of plant growth harmones (6-Benzylaminopurine, Indole-3-acetic acid) alone and in combination were used. For rooting of microshoots half strength of MS medium was taken supplemented with different plant growth hormones (Naphthalene Acetic Acid, Indole-3-butyric acid).

Sterilized explants were inoculated on sterilized media under aseptic condition in laminar air flow. All media were supplemented with 3% sucrose and 8.2 g/l of Mermaid^{TM} agar. The pH of all media was adjusted to 5.8 with 1 N NaOH or 1 N HCl prior to autoclaving at 1.05 kg/cm^2, 121^{o}C for 20 min. Cultures were maintained at 25^{0}C±2^{0}C air temperatures in a culture room

and light intensity of 1000 lux was provided using florescent tubes. Photoperiod of light and dark period of 16/8 hours was provided.

Shoot proliferation and multiplication

For bud sprouting and multiplication BAP concentration from 1.0-2.0 mg/l was taken along with the auxin IAA in different concentration from 0.2-0.5 mg/l. MS without any PGR was used as control. Data for percent bud sprout, days taken and number of shoots/explants were recorded on weekly interval.

Rooting

For root induction, micro shoots about 2-3 cm long were excised from mother explants and were transferred to half strength MS media containing different concentration of NAA from 1.0-2.0 mg/l and IBA ranging from 0.5-1.0 mg/l. Observation regarding percent rooting, days taken and no. of roots/micro shoots were recorded on weekly interval after culture. MS without PGR was taken as control.

Acclimatization

Well rooted regenerated plants were transferred to polybags containing two types of mixture, one treatment containing Sand: Soil: FYM (1:1:1 v/v) and in other treatment only soil for acclimatization of regenerated plants of rose. The plantlets were kept in polyhouse, where 25^{o}C and 80% RH was maintained.

Statistical analysis

The experiment was conducted two times and 3-4 replications were taken for every treatment. Data for pretreatments, shoot regeneration, multiplication, rooting and acclimatization were recorded on regular intervals. The result was analysed through simple CRD (Completely Randomised Design) method. Software used for this method was OPSTAT (Sheoran *et al.*, 1998).

Results and Discussion

The explants were treated with various concentrations of Bavistin and $HgCl_2$ and after that the treated explants were transferred to MS medium. Various duration of sterilization agents on nodal explants were compared (Table 1). Surface sterilization method in tissue culture may differ and variations have also reported for the different Rosa spp. (Farhani *et al.*, 2012) but common purpose is to obtain aseptic explants. Pre-treatment of explants with different sterilization agent had significant effect on survival of explants and microbial contamination (fig.1). Explants were treated with fungicides for different time period. In control explants were agitated for 20 min. in distilled water. The treatment T7 comprising Bavitin (0.2%) for 10 min + $HgCl_2$ (0.1%) for 6 min. agitation gave the highest explants survival (100%) and with minimum contamination (0%) followed by the treatment T3 that is Bavitin (0.2%) for 5 min + $HgCl_2$ (0.1%) for 6 min, here survival of explants was 75% and microbial contamination was 25%. When explants were treated with $HgCl_2$ (0.1%) for 10 min and 10 min along with Bavistin (0.2%) for 5 min and 10 min the explants were dried due to excessive treatment caused mortality of the explants. The explants survival and sprouting is better in pre-treated explants as compared to control. The fungicide used was systemic thus effective control of microbial infection was observed. Sub culturing of explants after 2-3 days of inoculation was done initially to control phenolic compound exudation because explants releases high amount of polyphenol

compounds in initial days of culturing (Salekjalati *et al.,* 2011).

Shoot regeneration

For shoot regeneration generally BAP has been found essential and it has been reported for shoot proliferation in rose (Ahmadian *et al.,* 2013) therefore MS media with different concentrations of BAP in combination with IAA were tried for shoot regeneration and multiplication. The maximum bud sprouting (90%) in explants was observed in T4 MS + BAP(2mg/l) + IAA (0.25mg/l) followed by (83%) the combination T3 MS + BAP(1.5mg/l) + IAA (0.25mg/l) (Table.2). The minimum response (8.3 days) was noted in hormone free MS medium (40%). The T4 also gave the earliest bud sprouting when compared with other treatments. Bud sprouting was maximum delayed (16 days) in hormone free media. The maximum number of shoots sprouted multiplication was with T4 (6.6) followed by T3 (4.0). Growth regulators at an optimum dose leads to good shoot proliferation.

The efficacy of BA + Auxin at various concentration either singly or in combination for shoot development has earlier been reported by Kumar *et al.,* (2006) and Zamir *et al.,* (2007). Superiority of BAP for shoot induction may be attributed to the ability of plant tissues to metabolize BAP more readily than other synthetic growth regulators or the ability of BAP to induce production of natural hormones such as Zeatin within tissue (Malik *et al.,* 2005).

Root formation

For in vitro rooting half strength MS medium supplemented with different concentration of NAA and IBA combinations were tried. When the shoots were multiplied they were transferred to rooting media. The best results for rooting of microshoots were obtained on T5 ½ MS+NAA (1.0mg/l)+IBA(1.0mg/l) which was significantly higher as compare to other treatments. The earliest root initiation (11.0 days) was observed on T5 followed by T2 ½ MS+NAA (1.0mg/l) (13.6 days) (Table 3). The time taken for root initiation was delayed in control (29.0 days) i.e. PGR free medium.

The maximum rooting (80%) along with highest no. of roots per plant (6.6) was observed on T5. The rooting on reduced based half strength medium was higher as compared to full strength (control) medium. Half strength MS media was effective for good rooting of microshoots (Youssef *et al.,* 2010; Saklani *et al.,* 2015, Shyama et.al. 2017). The effectiveness of IBA and NAA for promoting in vitro rooting had earlier reported by Shah *et al.,* 2008, Rai *et al.,* 2009). Although Metkasisko, 2011 observed that rooting media did not have the same effect on all tested genotypes of rose.

Survival

In the present investigation well rooted microshoots were washed with running tap water and transferred in polybags containing different combination of soil with high care avoiding damage to roots. The two types of mixture T1 and T2 containing Sand: Soil: FYM (1:1:1 v/v) and only soil respectively (Table 4) were used for acclimatization of regenerated plants of rose in polyhouse, where 78.5% survival of the tissue cultured plants were observed in Sand: Soil: FYM mixture as compared to only Soil where 44.7% survival was recorded (table.4). The responce of survival in Sand: Soil: FYM mixture was significantly higher as compare to only soil. Shyama *et al.,* 2017 also reported 86.24% survival in tissue cultured rose plants.

Table.1 Combinations of different concentration of Bavistin and $HgCl_2$ for Pre-treatments of explants

Treatments	(0.2%) Bavistin	$HgCl_2$ (0.1%)	Treatments	(0.2%) Bavistin	$HgCl_2$ (0.1%)
1	5 Min.	2 Min.	**6**	10 Min.	4 Min.
2	5 Min.	4 Min.	**7**	10 Min.	6 Min.
3	5 Min.	6 Min.	**8**	10 Min.	8 Min.
4	5 Min.	8 Min.	**9**	10 Min.	10 Min.
5	10 Min.	2 Min.	**10 (control)**	Agitation in water for 20 min.	

Table.2 Effect of growth regulators on *in vitro* bud sprouting and shoot proliferation in Rose

Treatments		Bud sprouting (%)	Days for bud sprouting	Number of shoots/ explants
T1	MS+BAP(1mg/l)+IAA (0.2mg/l)	64	14.2	2.5
T2	MS+BAP(1.5mg/l)+IAA (0.5mg/l)	66	15	3.3
T3	MS+BAP(1.5mg/l)+IAA (0.25mg/l)	83	12.2	4.0
T4	MS+BAP(2mg/l)+IAA (0.25mg/l)	90	8.3	6.6
T5	MS+BAP(2mg/l)+IAA (0.5mg/l)	63.3	13.3	3.0
T6 (control)	MS	40	16	3.0
C.D. (5%) **SE (m)**		**5.07** **1.6**	**1.7** **0.54**	**0.8** **0.28**

Table.3 Effect of basal medium strength and plant growth regulators on *in vitro* rooting of microshoots of Rose

Treatments		Days taken in root initiation	Rooting (%)	No. of roots/ plant
T1	1/2MS	20.8	31.6	2.5
T2	1/2MS+NAA (1.0mg/l)	13.6	72.3	4.8
T3	1/2MS+NAA(2.0mg/l)	17.6	53.3	3.5
T4	1/2MS+NAA(1.0mg/l)+ IBA (0.5mg/l)	14.	69.6	5
T5	1/2MS+NAA(1.0mg/l)+ IBA(1.0mg/l)	11	80	6.6
T6 control	MS	29	23.3	2.3
C.D. (5%) **SE (m)**		**1.4** **0.45**	**5.3** **1.7**	**0.6** **0.2**

Table.4 Effect of different soil mixture on survival of in vitro raised plants

Treatments		Survival of plantlets (%)
T1	Soil	44.7
T2	Soil: Sand: FYM	78.5
C.D. (5%) **SE (m)**		**3.9** **1.1**

Fig.1 Effect of different pre-treatments on contamination, survival and sprouting percentage of Rose explant

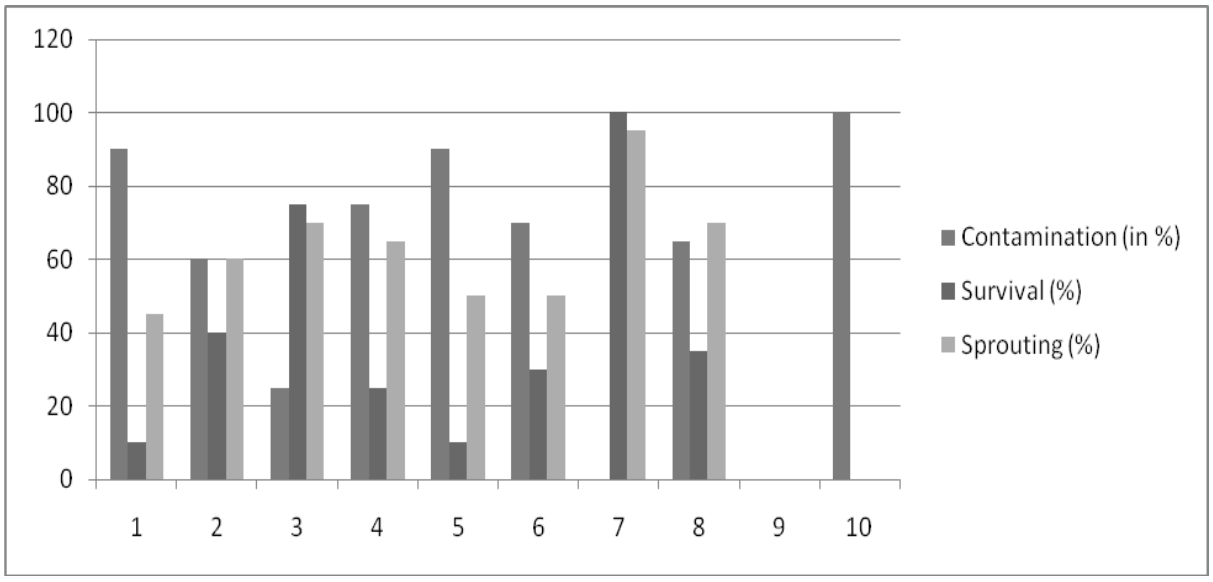

Fig.2 Various stages of *Rosa hybrid* L. micro-propagation: A-shoot initiation, B-multiplication, C- rooting, D- hardening

Anuja (2006) reported maximum survival of tissue cultured guava in Sand: Soil: FYM (1:1:1 v/v) potting mixture, aeration and nutrients to the regenerated plants was optimum for sufficient length of time. Saklani *et al.,* (2015) also reported that microshoots regenerated from nodal explants when transferred in sand: soil mixture, the plantlets showed healthy growth with 60% survival in ex-vitro condition whereas he found only 20% survival when used only soil for acclimatization.

A simple and efficient method of *in vitro* propagation for rose has been developed. This protocol help in producing disease free planting material of rose that may be adopted for other cultivars of rose easily.

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References

Ahmadian, E., Lolaei, A., Mobasheri, S. and Bemana, R. (2013). Investigation of importance parameters of plant tissue (review). *International journal of agriculture and crop sciences*. 5(8): 900-905.

Anuja, K. (2006). Comparative studies on in vitro and in vivo multiplication of guava (*Psidium guajava* L.) Ph.D. Thesis submitted to CCS Haryana Agricultural University, Hisar.

Dieleman, J., Verstappen, F., Nicander, B., Kuiper, D., Tillberg, E., Tromp, J. (1997). Cytokinins in Rosa hybrida in relation to bud break. *Physiologia Plantarum*., (99): 456–464.

Drefahl, A., Quoirin, M.G. and Cuquel, F.L. (2007). Micropropagation of Rosa × hybrida cv. Vegas via axillary buds. *Acta Horticulturae*. (751): 407-411.

Farahani, F. and Shaker, S. (2012). Propagation and growth from cultured single node ex plants of Rose (Rosa miniature). *African Journal of Plant Science*. 6(10): 277-281.

Halawani Eman M. (2014). Antimicrobial activity of Rosa damascena petals extracts and chemical composition by gas chromatog raphy-mass spectrometry (gc/ms) analysis. *African Journal of Microbiology Research*. 8(24): 2359–67.

Hameed, N., Shabbir, A., Ali, A. and Bajwa, R. (2006). In vitro micropropagation of disease free rose (Rosa indica L.). *Mycopathologia*. (4): 35-38.

Ibrahim, R. and Debergh, P.C. (2001). Factors controlling high efficiency of adventitious bud formation and plant regeneration from in vitro leaf explants of roses (Rosa hybrida). *Scientia Horticulturae*. (88): 41-57

Metka ŠiŠKo. (2011). Micropropagation of roses (Rosa spp.): The effects of different media on in vitro rooting. *Agricultura*. 8(2): 19-22

Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*. (15):473– 97.

Ozel, C.A. and Arslan, O. (2006). Efficient micropropagation of English rose 'Heritage' under in vitro conditions. *International journal of Agricultural & Biology*. pp. 626-629.

Pati, P.K., Rath, S.P., Sharma, M., Sood, A. and Ahuja, P.S. (2006). *In vitro* propagation of rose: a review. *Biotechnology Advances*, (24): 94–114.

Previati, A., Benelli, C., Da Re, F., Ozudogru, A. and Lambradi, M.

(2008). Micropropagation and in vitro conservation of virus-free rose germplasm. Propagation *of Ornamental Plants*. (8): 93-98.

Rai, M.K., Jaiswal, V.S. and Jaiswal, U. (2009). Shoot multiplication and plant regeneration of guava (*Psidium guajava* L.) from nodal explants of in vitro raised plantlets. *Journal of Fruit and Ornamental Plant Research*. (17):29-38

Saklani, K., Pant, H. and Rawat, V. (2015). Micropropagation of rose cultivars: biotechnological application to floriculture. *Journal of Environmental Research and Development*. (10):40-46

Salekjalati, M., Jafari, B. and Tarinijad, A. (2011). In vitro multiplication of rose (*Rosa hybrid* cv. Baccarat). *American-Eurasian journal of agricultural and environmental sciences*. 11(1): 111-116

Sheoran, O.P, Tonk, D.S., Kaushik, L.S., Hasija, R.C. and Pannu, R.S. (1998). Statistical Software Package for Agricultural Research Workers. Recent Advances in information theory, Statistics & Computer Applications by D.S. Hooda & R.C. Hasija Department of Mathematics Statistics, CCS HAU, Hisar. pp. 139-143.

Shyama Kumari, Singh, K.P., Raju, D.V.S., Panwar S. and Sarkhel, S. (2017). Establishment of in vitro micropropagation protocol for Rose (Rose x hybrid) cv. Raktgandha. *Indian journal of ecology*. 44 (4):166-170.

Soomro, R., Yasmin, S. and Aleem, R., (2003). In vitro propagation of Rosa Indica. *Pakistan Journal of Biological Sciences*. 6 (9): 169-177.

Yousssef, M., El-Helw M.R.., Taghian A.S. and El-Aref, H.M. (2010). Improvement of *Psidium guajava* L. using Micropropagation. *Acta Horticulturae*. (84): 223-230.

Zamir, R., Ali, N., Shah, S.T., Muhammad, T. and Shah, S.A. (2007). In vitro regulation of guava (*Psidium guajava* L.) from shoot tips of mature trees. *Pakistan Journal of Botany*. 39(7): 2395-2398.