American-Eurasian J. Agric. & Environ. Sci., 11 (1): 111-116, 2011
ISSN 1818-6769

In vitro Multiplication of Rose (*Rosa hybrida.* cv. Baccara)

[1]*Morteza Salekjalali,* [1]*Behboud Jafari and* [2]*Alireza Tarinejad*

[1]Department of Biology, Ahar Branch, Islamic Azad University, Ahar, Iran
[2]Department of Agriculture, Tarbiat Moallem University of Azarbaijan, Tabriz, Iran

Abstract: This research was performed to investigate the effects of BAP, NAA, IBA and GA_3 plants growth regulators on micropropagation of Rose (*Rosa hybrida.* cv. Baccara). Young healthy shoots containing axillary buds were taken from fields and then surface sterilized and cultured on Murashige and Skoog (MS) medium supplemented with different combinations of 6-Benzylaminopurine (BAP), 1-Naphtalene acetic acid (NAA) and Gibberellic acid (GA_3). Finding results were revealed BAP and NAA significantly affected on number of green leaves, brown leaves and induced axillary shoots per explants. High growth and multiplication rates of Rose (*Rosa hybrida* cv. Baccara) were achieved using full-strength of MS culture medium supplemented with 1 mg l^{-1} BAP without NAA and GA_3. Rooting of shoots was achieved by ½ strength of MS medium containing 3-Indolebutyric acid (IBA) at the concentration of 2 mg l^{-1} with up to 90% rooting. Plantlets were acclimatized in a soil mixture consisting of mineral perlite and peat mass (2: 1 v/v) and successfully transferred to the greenhouse after 4 weeks.

Key words: Proliferation · Rooting · BAP · NAA · GA_3 · IBA · *Rosa hybrida* cv. Baccara

INTRODUCTION

Rose "Queen of Flowers" is a beautiful flower of an immense horticultural importance. The genus *Rosa*, member of the family Rosaceae, comprises more than 100 species [1]. There are more than 20,000 commercial cultivars, which are collectively based on only 8 of the approximately 200 wild species in the genus *Rosa* [2]. Roses have been one of the world's most popular ornamental plants for a long time. They are grown worldwide as cut flowers and potted plants and in home gardens. The flowers vary greatly in size, shape and color.

Plant tissue culture refers to the *in vitro* culture of plants from plant parts (tissues, organs, embryos, single cells, protoplasts, etc.) on nutrient media under aseptic conditions [3]. *In vitro* cultures are now being used as tools for the study of various basic problems in plant sciences. It is now possible to propagate all plants of economic importance in large numbers by tissue culture. Significant features of *in vitro* propagation procedure are its enormous multiplicative capacity in a relatively short span of time; production of healthy and disease free plants; and its ability to generate propagules around the year [4].

Rose is generally propagated by vegetative methods like cutting, layering, budding and grafting. Seeds are used for propagation of species, new cultivars and for production of rootstocks. Although propagation by vegetative means is a predominant technique in roses, yet it does not ensure healthy and disease-free plants. Moreover, dependence on season and slow multiplication rates are some of the other major limiting factors in conventional propagation [5].

Plant tissue culture is the most efficient and reliable method for rapid and mass scale production of disease free and identical plants of roses through out the year. The first report of shoot proliferation and rooting of a species rose in culture was made for *R. multiflora* [6, 7].

The objective of this study was to examine the effect of BAP, NAA and GA_3 on shoot proliferation and IBA on rooting and finally, develop a protocol for micropropagation of *Rosa hybrida* cv. Baccara.

MATERIALS AND METHODS

Plant Materials: Nodal explants containing axillary buds of actively field-grown 'Baccara' rose were used for micropropagatin experiments. They were cut in 3-4 cm

Corresponding Author: Morteza Salekjalali, Department of Biology, Ahar Branch, Islamic Azad University, Ahar, Iran
Tel: +98-426-2232495, Fax: +98 -426-2237718.

length segments and hold in sterilized distilled water containing few drop of Tween 80 for few minute. Then surface-disinfested using 70% ethanol for 60 sec and then immersed in 10% of commercial Laundry bleach (5.25% NaOCl) for 20 min. All of this stages followed by three rinses in sterilized distilled water. They were trimmed down to 1 cm long prior to transferring to establishment medium.

Establishment Medium: In establishment stage, all explants cultured on a MS medium containing salts, vitamins and sucrose and without any hormones. Explants were sub cultured to the fresh medium after 3 days because it was released phenol compounds.

Proliferation Medium: The basal nutrient medium containing MS (Murashige and Skoog, 1962) salts and vitamins was used with NAA, BAP and GA_3. Two experiments were separately designed. In the first experiment, NAA at the concentrations of 0, 0.005, 0.01 mg l^{-1} was combined with BAP at the concentrations of 0, 1, 2, 3 mg l^{-1}. After that better hormones and compositions of them were selected, in the second experiment, the effects of BAP with 0, 1 and 2 mg l^{-1} and GA_3 with 0 and 1 mg l^{-1} were examined. Explants were sub cultured to the fresh medium every 4 weeks. Finally, excised single shoot from multiple shoots were transferred to the fresh medium for root induction.

Rooting Medium: To establish root proliferation, green and normal adventitious shoots from shoot proliferation cultures were excised and cultured on MS medium containing full and ½ strength of MS salts. Two additional treatments (with or without IBA at the concentration 2 mg l^{-1}) were tested in the both above mediums. Cultures were maintained at 22°C in a culture room with a 16 hrs photoperiod light.

Acclimatization and Field Establishment: Plantlets were removed from the culture flasks and washed with water to remove all medium attached to the roots. They were then transplanted to small plastic pots containing mineral perlite kept in a growth chamber and maintained at 22°C and 90% Relative Humidity (RH) under a 16 hrs photoperiod. After 3 days, RH was reduced to 80% and 7 days later reduced to 70%. After 2 weeks the plants were transferred to large plastic pots containing mineral perlite and peat mass (2: 1 v/v) and maintained in growth chamber with 22°C and 60% RH. After 4 weeks the pots were transferred to the greenhouse for further growth.

Statistical Analysis: For evaluation of the studied hormones effects and selection of the best hormones compositions, two experiments were designed. 12 different treatments from composition of NAA and BAP hormones and 6 treatments from composition of BAP and GA_3 hormones were used in the first and second experiment, respectively. In the both experiment, three traits of the grown plantlets (number of green leaves, number of brown leaves and number of axillary shoots) were measured. All experiments were conducted twice as a completely randomized design with 9 replications. Variance of Data was analyzed using Multivariate GLM (General Linear Model) and comparisons of means were conducted using Duncan's Multiple Range Test at 0.05 and 0.01 significant levels.

RESULTS

In the establishment stage, cultured explants were nicely grown in the free hormones MS medium during two subcultures with 3 weeks interval (Fig 1-A). Because explants released high amount of polyphenol compounds in the initial days of culturing, explants sub cultured to fresh medium after 3 days. Phenol compounds were prevented from growing of explants. Sub culturing of explants to fresh medium satisfactorily solved this problem.

In the first experiment of the proliferation stage, analysis of variances was shown there was significant difference among of 12 treatments into green leaves, brown leaves and axillary shoots. Through results of Duncan tests, maximum growth and multiplication rates were statistically obtained in the treatments of T2 (0 mg l^{-1} NAA + 1 mg l^{-1} BAP) and T3 (0 mg l^{-1} NAA + 2 mg l^{-1} BAP) and they were selected as the best compositions and introduced to the second experiment (Table 1). However, the highest number of green leaves and axillary shoots were produced with 0 mg l^{-1} NAA and 1 mg l^{-1} BAP.

Results of analysis were shown NAA had significantly effect on all studied traits and BAP had too (Tables 2 and 3). Mean comparisons were revealed decreasing of number of green leaves and axillary shoots but increasing of number of brown leaves by increasing on NAA concentrations (Table 2). Also, Mean comparison were showed number of green leaves, brown leaves and axillary shoots significantly varied with BAP at the concentration 1 and 2 mg l^{-1} in comparison with free BAP medium but green leaves and axillary shoots decreased and brown leaves increased when concentration increased to 3 mg l^{-1} (Table 3).

Table 1: The effect of plant growth regulator compounds on numbers of green leaves, brown leaves and axillary shoots of *Rosa hybrida* cv. Baccara in the first experiment of proliferation stage.

Treatment (NAA + BAP)	Green leaves	Brown leaves	Axillary shoots
T1(0+0)	22.67±5.81**cd	6.44±2.43**b	4±1.41**b
T2(0+ 1)	34.89±9.70a	2.44±2.81a	5.55±1.16a
T3(0+ 2)	29.22±3.52ab	3.78±1.63a	5.33±1.33a
T4(0+ 3)	15.89±8.75ef	12.78±3.79e	3.56±1bc
T5(0.005+ 0)	20.33±8.06de	9.56±3.21cd	3.33±0.98c
T6(0.005+ 1)	24.89±11.16bc	6.56±2.35b	3.56±0.87bc
T7(0.005+ 2)	23.67±5.58bc	8.67±3.38cd	3.44±1.12bc
T8(0.005+ 3)	14.22±7.76ef	15.78±3.09f	3.33±0.64c
T9(0.01+ 0)	17.78±6.95ef	12.78±3.97e	3±0.75c
T10(0.01+ 1)	23.33±10.19bc	7.67±2.45bc	3.22±0.45c
T11(0.01 +2)	19.78±7.26de	10.89±3.31de	3±0.88c
T12(0.01+ 3)	11.44±8.12f	17.33±3.73f	3.22±0.75c

In each column, means followed by the same letters are not significantly different using Multiple Dunkan Test. * and ** are significant at 5% and 1% respectively and ns is not significant

Table 2: The main effect of NAA hormone on numbers of green leaves, brown leaves and axillary shoots of *Rosa hybrida* cv. Baccara

NAA	Green leaves	Brown leaves	Axillary shoots
0	25.67±8.15**a	6.36±2.35*a	4.61±1.16**a
0/005	20.78±7.20b	10.14±3.65b	3.42±0.84b
0/01	18.08±7.76b	12.17±3.23c	3.11±0.75c

In each column, means followed by the same letters are not significantly different using Multiple Dunkan Test. * and ** are significant at 5% and 1% respectively and ns is not significant

Table 3: The main effect of BAP hormone on numbers of green leaves, brown leaves and axillary shoots of *Rosa hybrida* cv. Baccara

BAP	Green leaves	Brown leaves	Axillary shoots
0	20.26±9.34*b	9.59±3.49**c	3.44±0.96**b
1	27.70±11.32a	5.56±3.43a	4.11±1.36a
2	24.22±8.76a	7.78±2.62b	3.92±1.03a
3	13.85±7.47c	15.30±3.71d	3.37±0.86b

In each column, means followed by the same letters are not significantly different using Multiple Dunkan Test. * and ** are significant at 5% and 1% respectively and ns is not significant

Table 4: The effect of plant growth regulator compounds on numbers of green leaves, brown leaves and axillary shoots of *Rosa hybrida* cv. Baccara in the second experiment of proliferation stage

Treatment (BAP + GA_3)	Green leaves	Brown leaves	Axillary shoots
T1(0+0)	9.61±4.68**c	11.28± 3.45^{ns}a	4.06±1.23**c
T2(0+ 1)	9.61±5.21c	9.61±3.18a	4.06±1.10c
T3(1+ 0)	23.94±8.32a	10.50±4.41a	5.50±1.34a
T4(1+ 1)	15±8.90bc	9.72±2.38a	4.94±1.29b
T5(2 + 0)	15.33±7.14bc	10±3.13a	5±1.52b
T6(2 + 1)	17.94±6.22b	7.72±2.57a	4.89±1.27b

In each column, means followed by the same letters are not significantly different using Multiple Dunkan Test. * and ** are significant at 5% and 1% respectively and ns is not significant

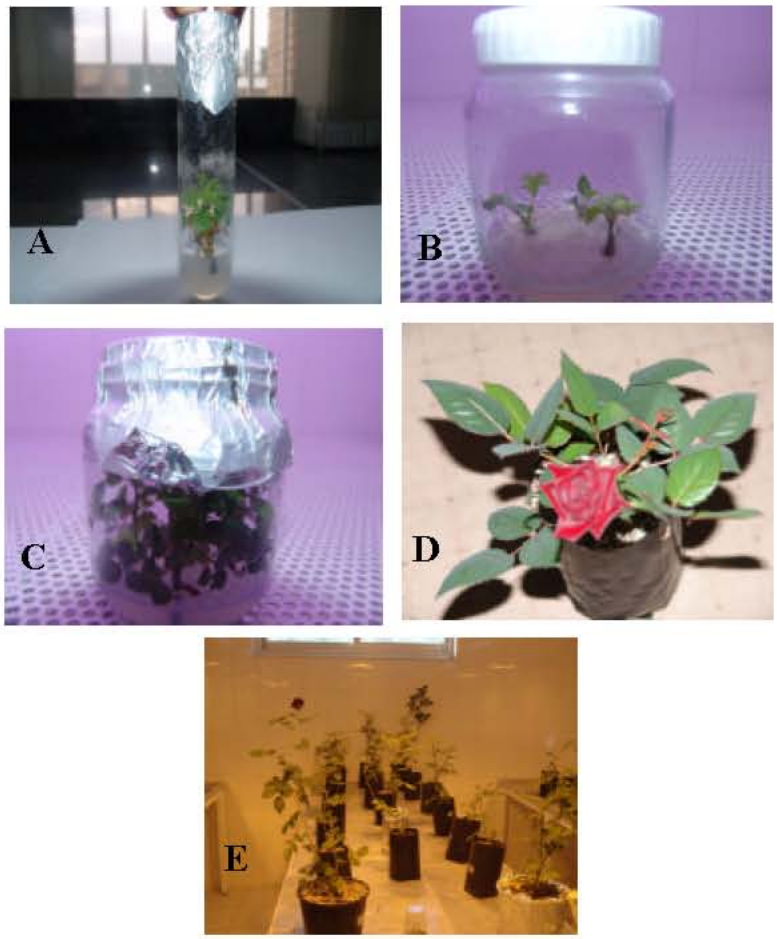

Fig. 1: Micropropagation of *Rosa hybrida* cv. Baccara: (A) growth of explants in the establishment medium (B) and (C) different steps in the proliferation stage (D) and (E) growing plants in greenhouse

In the second experiment, statistical analysis of collected data was shown GA_3 not significantly affected on any studied traits (Data are not presented). Also, treatments showed significant effect on the green leaves and axillary shoots and no significant effect on brown leaves. On the base of mean comparison, treatment of 3 (1 mg l^{-1} BAP + 0 mg l^{-1} GA_3) was selected as desirable composition (Table 4). Figs 1-B, C show proliferation stage and shoot multiplication.

In rooting of multiplication shoots, ½ strength of MS medium containing IBA at the concentration 2 mg l^{-1} with up to 90% rooting were more suitable.

After 2 weeks, in order to acclimatization plantlets were transferred to the small pots containing perlite in a growth chamber and then, the plants were transferred to large plastic pots containing perlite and peat mass and maintained in growth chamber after 2 weeks (Fig. 1-D,E).

DISCUSSION

Some explants, especially those from the shoot apex itself, released a brown substance from the cut surface into the establishment medium. These substances noticeably decreased the bud growth of shoots. The brown substance was possibly a polyphenol that is toxic to the explants [7]. This problem was resolved with sub culturing of explants to the fresh medium after 3 days of cultures. [8] reported that explants exuded less brown material when transferred to fresh medium after 3-5 days.

In the present study, significant effect of BAP hormone was revealed in proliferation stage on growth and multiplication of *Rosa hybrida* cv. Baccara. *In vitro* shoot proliferation and multiplication are largely based on media formulations containing cytokinins as a major PGR. Inclusion of BAP in the culture medium was essential for bud break and shoot multiplication of *R. hybrida* [9,10]. [11] reported that BAP was the most effective growth

regulator in stimulating shoot proliferation. BAP has approximately been used as important and basic hormone in the all of the previous research about micropropagation of Rose. Optimal amounts of BAP for multiplication of *Rosa hybrida* cv. Baccara obtained in concentration of 1 and 2 mg l^{-1} but by supplemental experiment, concentration of 1 mg l^{-1} resulted better than other. By increasing amount of BAP to 3 mg l^{-1}, growth and multiplication rates of plantlets significantly decreased (Table 3). [8] reported that low concentration of BAP stimulated development of axillary buds, but higher BAP inhibited shoot proliferation.

Presence of NAA in the proliferation medium significantly reduced multiplication rate and growth indexes of explants (Table 2). The results of our experiment are supported by the findings of [8], who demonstrated that the addition of NAA generally reduced shoot proliferation. Also, [12, 13] reported that NAA had no significant effect in proliferation of rose plants.

Addition of GA_3 to the medium did not significantly affect on proliferation in comparison with medium without GA_3 (Data are not presented). Some micropropagation media contain gibberellins, but it can reduce shoot survival and leaf expansion [7]. GA_3 has rarely used in the previous researches about micropropagation of roses. Nevertheless, [14] reported that incorporation of GA_3 at low concentrations (0.1-0.25 mg l^{-1}) in the BAP supplemented medium improved explant response up to 95% with more than seven shoots per explant.

Through Duncan tests from two experiments in the proliferation stage, MS medium containing BAP at the concentration of 1 mg l^{-1} treatments were selected as desirable composition for shoot proliferation (Table 1 and 4).

Although it appears to be relatively easy to proliferate rose shoots *in vitro*, rooting is frequently difficult. [15] suggested that rooting is affected by genotype, MS medium salts concentration, cold dark treatment and auxin type. In this research, reduced salt concentration increased rooting in MS medium, in accordance with [7] who reported that the reduced salt concentration generally increased rooting in MS medium. In most of the earlier reports, varying concentrations of different auxins have been used for root induction [16-19].

In the base of our finding, the most roots and high survival percentage of plantlet (up 90 %) were initiated per explants on half-strength MS medium containing IBA at the concentration 2 mg l^{-1}.

Our investigations showed that the best shoot proliferation was obtained at 1 mg l^{-1} BAP in full-strength MS medium, while rooting of shoots improved with half-strength MS medium containing of IBA at the concentration of 2 mg l^{-1}.

ACKNOWLEDMENTS

The authors are gratefully indebted to Sevil Farhadi, Javad Rezapour and Mahmoud Reza Saeidi for their technical assistance in the laboratory and greenhouse.

REFERENCES

1. Horn, W.A.H., 1992. Micropropagation of rose (*Rosa L*). In: Bajaj Y.P.S. (Ed.) Biotechnology in agriculture and forestry. Springer. Germany, pp: 320-342.
2. Roberts, A.V. and E.F. Smith, 1990. The preparation of in vitro chrysanthemum for transplantation to soil. Plant Cell Tiss. Org. Cult., 21:129-132.
3. Altman, A., 2000. Micropropagation of plants: principles and practices. In: Spier R. (Ed.) Encyclopedia of Cell Technology. John Wiley and Sons Inc. New York, pp: 916-929.
4. Dhawan, V. and S.S. Bhojwani, 1986. Micropropagation in crop plants. Glimpses in Plant Res., 7: 1-75.
5. Pati, P.K., S.P. Rath, M. Sharma, A. Sood and P.S. Ahuja, 2006. *In vitro* propagation of rose-a review. Biotech. Advances, 2: 94-114.
6. Elliott, R.F., 1970. Axenic culture of meristem tips of *Rosa multiflora*. Planta, 95: 183-186.
7. Skirvin, R.M., M.C. Chu and H.J. Young, 1990. Rose. In: Ammirato, P.V. D.R. Evans, W.R. Sharp and Y.P.S. Bajaj, (Eds.) Handbook of Plant Cell Culture. Springer. New York, pp: 716-743.
8. Yan, M., D.H. Byrne and C. Jing, 1996. Propagation of rose species *in vitro*. *In vitro* Cell Dev. Biol. Plant, 32: 103-108.
9. Hasegawa, P.M., 1980. Factors affecting shoot and root initiation from cultured rose shoot tips. J. Am. Soc. Hortic. Sci., 105: 216- 20.
10. Wulster, G. and J. Sacalis, 1980. Effects of auxins and cytokinins on ethylene evolution and growth of rose callus tissue in sealed vessels. Horti. Sci., 15: 736-7.
11. Vijaya, N., G. Satyanarayana, J. Prakash and R.L.M. Pierik, 1991. Effect of culture media and growth regulators on *in vitro* propagation of rose. Curr. Plant Sci. Biotechnol. Agric., 12: 209-14.
12. Cai, J.M.Y. and D.L. Qian, 1984. Induction of multiple shoots and rapid propagation of clones of china rose [*Rosa Chinensis*]. Plant Physiol., 5: 37-8.

13. Nikbakht, A., M. Kafi, M. Mirmasoumi and M. Babalar, 2005. Micropropagation of Damask Rose (*Rosa damascena* Mill.) cvs Azaran and Ghamsar. Int. J. Agri. Biol., 7: 535-538.
14. Rout, G.R., B.K. Debata and P. Das, 1990. *In vitro* clonal multiplication of roses. Proc. Indian Natl. Sci. Acad., 60: 311-8.
15. Kim, C.K., J.D. Chung, S.K. Jee and J.Y. Oh, 2003. Somatic embryogenesis from *in vitro* grown leaf explants of *Rosa hybrida* L. Plant Biotechnol. J., 5: 161-4.
16. Collet, G.F., 1985. Enracinment ameliore lors de la production *in vitro* de rosiers. Rev. Suisse Vitic. Arboric. Hortic., 17: 259-63.
17. Kirichenko, E.B., T.A. Kuz'-Mina and N.V. Kataeva 1991. Factors in optimizing the multiplication of ornamental and essential oil roses *in vitro*. Bull. Gl. Bot. Sada, 159: 161-7.
18. Chu, C.Y., S.L. Knight and M.A.L. Smith, 1993. Effect of liquid culture on the growth and development of miniature rose (*Rosa chinensis*). Plant Cell Tiss. Org. Cult., 32: 329-34.
19. Arnold, N.P., M.R. Binns, D.C. Cloutier N.N. Barthakur and R. Pellerin, 1995. Auxins, salt concentrations and their interactions during *in vitro* rooting of winter-hardy and hybrid tea roses. Hort. Sci., 30: 1436-40.