JOP
Journal of Ornamental Plants
Available online on: www.jornamental.com
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

In Vitro Flower Bud Formation, Plant Regeneration and Morphogenetic Studies in Local Scented Cultivar of *Rosa indica*

Raageeva Bimal and Niti Kiran*
Biotech Lab, University Department of Botany, B.R.A. Bihar University, Bihar-842001 (India).

Received: 23 September 2013 Accepted: 14 January 2014
*Corresponding author's email: niti_kiran2004@yahoo.co.in

f SID

Abstract

In vitro propagation of rose (*Rosa indica*) as well as rose breeding is a valuable economic enterprise and has received highest importance globally including Indian subcontinent. The development of new cultivars for commerce is faced with new challenges. *In vitro* micropropagation protocols of varieties of roses have been established but very few reports on the clonal propagation of local cultivars of Indian scented rose has been made. In the present paper we report the dedifferentiation and redifferentiation of stem nodal, leaf disc, petals well as ovary explants cultured on MS medium supplemented with various growth adjuvants such as coconut water (CW 5%-20%v/v), auxins (IAA, NAA, 2,4-D and IBA) and cytokinins (BAP and Kn) in varying concentrations. Vigorous callus formation was observed showing embryogenic clusters in both the nodal and leaf disc explants on MS medium supplemented with CW(10% v/v) + 2,4- D (11.3 µM) + BAP (3.55 µM). The proliferation of 4-7 multiple shoots (mean number of shoots 5 ± 1.24) as well as *in vitro* flower bud formation on *in vitro* regenerated rose plants from nodal explants cultured on MS medium supplemented with CW + 2,4-D + BAP have been observed. The regenerated shoots treated with 'Quic Root' (commercially available rooting chemical) produced well developed root system.

Keywords: Multiple shoots,'Quic Root', Rose, *Rosa indica*, Tissue culture.

www.SID.ir

INTRODUCTION

Plant tissue culture is a novel technique widely used for micropropagation of plants under aseptic condition. Rose,being the queen of flowers is a symbol of beauty, love and tranquility and has been a favorite object of research for plant scientists for morphogenetic studies. Well established and reliable protocols for micropropagation of rose have been developed for hybrid cultivars using shoot tip and axillary bud and petal explants (Hasegawa, 1979; Bressan *et al.*, 1982; Burger *et al.,* 1990; Skirvin *et al.,* 1990; Rout *et al.*, 1991; Chatani *et al.*, 1996; Murali *et al.*, 1996; Pati *et al.,* 2004, 2005; Zabbarzadeh and Khos-khui, 2005; Senapati and Rout, 2008; Nak-Udom *et al.*, 2009; Kermani *et al.*, 2010; Mamaghani *et al.*, 2010; Ebrahimi and Mohammadi-Nejad, 2011; Hegde *et al.*, 2011; Mukhambetzhanov *et al.*, 2011; Pawlowska, 2011; Farahani and Shaker, 2012; Moallem *et al.*, 2012; Shadparvar, 2012; Shirdel *et al.*, 2013; Zeng *et al.*, 2013). Earlier attempts to induce organogenesis from callus of rose were unsuccessful (Khosh- Khui and Sink, 1982). Later, adventitious differentiation in callus culture obtained from leaf, stem, internodes and zygotic embryo segments have been demonstrated by (Tweedle *et al.*, 1984; Lloyd *et al.*, 1988; Burger *et al.,* 1990). Recently, organogenesis and plant regeneration from petal explants have also been reported in hybrid roses (Chatani *et al*., 1996; Murali *et al*., 1996; Nanomura *et al.*, 2001). Flower formation is a very important morphogenetic event depending on the genetic makeup, physiological stage of explants and environmental factors. *In vitro* flower initiation is not a very common phenomenon. *In vitro* induction of flower bud has been reported in different varieties of *R.hybrida* L. (Wang *et al*., 2002; Kanchananpoom *et al.,* 2009), hybrid tea rose cv.'First Prize'(Vu *et al*., 2006). Tissue culture provides a convenient tool to study the biological mechanism of transition from vegetative to reproductive phase. However, no systematic studies have been made on *In vitro* flower or bud formation in scented variety of *R.indica* and also there are very few reports on the morphogenetic potentials of vegetative and reproductive parts of Indian scented rose (Soomro *et al.,* 2003; Hameed *et al.,* 2006). Rose propagation which is usually practiced by cutting and grafting is labour intensive (Horn, 1992) and dependent on season. The slow multiplication rate in roses is another constrain which may be overcome by using the plant tissue culture technique for efficient and reliable mass production of identical plants of rose throughout the year. In the present paper multiple shoot, plant and flower bud formation in local scented cultivar of rose (*Rosa indica*) followed by 'Quic Root'(a commercially available rooting chemical) induced *In vitro* rooting in shoots have been established and the protocol has potentials for application in tissue culture nursery both for raising planting materials as well as rose flower (buds).

MATERIAL AND METHODS

Fresh explants were collected from Botany department of B.R.A. Bihar University. The explants such as node, inter node, pollinated flower buds, leaf, petals and embryo were washed thoroughly under running tap water for half an hour followed by washing in liquid detergent for next half an hour. Later the explants were again washed under running tap water for 10 min. The explants were surface sterilized with 0.1% $HgCl_2$ solution for 5 min and finally washed two to three times with double distilled sterile water. These explants were cultured on Murashige and Skoog's medium (1962) supplemented with coconut water (CW 10%v/v), IAA (0.57- 28.5 μM), 2,4-D (0.45-22.6 μM), NAA (0.54-26.8 μM), BAP (0.44-22.2 μM), Kn (0.46-23.2 μM), IBA (0.49-24.60 μM) either singly or in various combinations. The regenerated shoots were rooted *in vitro* by treating the lower ends of shoots by 'Quic Root' (Ashwin Chemical, Banglore, India) for 10-30 sec and culturing in MS liquid medium (half strength of macro, micro, iron and vitamins) and 3% sucrose. The cultures were incubated at 25ºC ± 5ºC under cool fluorescent light and 16 h photoperiod in culture room. The experiments were repeated at least three times to substantiate the reproducibility of the observations and data presented in table 1 show the mean value of three sets of experiments.

Archive of SID

www.SID.ir

RESULT AND DISCUSSION

In local scented cultivar of *R.indica* the explant taken from both vegetative and reproductive organs responded in culture and showed dedifferentiation into callus as well as regeneration of shoots. The explants cultured on MS medium without any growth adjuvants did not show any response.

Table 1 shows relationship between the number of nodal explants responding by proliferating shoot(s) on different nutrient media supplemented with various growth promoters. The nodal explants cultured on MS + BA (2.22 µM) showed callus formation from all over the surface of the explants. However, the nodal explants cultured on MS + BAP (22.20 µM) + Kn (2.32 µM) showed proliferation of axillary buds, into 2 shoots/node and very poor callus formation was observed. Rooting in the cultured explants was observed on MS medium supplemented with BAP (22.20 µM) + Kn (2.32 µM), but these roots started dedifferentiating into callus in 4-6 days of culture. The nodal explants cultured on MS + BAP (11.1 µM) + Kn (4.6 µM) also produced two shoots per explant but the frequency of response was better. In another experiment, the nodal explants cultured on MS + BAP (3.55 µM) + 2,4-D (11.31 µM) + CW10%v/v produced 4-7 (mean number of shoot 5 ± 1.24) multiple shoots (Figs.1-3). However, the nodal explants cultured on MS + 2,4-D (2.26 µM) + Kn (4.46 µM) and 2,4-D (4.5 µM) + Kn (4.6 µM) promoted the growth of only one shoot in 7.6% and 16.9% explants, respectively. The regenerated shoots treated with 'Quic Root' and cultured on half strength MS constituents containing 3% sucrose produced well developed root system and the protocol proved to be highly effective (Figs. 4-6). The 'Quic Root' treated ends turned dark (Fig. 4) and in 2-3 days rupturing of the surface due to bulging at the treated end was observed (Fig. 5). The root initiation was also observed in 5- 6 days of culture followed by formation of 3-4 well developed roots in about 12 -15 days of culture (Fig. 6).

The stem pieces (Figs. 7-8), petal (Fig. 9), leaf discs (Fig. 10) and fertilized ovary pieces (Figs. 11-12) cultured on the same medium (MS + BAP + 2,4-D + CW) showed callus formation. The regenerated shoots sub-cultured on MS medium containing NAA (5.37 µM) + BAP (22.20 µM) induced formation of flower buds on tiny rose shoots (Plate 2 Figs. 13-14 and 16). The first flower bud was observed in 35 days of culture, while the other bud appeared in 62 days of culture. The first one was miniature flower bud with green sepals and enclosing dark violet petals, the buds measured 2-3 mm in length but did not open. The peduncle of the first bud did not show clearly formed adnate stipule. The second flower bud was comparatively elongated and showed green sepals and dark violet petals. The stalk of the bud very clearly showed adnate stipules probably indicating the transformation of leaf into flower bud (Plate 2, Fig. 16).

The flower bud (Fig. 15) also produced on MS + BAP (0.89 µM) + CW (10%v/v). The IBA was used for rooting of the regenerated shoots and the shoots were cultured on MS medium supplemented with IBA (4.90 µM) developed roots. Callus formation was observed in 6-8 weeks with on MS medium supplemented IBA and the callus growth was average. In some of the shoots

Archive of SID

Table 1. Correlation between number of nodal explants responding by proliferating shoot(s) on different nutrient media containing various growth promoters

Sl.No.	Different hormones supplemented in MS basal medium	Number of responsive explants in culture in term of shoot proliferation/130 explants in each set of medium*	Response in terms of number of shoot(s)/explants
1.	2,4-D (11.3 µM) + BAP (3.55 µM) + CW (10%)	60.10±0.9	4-7 (mean shoot number 5±1.24)
2.	BAP (22.2 µM) + Kn (2.3 µM)	17.09±0.9	2
3.	BAP (11.1 µM) + Kn (4.6 µM) + NAA (5.3 µM)	21.18±0.9	2
4.	2,4-D (2.26 µM) + Kn (4.46 µM)	10.40±1.6	1
5.	2,4-D (4.5 µM) + Kn(4.6 µM)	22.09±1.6	1

*(mean ± SD)

www.SID.ir

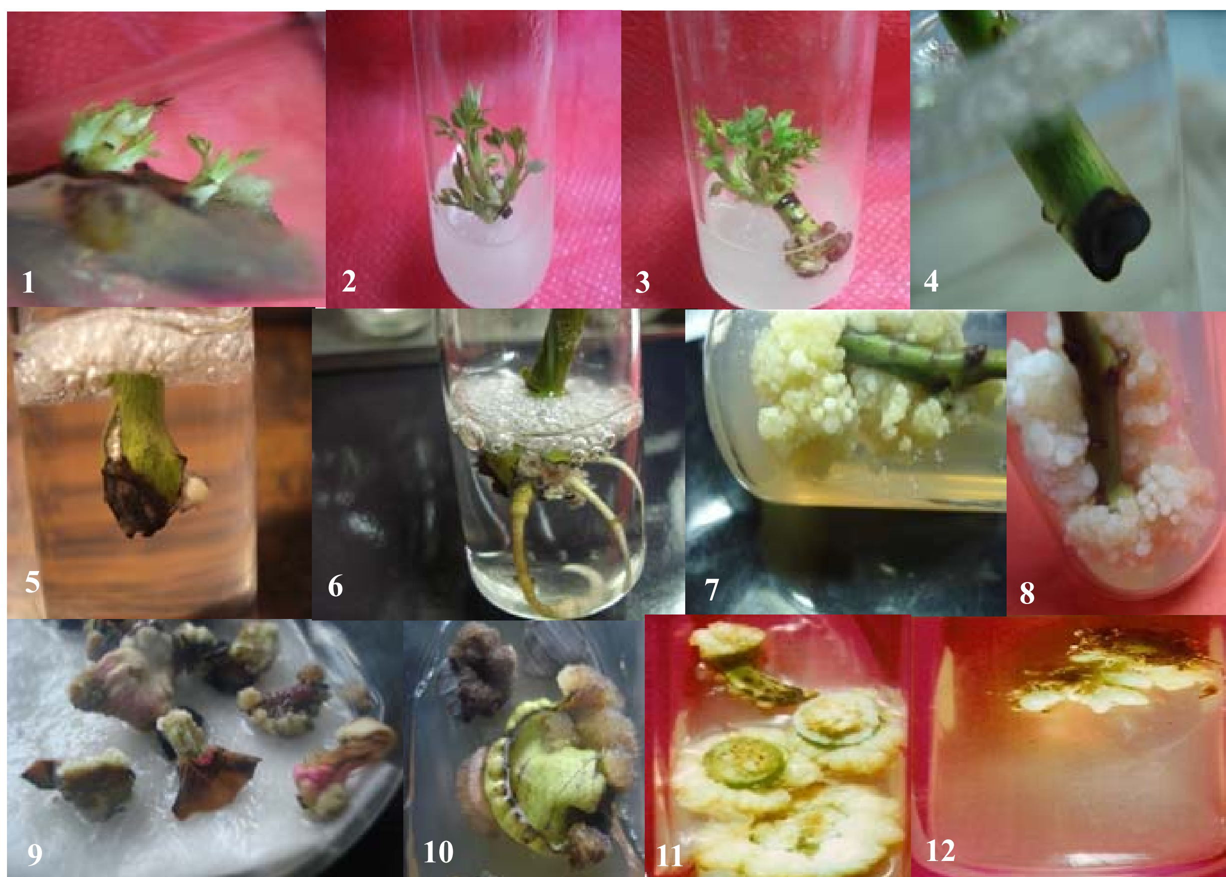

Plate 1. Morphogenetic studies in *Rosa indica*. Figs.1-3. Multiple shoots developing from nodal explants on MS + 2,4-D (11.3 µM) + BAP (3.5 µM) + CW (10%v/v). Figs. 4-6. Different stages of 'Quic Root' induced root formation. Figs.7-8. Embryogenic callus showing globular embryoids from nodal explants. Figs.9-12. Callus proliferation from petals, leaf discs unfertilized and fertilized ovary pieces.

in vitro differentiation of flower bud was observed in presence of IBA but did not blossom. The nodal explants cultured on MS medium containing 9.80 µM IBA showed profuse callus formation in 5-6 days as well as also root formation in 20-25 days of culture. When the nodal explants were cultured on MS medium supplemented with IBA (4.90 µM) and Kn (5.71 µM) both callus formation and root initiation was observed in 10-12 days of culture. Callus formation was observed in the nodal explants cultured on MS medium containing IBA (9.80 µM) + CW (10%v/v) in 6-7 days. The half strength MS medium supplemented with 2.46 µM IBA induced callus formation from nodal explants in just 2- 3 days of culture. Petal disc cultured on MS medium supplemented with BAP (0.44 µM) + NAA (1.07 µM) and incubated in dark produced callus in 80% of the explants but no differentiation or shoot bud was observed.

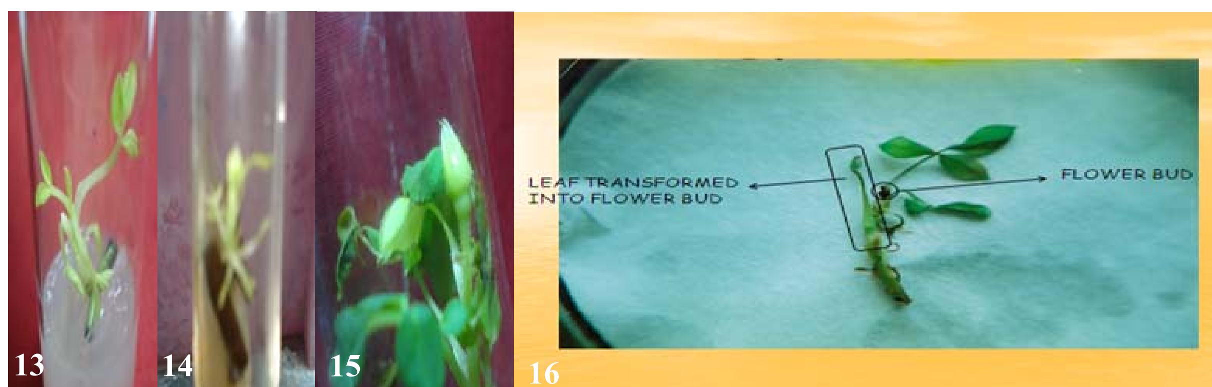

Plate 2. Morphogenetic studies in *Rosa indica.* Fig.13. A regenerated shoot. Figs. 14, 16. Differentiation of flower buds in vitro. Fig.15. A well developed flower bud was formed on MS + BAP (0.89 µM) + CW (10%v/v).

www.SID.ir
Archive of SID

R.indica (local cultivar) bearing the most attractive flowers, is strongly scented flowering plant and is widely used in perfumery, cosmetic products, medicine and food products. *In vitro* mcropropagation protests in any of rose cultivars have been developed in the past using shoot tip and nodal explants (Rout *et al*., 1989, 1990; Skirvin *et al.*, 1990; Pati *et al.*, 2005; Zabbarzadeh and Khosh-Khui, 2005; Sengupta and Rout, 2008; Canali and Kazaz, 2009; Kermani *et al*., 2010; Hegde *et al*., 2011; Pawlowska, 2011; Ebrahimi and Mohmmadi-Nejad, 2011; Farahani and Shaker, 2012; Moallem *et al*., 2012; Shadparvar, 2012; Shirdel, 2013; Zeng *et al*., 2013). However, the slow rates of multiplication through conventional method of cutting as well as its dependence on the season are greatest disadvantages for establishment of rose nursery for commerce. In the present paper we report the establishment of an efficient method for shoot and root regeneration in tissue culture of local scented cultivar of experimental system *R.indica* L. 2,4-D, BAP and CW induced maximum number of 4-7 shoot buds from node each node of the explants.The hormonal combination BAP + Kn produces only 2 shoots/explants,while MS medium supplemented with 2,4-D + Kn produced only one shoot per nodal explant. Soomro *et al*. (2003) reported plant regeneration in *R. indica* on MS medium supplemented with IBA and NAA. Rout *et al*. (1991) reported the induction of callus and somatic embryognesis in *R.hybrida* var.Landora only in the presence of three adjuvants. Rout *et al.* (1990) observed that BAP is the most effective growth adjuvant for shoot proliferation. They also reported similar observations in *R.canina* and *R.damascena.* The formation of globular callus along with rooting has been observed in present system which shows similarity with the observation made by Rout *et al*. (1990). In *R. damascena* direct shoot regeneration from leaf in the presence of TDZ (thidiazuron) and $AgNO_3$ (silver nitrate) was observed (Pati *et al.,* 2004). In *R. hybrida* L. cv. 'Perfume Delight' 3 shoots were regenerated from nodal explants on BA+NAA combination (Nak- Udom *et al.*, 2009). However, Senapati and Rout (2008) reported high frequency of shoot multiplication in IAA + cytokinin supplemented medium in three cultivars of *R. hybrida* viz. *R. hybrida* 'Cri Cri', 'Pariser Charme' and 'First Red'. The rose cultivar'Pareo' the nodal explants produced multiple shoots on MS medium containing BAP (Mukhambetzhanov *et al.,* 2011). The proliferation of 4±7 multiple/shoots explants was obtained on MS + 2,4-D + BAP + CW, while Soomro *et al*. (2003) reported regeneration of 2-6 shoots per explants in presence of IBA and NAA. Shirdel *et al*. (2013) higher concentration of BAP as the most important factor for multiple shoot induction in *R. canina*. In present system, BAP alone induced callus formation but when supplemented with 2,4-D and CW proved to be important for proliferation of multiple shoots. Shadparvar (2012) reported callus mediated shoot formation in *R.miniature* 'Green Ice' in the presence of IBA and 2iP. In R. foetida, BA + IBA combination was found essential for highest number of shoots (4.8 mean shoot number per explants) (Ebrahini and Mohammadi-Nejad, 2011). Farahami and Shaker (2012) recorded maximum no.of 4 shoots per node on MS + BAP + IBA in miniature. In *R. hybrida* cv.'Black Baccara' Bayanati and Mortazavi (2013) reported proliferation in the presence of BAP. Kermani (2010) also found BAP inducing maximum of 4.66 shoots on VS medium in *R. persica*. In *R. damacena*, BAP + TDZ containing MS medium induced maximum number of shoots (Mamaghani *et al.,* 2010). In the present study, the rose explants (nodal, leaf, ovary and petals) treated individually with different auxins such as IAA, NAA, 2,4-D, IBA did not show any noticeable morphogenetic effect. Although auxin (2,4-D) exerts control at transcriptional and translational level (Sengupta and Raghavan,1980 a,b) the auxin applied individually did not favour redetermination of a cell for morphogenesis in the present experimental system. The physiological role of IBA in morphogenesis in rose is remarkable. The half strength MS containing IBA induced callus formation but supported healthy shoot development only when IBA was added in the full strength MS medium. Low concentration of IBA has been found to promote shoot development in Macrotyloma uniflorum (Tejavathi *et al.*, 2010). Under normal growth conditions differentiation of flower takes place when a plant attains maturity, since the age of the plant is genetically determined and is species specific. A flower bud or flower

Archive of SID

www.SID.ir

is produced when physico-chemical and environmental milieu are congenial. However, these conditions can be regulated for transformation of vegetative phase of a plant into reproductive phase. The pioneer work on *in vitro* flowering was reported in Cassava without hormones in culture medium (Tang *et al.*,1983). *In vitro* inflorescence had been induced from axillary buds of Morus alba (Naik and Latha, 1996). Pai *et al*. (1986) induced inflorescence through inflorescence culture. The precocious flowering in bamboos has been reported through inflorescence culture (Nadgauda *et al*., 1990). *In vitro* flowering from cotyledon cultures of groundnut was reported by Narasimhalu and Reddy (1984). *In vitro* flowering was produced on callus derived plantlets of Papaver somnifera (Yoshikama and Furaya, 1983) and during somatic embryo formation in Brassica nigra (Mehta *et al.,* 1993). Very recently, *in vitro* flower induction from callus in juvenile explants of sugarcane, Saccharum officinarum var. CoC 671 has been reported (Virupakshi *et al.*, 2002). A tiny spathe like formation has been reported in banana from cultured male floral primordia (Bimal and Jha, 2008). In the present experimental system, flower bud formation in the regenerated plant took place in 6-8 weeks in the presence of hormone IBA or in combination of NAA+BAP. Flowering from vegetative tissue was observed *in vitro* on long-term callus culture of sugarcane meristematic tissue (Virupakshi *et al.*, 2002). It seems probable that osmotic and nutritional stress to the growing tissue resulted in flower bud formation in 6-8 weeks of culture on the medium without subculturing. *In vitro* flower bud or flower formation is not a spontaneous developmental decision of the plant rather it is the culmination of closely integrated programmed physiological and developmental changes which evocate flowering (Govil, 2004). The flower buds formed in culture did not open, it is likely that the appropriate signal to induce opening of the *in vitro* produced flower buds might be lacking in the scented variety of *R.indica.* There are many reports indicating the synergistic action of auxin and coconut water (Bapat and Narayan Swami, 1977; Bimal and Jha, 1986). Wang *et al.* (2002) studied *in vitro* flowering in six cultivars of rose and the highest percentage of flower bud induction (49.1%) was obtained on media supplemented with TDZ and NAA for the cultivar Orange Parade. TDZ or ZT was the best choice for flower induction in all six cultivars studied. The culture and subculture duration were also found to be important for flower induction. Age of plantlets in culture and age of mother plant providing explants influence the induction of flower formation and generating a consistent system of large scale production. Five Parade rose hybrids (Binanea Parade, Fiesta Parade, Orange Parade, Scarlet Parade and Viva Parade, from the Netherlands) and a bigger red rose cultivar (RF) from Malaysia were tested and a period of 45 days in induction medium was responsible for highest percentage of plantlets forming flower buds (Wang *et al*., 2002). It is likely that prolonged vegetative growth including elongation of stem and formation of roots in culture might have made transition to the reproductive stage. Vase life of *in vitro* induced flower was about one month at room temperature or below 25℃. 'First Prize' variety of rose (hybrid tea) differentiated flower shoots cultured on MS medium + BAP + NAA + 3% sucrose (Vu *et al.*, 2006). According to them sucrose is a key factor in induction, cytokines increase the flowering percentage and normal development and reduced inorganic and organic salt concentration in MS medium had a positive effect on *in vitro* flowering. Kanchanapoom *et al*. (2009) reported proliferation of 5 shoots per explants on MS + BAP (13.30 μM) + Kn (4.60 μM), followed by flower induction on MS + BAP (8.90 μM) in 9 weeks in *R. hybrida* L.cv. 'Red Master Piece'. Although *in vitro* flower bud has been induced, more reliable culture conditions for *in vitro* flower induction need to be elucidated. In our experiments flower bud formation in local scented variety of rose (*R. indica*) has been observed. Stimulation of cell division by coconut water has been reported earlier (Morel, 1950; Bakely and Steward, 1961). However, in the present study, besides coconut water the presence of 2,4-D and BAP seems to be a prerequisite for stimulation of cell division. Although, there are reports on the induction of callus, shoot etc. in the presence of either auxin or cytokinin individually, auxin or cytokinin did not play a role in any type of morphogenesis in *R. indica* var. local scented except IBA. However, IBA in combination with BAP was found to

Archive of SID

www.SID.ir

be the most suitable treatment in damask rose, *R. damascena* Mill. (Zabbarzadeh and Khosh-Khui, 2005). Sisko (2011) reported genotype dependent rooting response in *Rosa* spp.The regeneration of roots is regulated at least in part by auxin and the localization of the differentiation of roots at the basal end of the cuttings is due to the polar movement of auxin towards the physiologically lower end. Mukhambetzhanov *et al*. (2011) reported 100% rooting in rose cultivar' Pareo' in the presence of IBA + IAA. The effect of rooting chemical 'Quic Root' has been studied in *R. indica* for the first time. The efficiency of root induction and morphogenesis observed in *R. indica* was significant. Altogether 3-7 roots differentiated and attained proper growth. The rooting chemical has been reported to enhance cambial activity by mobilizing reserve food materials to the site of root formation (Gurumurithi *et al.*, 1984; Purohit *et al.,* 2008). Although spring or the season when the fresh flush starts has been found to be a favorable period for rooting experiment, in the present experiment rooting can be induced round the year.

CONCLUSION

Results obtained in the present study report the establishment of a two step protocol for rapid micropropagation of the local scented cultivar of *R.indica* L. and *in vitro* proliferation of rose flower buds.The protocol using MS nutrient Medium supplemented with 2,4-D, BAP and CW has been successfully established for regeneration of multiple shoots from nodal explants followed by 'Quic Root' mediated rooting of rose micro-shoots which has potentials for development of tissue culture nursery supplying tissue culture raised planting material to the market.

ACKNOWLEDGEMENTS

Authors thank Head of the University Department of Botany for facilities. One of us (NK) thanks UGC for financial assistance (No.F.15-21/12 (SA-II).

Archive of SID

Literature Cited

Bakley, L.M. and Steward, F.C. 1961. Growth induction of cultures of Haplopappus gracilis the behavior of cultured cells. American Journal of Botany, 48:351-358.

Bapat,V.A. and Narayanswamy, S. 1977. Mesocarp and endosperm culture of Achrassapota Linn. *In vitro*. Indian Journal of Experimental Biology. 19:294-298.

Bayanti, M. and Mortazavi, S.N. 2013. Micropropagation from cultured nodal explants of *Rosa hybrida* c.v.'Black Baccara'. International Journal of Agronomy and Plant Production, Vol. 4(6); 1381-1385.

Bimal, R. and Jha, K.K. 1986. Direct flower neoformation from excised ovary pieces of *Cestrum diurnum* L. Journal of Plant Anatomy and Morphology. 3 (1) : 9-11.

Bimal, R. and Jha, L.K. 2008. *In vitro* plant regeneration from male floral primordia in endemic and endangered banana var. Malbhog. In : I.D Arya and Sarita Arya (eds.) Utilization of Biotechnology in Plant Sciences. Forest Research Institute, Dehradun (India).pp 255-263.

Bressan, P.H., Kim, Y.J., Hyndman, S.E., Hasegawa, P.M. and Bressan, R.A. 1982. Factors affecting *in vitro* propagation of Rose. Journal of American Society of Horticultural Science, 107(6): 979–90.

Burger, D.W., Liu, L., Zary, K.W. and Lee, C. I. 1990. Organogenesis and plant regeneration from immature embryos of Rosa hybrid L. Plant Cell Tissue Organ Cult. 21;147 -152.

Canali, F.A. and Kazaz, S. 2009. Biotechnology of roses: progress and future prospects. Suleymani Demirel Universitesi Orman Fakuttesi Dergisi Seri: A, Sayi: 1, Sayfa:167-183.

Chatani, K., Toyoda, H., Morikawa, Y., Ogata, Y., Koreeda, K.,Yoshida, K., Hagi, T., Matsuda, Y. and Ouchi, S. 1996. Callus induction and plant regeneration from petal explants of Rose (*Rosa hybrida*). Plant Tissue Culture Lett., 13 (2),185-188.

Ebrahimi, F. and Mohammadi-Nejad, G. 2011. Study of microprogation of Eglantine (*Rosa foetida*)

www.SID.ir

in vitro condition. Advances in Environmental Biology 5(10); 3203-3206.
Farahani, F. and Shaker, S. 2012. Propagation and growth from cultured single node explants of rosa (*Rosa miniature*). African Journal of Plant Science, Vol.6(10); 277 -281.
Govil, S.M. 2004. Mystery of flower. Proc. Indian Science Congress 91th Session Sectional Presidential Address Plant Sciences pp. 1-24.
Gurumurthi, R., Gupta, B. and Kumar, A. 1984. Hormonal regulation of root formation. In: Purohit, S.S. (ed.) Hormonal regulation of plant growth and development. Agrobotanical Publishers (India) pp. 387-400.
Hameed, N., Shabbir, A., Ali, A. and Bajwa, R. 2006. *In vitro* micropropagation of disease free rose (*Rosa indica* L.). Mycopath, 4(2):35-38.
Hasegawa, P.M. 1979. *In vitro* propagation of rose. Hort Science, 14 (5): 610-612.
Hegde ,V., Kag, B., Sathyanarayana, B.N. and Andsharath, R. 2011. Rapid *in vitro* multiplication and shoot regeneration from nodal explants of Rose. Journal of Cell and Tissue Culture, Vol. 11(3): 2961-2965.
Horn, W.A.H. 1992. Micropropagation of rose (*Rosa* L.). In: Bajaj, Y.P.S.(ed) Biotechnology in Agriculture and Forestry (Vol. IV). Springer Verlag Germany pp. 320-324.
Kanchanapoom, K., Posayapisit, N. and Kanchanapoom, K. 2009. *In vitro* flowering from cultured nodal explants of Rose (*Rosa hybrid* L.). Notulae Botanicae Horti Agrobotanici Cluj- Napoca Journal, 37(2) :261-263.
Kermani, M.J., Khosravi, P. and Kavand, S. 2010. Optimizing *in vitro* propagation of *Rosa persica*. Iranian Journal of Genetics and Plant Breeding. Vol.1. No.1, pp 44-51.
Khosh–Khui, M. and Sink, K.C. 1982. Rooting enhancement of *Rosa hybrida* for tissue culture propagation. Scientia Horticulturae, 17:371-376 .
Lloyd, D., Roberts, A.V. and Short, K.C. 1988. The induction *in vitro* of adventitious shoots in *Rosa.* Euphytica 37:31-36.
Managhani, B.A., Ghorbani, M., Assareh, M.H. and Zare, A.G. 2010. *In vitro* propagation of three damask roses accessions. Iranian Journal of Plant Physiology, 1(2):85-94.
Mehta, U.J., Hazra, S. and Mascarenhas, A.F. 1993. Somatic embryogenesis and *in vitro* flowering in *Brassica nigra*. *In vitro* Cellular & Developmental Biology Plant, 29: 1-4.
Moallem, S., Behbahani, M., Mouavi, E. and Karini, N. 2012. Direct regeneration of *Rosa canina* through culture. Trakia Journal of Science,Vol.10(3); 23-25.
Morel, G. 1950. Surla culture des tissue de deux monocotyledons. Comptes Rendus del Academine Sciences, 230:1099-1101.
Mukhambetzhanov, S., Mursalieva, V. and Rakhimbaev, I. 2011. Micropropagation of rose cultivar 'Pareo'. African Journal of Biotechnology, Vol. 10 (46): 9344 -9346 .
Murali, S., Sreedhar, D. and Lokeswari, T.S. 1996. Regeneration through somatic embryogenesis from petal –derived calli of *Rosa hybrid* L. cv. Arizona (hydrid tea). Klumer Academic Publishers. Printed in Netherlands. 271-275.
Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15 : 473-497.
Nadgauda, R.S., Parasharami,V.A. and Mascarenhas, A.F. 1990. Precocious flowering and seedling behaviour in tissue cultured bamboos. Nature 344 :335-336.
Naik, G.R. and Latha, K. 1996. *In vitro* flowering in *Morus alba*. Bullentin of Pure Applied Science, 15B (2)-129-132.
Nak-Udom, N., Kanchanapoom, K. and Kamnoon, K. 2009. Micropropagation from cultured nodal explants of rose (*Rosa hybrida* L. cv. 'Perfume Delight'). Songklanakarin Journal of Science and Technology, 31 (6):583-586.
Narasimhalu, S.B. and Reddy, G.M. 1984. *In vitro* flowering and plant formation from cotyledons of groundnut (*Arachis hypogea* L.). Theoretical and Applied Genetics, 69:87 -91.

Archive of SID

www.SID.ir

Nonomura, T., Ikegami, Y., Morikawa, Y., Matsuda, Y. and Toyoda, H. 2001. Induction of morphologically changed petals from mutagen treated apical buds of rose and plant regeneration from varied petal derived calli. Plant Biotechnology 18(3) :233-236.

Pai, V., Thejavathi, G.H. and Nijaligappa, B.H.M. 1986. International Congress of Plant Tissue Cell Culture 6th Meeting , Minnesota (USA) p.176.

Pati, P.K., Rath, S.P., Sharma, M., Sood, A. and Ahuja, P.S. 2005. *In vitro* propagation of rose - a review. Biotecnology Advances, pp 95-111.

Pati, P.K., Sharma, M., Sood, A. and Ahuja, P.S. 2004. Direct shoot regeneration from leaf explants of *Rosa damascene* Mill. *In vitro* Cellular & Developmental Biology Plant ,40:192-195.

Pawlowska, B. 2011. The effect of BA and GA_3 on the shoot multiplication of *in vitro* cultures of polish wild roses. Folia Horticulture, 23 (2):145-149.

Purohit, V.K., Negi, V., Phondani, P.C., Rawat, L.S., Maikhuei, R.K. and Joshi, S.C. 2008. Assessmnt of root formation to stem cutting of *Spondias pinnata*. National Academy Science Letters (India) 31(1&2) :17-22.

Rout, G.R. Debata, B.K. and Das, P. 1989. Micropropagation of *Rosa hybrida* L. cv. 'Queen Elizabeth' through *in vitro* culture of axillary buds. Orissa Journal of Horticulture. Vol. 16(182):1-9.

Rout, G.R., Debata, B.K. and Das, P. 1990. *In vitro* clonal multiplication of roses, Proceedings of the National Academy of Sciences (India), 60(B) : 311-318.

Rout, G.R., Debata, B.K. and Das, P. 1991. Somatic embryogenesis in callus cultures of *Rosa hybrida* L. cv 'Landora'. Plant Cell Tissue and Organ Culture. 27:65-69.

Senapati, S.K. and Rout, G.R. 2008. Study of culture conditions for imporved micropropagation of hybrid rose. Hort Science, 35(1); 27-34.

Sengupta, C. and Raghavan, V. 1980a. Somatic embryogenesis in carrot cell suspension .I. Pattern of protein and nucleic acid synthesis. Journal Experimental Botany, 31:247 -258.

Sengupta, C. and Raghavan,V. 1980b. Somatic embryogenesis in carrot cell suspension .II. Synthesis of ribosomal RNA and poly (A) RNA. Journal Experimental Botany, 31: 259 -268.

Shadparvar, V. 2012. Micropropagation of rosa miniature'Green Ice' with IBA and 2ip. European Journal of Experimental Biology, Vol. 2 (3); 832-835.

Shirdel, M., Motallebi-Azar, A., Matloobi, M. and Zaare - Nohandi, F. 2013. Effect of nodal position and growth of dog rose (*Rosa canina*). Journal of Ornamental and Horticultural Plants 3(1): 9-17.

Sisko, M. 2011. Micrpropagation of roses (*Rosa* spp.): The effects of different media on *in vitro* rooting. Agriculture, Vol.8(2); 19-22.

Skirvin, R.M. and Chu, M.C. 1979. *In vitro* propagation of 'Forever Your' Rose. Horticultural Sciences, 14(5): 608-610.

Skirvin, R.M., Chu, M.C. and Young, H.J. 1990. Rose. *In*: Ammirato, P.V., Evans, D.R., Sharp, W.R. and Bajaj, Y.P.S. (Eds.) Handbook of Plant Cell Culture (Vol.5). McGraw Hill Publishing Co. New York. pp. 716-743.

Soomro, R., Yasmin, S. and Aleen, R. 2003. *In vitro* propagation of *Rosa indica*. Pak. J. of Biol. Sci. 6(9):826-830.

Tang, A.F., Cappadocia, M. and Byrne, D. 1983. *In vitro* flowering in Cassava (*Manihot escueleuta* Crantz). Plant Cell Tissue Organ Culture, 2 :199 -206.

Tejavathi, D.H., Devaraj, V.R., Murthy, S.M., Anitha, P. and Nijagunaiah, R. 2010. Regeneration of multiple shoots from the callus cultures of *Macrotylemauni florumlann* Verdc. Indian Journal of Biotechnology, 9(1):101 -105.

Tweedle, D., Roberts, A.V. and Short, K.C. 1984. *In vitro* culture of roses. In: Novak, F.J., Havel, L. and Dolezel, L. (eds.) Plant tissue and cell culture application to crop improvement. Czechoslovak Academy of Sciences (Prague). pp 529 -530.

Virupakshi, S., Manjunatha, B.R. and Naik, G.R. 2002. *In vitro* flower induction in callus from a

Archive of SID

www.SID.ir

Juvenile explants of sugarcane, *Saccharum officinarum* L.var. Coc 671. Current Science 83(10): 1195 -1197.
Vu, N.H., Anh, P.H. and Nhut, D.T. 2006. The role of sucrose and different cytokinins in the *in vitro* floral morphogenesis of rose (hybrid tea) cv.'First Prize'. Plant Cell Tissue Organ Culture, 87:315 -320.
Wang, G.Y., Yuan, M.F. and Hong, Y. 2002. *In vitro* flower induction in roses. *In vitro* Cellular & Developmental Biology Plant, 38:513 -518.
Yoshikawa, T. and Furuya, T. 1983. Regeneration and *In vitro* flowering of plants derived from callus cultures of opium poppy (*Papaver somniferum*). Experientia 39:1031-1033.
Zabbarzadeh, Z. and Khosh–Khui, M. 2005. Factors affecting tissue culture of damask rose (*Rosa damascence* Mill). Scientia Horticulturae, 105(1) : 475-482.
Zeng, S.J., Liang, S., Zhang, Y.Y., Wu, K.L., Da Silva, T. and Duan, J. 2013. *In vitro* flowering red miniature rose. Biologia Plantarum. 57(3):401-409.

Archive of SID

www.SID.ir