



A REVIEW ON IN VITRO MICROPROPAGATION OF ROSA DAMASCENA MILL L.

Uma Maheswari N.* and Vaishnavi K

P.G. and Research Department of Microbiology S.T.E.T Women's College, Sundarakkottai,
Mannargudi-614 016, Tamil Nadu, India.

***Corresponding Author: Uma Maheswari N.**

P.G. and Research Department of Microbiology S.T.E.T Women's College, Sundarakkottai, Mannargudi-614 016, Tamil Nadu, India.

Article Received on 18/03/2018

Article Revised on 08/04/2018

Article Accepted on 29/04/2018

ABSTRACT

In tissue culture such as rapid multiplication, cultivar development via somaclonal variation and genetic transformation. Tissue culture allows overcome some of the sterility problems through direct regeneration. *In vitro* propagation rose has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants. During the last several years, different approaches have been made for *in vitro* propagation of rose. Micropropagation using apical buds or nodal segments and understanding the specific requirements at different stages has been comprehensively covered in literatures. Roses are the most economically important flowers in the world. 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*. Traditionally, most ornamental roses are heterozygous and do not breed true to type, they are therefore propagated vegetatively. Miniature roses are usually propagated by cuttings but the roses are usually propagated by budding are bench-grafting onto rootstocks of species such as *Rosa canina* 'Inermis' and *Rosa multiflora* 'simplex'. The conventional propagating methods are very slow, time consuming and tiring.

KEYWORDS: *Rosa damascena* Mill L., *in vitro*, Micropropagation, Shoot multiplication, BAP, Root induction.

INTRODUCTION

Rose (*Rosa hybrida* L.) is the most popular of the flowers because of its beauty and fragrance that is why it is rightly called the queen of flowers. The genus *Rosa* contains more than 1400 cultivars and 150 species^[1], which are grown for rootstocks, curiosity value and striking floral display. Apart from its ornamental values, it is also used for the production of essential oil and vitamin C.^[2-3] These characters make it an important cash crop. Conventionally, it is propagated asexually through cuttings, budding or grafting scion cultivars on specific rootstocks in the particular seasons. These methods are laborious and time taking with very low percentage of success. It has also been observed that plants raised from these methods are infected with different diseases that affect flower production and quality, and ultimately their market value is decreased.^[4] In general cutting of hybrid roses are difficult to root.

Tissue culture methods have been developed as a potential tool for rapid and mass propagation in number of plant species. The central concept of tissue culture is totipotency i.e., every living cell has the genetic information needed to develop into complete organism.^[5] Thus, new avenues became an important alternative of conventional propagation procedures for the plants, especially those propagated vegetatively.

Micropropagation offers not only quick propagation of plants, but also eliminates diseases and provides scope for development of new cultivars.^[6] Tissue cultures system in roses have already been established.^[7-8] To establish a flowering research system *in vitro*, it is necessary to develop a reliable and rapid shoot organogenesis protocol. In this context we describe an efficient protocol. In this context we describe an efficient protocol for the culturing and flowering of *Rosa damascena* L. cv. 'Heirloom' *in vitro*. The application of tissue culture techniques to the regulation and commercial propagation of hybrid roses is more recent developed. The major commercial use of tissue culture techniques in vegetative propagation of hybrid roses is the combination of multiplication and regeneration. Through *in vitro* techniques the small quantity of source material has promoted such research in applying the potential for the purpose of plant propagation. Roses are the most economically important flowers in the world. 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*.^[9] 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. Traditionally, most ornamental roses are heterozygous and do not breed true

to type, they are therefore propagated vegetatively. Miniature roses are usually propagated by cuttings but the roses are usually propagated by budding or bench-grafting onto rootstocks of species such as *Rosa damascena* 'simplex'^[10] The conventional propagating methods are very slow, time consuming, and tiring. Tissue culture on the other hand is becoming increasingly popular as an alternative means of plant vegetative propagation. Roses "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. Rose is generally propagated by vegetative methods like cutting, layering, budding and grafting. Seeds are used for propagation of species and also for production of rootstocks.^[11] 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.^[12] In the last few years, *in vitro* propagation has revolutionized commercial nursery business.^[13] The history of rose tissue culture dates back to 1945, when Nobecourt and Kofler succeeded in obtaining callus and roots on the explanted buds. In the year 1946, Lamments for the first time reported the use of embryo culture in rose breeding. Studies were initiated by^[14] to culture cells, cell suspension and calli with a view to understand differentiation and regeneration. The first shoot organogenesis from callus tissue was reported by^[15] in a climbing Hybrid Tea rose 'The Doctor'. The earliest references of rose micropropagation were those of^[16] and^[17] in *R. hybrida* cv. Superstar and *R. damascena* Mill, respectively.

Although the presence of a cytokinin is almost always advantageous and is often all that is required, optimum rates of shoot initiation generally occur with combinations of auxin and cytokinin.^[18] Natural and synthetic auxins have been used extensively *in vitro* in plant cell tissue and organ culture to obtain specific morphogenic responses. One of the most important applications of auxins is the induction of adventitious root formation. Induction of adventitious roots in roses has been demonstrated by workers such as.^[19] But the presence of auxin in defined combinations with cytokinins in the culture medium is also necessary to obtain adventitious shoot formation.^[20] 'Iceberg', also known as Fee des 'Neiges', a repeat blooming floribunda, was bred by Kordes in Germany and is the result of a cross between 'Robin Hood', a Pemberton bred hybrid musk and 'Virgo' a large flowered hybrid tea rose. It's ever present double white flowers, often with a flush of pink in spring and fall, are lightly fragrant. This rose has won many awards including the National Rose Society Gold Medal in 1958, the Baden-Baden Gold Medal in 1985, the ADR Anerkannte Deutsche Rose in 1960, the world's Favorite Rose in 1983 and the Royal Horticulture Society Award of Garden Merit in 1993. Although tissue culture of roses has been reported by

many authors but procedures for mass production at commercial level has not been reported in the scientific publications. In the present study, attempts were made to assess the interactive influence of BAP (6-benzylaminopurine) and NAA (1-naphthalene acetic acid) concentrations on the growth rate of *Rosa hybrida* cv. Iceberg for the first time, and thus increase growth rate to a level suitable for commercial use. We also describe a procedure for root initiation, root growth and acclimatization of plantlets to *in vitro* conditions.

Micropropagation

Micropropagation has five major advantages compared to the conventional methods of plant propagation; (i) it is an invaluable aid in the multiplication of elite clones of intractable/recalcitrant species; (ii) it is important in terms of multiplying plants throughout the year, with control over most facets of production; (iii) it is possible to generate pathogen-free plants even from explants of infected mother plants; (iv) plant materials such as male sterile, fertility maintainer and restorer lines can be cloned; and (v) it enables the production of a large number of plants in a short time from a selected number of genotypes.^[21] Micropropagated explants require less space and labor inputs for the maintenance of germplasm collections.^[22] The *in vitro* conservation methods are considered particularly interesting for those horticultural species that are propagated by vegetative methods as well as those with recalcitrant seeds.^[23] A variety of approaches have been used separately or in combination to reduce the growth rate of *in vitro* plant tissues. Probably, the most successful strategies have involved temperature reductions, but the responses vary significantly between and within species. Slow growth is used as a short-term to medium-term conservation in many laboratories, including in the Centro Internacional de la Papa (CIP) and Centro Internacional de Agricultura Tropical (CIAT).^[24] More than 200 species are present in the genus *Rosa*^[25] from which 14 wild species are present in Iran. *Rosa pulverulenta* is a small bush (50 to 100cm) with small pink (and rarely white) solitary flowers (10-25 mm in diameter) or cluster with 2-4 flowers. *R. pulverulenta* usually grows in Asia, South east Europe including West Syria, Caucasus, Azerbaijan, Armenia, Iran and Afghanistan.^[26] Traditionally, most roses are heterozygous and do not breed true type. Therefore, they are propagated by vegetative methods. Since most rose species are difficult to root, conventional propagating methods are very slow, time consuming, and tiring. Tissue culture on the other hand is becoming increasingly popular as an alternative to the conventional plant propagation methods.^[27] In Iran, a large number of *Rosa* species have been exposed to extinction and therefore preservation techniques are necessary to safeguard their future. The most important technique in micropropagation is meristem proliferation where in apical buds or nodal segments harbouring an axillary bud are cultured to regenerate multiple shoots without any intervening callus phase.

Stages involved in micropropagation

A successful micropropagation protocol proceeds through a series of stages, each with a specific set of requirements. These are (i) initiation of aseptic cultures, (ii) shoot multiplication, (iii) rooting of microshoots, (iv) hardening and field transfer of plants raised from tissue culture.^[12]

Initiation of aseptic cultures

Choice of explants: The choice of explants for initiation of culture is largely dictated by the method to be adopted for *in vitro* propagation. Explants with vegetative meristem are often suitable for enhanced axillary branching. The most commonly used explants is a nodal stem segment, wherein the axillary bud is made to proliferate to form multiple shoots. The performance of nodal segments is much better than the shoot tips.^[11]

Sterilization

The middle part including axillary buds of *R.damascena* Mill L. from vegetative shoots were excised and collected. The explants were surface sterilized by dipping in 70% ethanol for 30s, then incubated in 10% Clorox (sodium hypochlorite 5.25%) with 3 drops of tween-20 for 10 min, followed by 5% Clorox for 5 min and subsequently rinsed three to four times with sterile distilled water. Axillary node (1 cm long) sections were excised and transferred to glass bottles with plastic closures (1 explant per flask).

The nodal explants were cultured on semisolid MS^[28] basal medium supplemented with different concentrations 6-benzylaminopurine (BAP) indole-3-acetic acid (IAA) or 1-naphthalene acetic acid (NAA) alone or in combination. Agar-agar was added to the medium as gelling agent after adjusting the pH to 5.7-5.8 using 0.1N NaOH or 0.1N HCL before autoclaving. 20 ml of the molten medium was dispersed into the culture tube (25×15mm) plugged with non-absorbent cotton wrapped in one layer of cheesecloth and autoclaved at 121⁰c or 1.06 kg/cm² for 15 min. The explants were inoculated aseptically. The cultures were maintained at 25±2⁰c under 16 hrs photoperiod. Subsequently, the cultures were maintained by regular subculture at four week intervals on fresh medium with the same composition.

Browning of the medium

Browning of the medium is the result of oxidation of polyphenols exuded from the cut surface of the explants which could be overcome by adding substances such as PVP (polyvinylpyrrolidone), citric acid or ascorbic acid or resorting to frequent subculturing^[29] or incubating cultures for a day or two in total darkness after inoculation as polyphenols oxidase activity was found to be induced by light.^[30] Stated that 3 days of culture on the medium containing charcoal followed by transfer to a fresh medium was highly effective in enhancing the growth of primary explants.^[31]

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. MS^[28] basal medium (without hormone) was used for the *in vitro* of induction of explants in culture; the pH of the medium was adjusted to 5.8 before adding 8g l⁻¹ plant agar. Media were autoclaved for 15 min at 121⁰c and 1.2kPa pressure. Cultures were placed under high pressure metal halide lamps on a 16/8 hour light/dark cycle in a culture room maintained at 21±1⁰c. Axillary shoots were detached and transferred to MS medium^[32] in which FeNaEDTA was replaced by FeEDDHA as iron source after 14 days.

Proliferation Medium

The basal nutrient medium containing MS^[28] salts and vitamins was used with NAA, BAP and GA₃. 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 2mg l⁻¹ and GA₃ 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.

Shoot proliferation

The shoot proliferation media contained full strength MS salts and vitamins with various levels of BAP (0, 2, 4 and 8µM) in combination with NAA (0, 0.05, 0.25 and 0.5µM). Each treatment involved 5 repeats with 5 explants (25 explants). Number of axillary shoots and number of new leaves were recorded after 21 days for three subsequent subcultures and the averages were calculated.

This is the most crucial stage of micropropagation. The success of a micropropagation protocol, to a large extent, depends on the rate and mode of shoot multiplication. Various factors that influence *in vitro* shoot multiplication in rose are listed below.

Media

MS^[28] medium was found to be the most commonly used for rose propagation.^[33] Reported that the standard MS medium induced the best rates of shoot proliferation in different rose cultivars. However, the use of other media has also been reported.^[30] Used Linsmair and Skoog's medium supplemented with BAP (0.5 mg/l) and IBA (0.1 mg/l) for shoot initiation.^[34] Also used Linsmair and skoog's medium and obtained faster rates of proliferation with BAP (0.1-2.5 mg/l). Other media like Gamborg's and Lee and de Fossard's were used by Alekhno and Quorine Lepoivre (QL) and woody plant medium (WPM) were used for micropropagation of *R.hybrida* cv. Moneyway (van der salm *et al.*, 1994).^[32]

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. For rooting, shoots were transferred to the MS medium supplemented 3mg/l active charcoal and of 0.25 mg/l BAP, 1 mg/l NAA, 0.5 mg/l NAA+0.25 BAP, 1 mg/l NAA+0.25 BAP and 1.5 mg/l NAA+0.25 MG/L BAP. Root length and percentage of roots induction were recorded after 40 days.

Rooting of microshoots

For any micropropagation protocol, successful rooting of microshoots is a pre-requisite to facilitate their establishment in soil. Considerable work has been done to enhance rooting efficiency in different rose varieties. Rooting of microshoots can be accomplished both under *in vitro* and *ex vitro* conditions.

Induction of rooting from microshoots

After successful multiplication, the microshoots were excised and transferred to different rooting media for induction of roots. The rate of rooting varied in different varieties and types of media. The result showed that the development of roots from microshoots was obtained on half-strength MS medium supplemented with IBA or NAA or IAA with 2% sucrose. It was also observed that among the three auxins tested, IBA favored a good response as compared to IAA or NAA. The *in vitro* rooting capacity depends on the interaction of internal and external factors.^[35]

Media

^[36]Reported the use of MS medium with major elements reduced to one quarter to one third strength for root induction.^[37] Reported that half-strength MS medium supplemented with NAA (0.54 µM) was adequate for inducing rooting in cv. Bridal Veil of hybrid rose.^[38] Reported that 10 different rose cultivars rooted within 10-14 days on one third strength of MS medium supplemented with IAA at 5.7 µM. Subsequently,^[39] achieved very high percentages of rooting in cv. Queen Elizabeth using long shoots and by dilution of MS medium to one fourth strength without a growth regulator.

Root Initiation^[40]

Shoots were cultured on shoot elongation medium (MS mineral salts and vitamins without hormones) for 21 days prior to rooting treatments. For rooting, three concentrations of MS mineral salts and vitamins (full-, half- and quarter-strength) containing IBA (3-Indolebutyric acid) (0.25 µM) and NAA (0.25µM) were tested in semi-solid and liquid media. For liquid medium, sorbarods (Cellulose support plugs; Sorbarod, Ilacon, UK) were used. Each treatment involved 5 repeats with 5

explants (25 explants). After 21 days, number of roots and their lengths were recorded and data for different concentrations of MS media (full, 1/2 and 1/4) and state of media (semi-solid and liquid) were recorded.

Transferring plantlets to soil

Shoots (3-4cm long) was separated individually and transferred to rooting medium obtained from the first experiment. After 4 weeks, the number of rooted shoots, and number of roots per shoot were recorded. The plantlets (6-7 cm in length) were washed in sterile distilled water to remove traces of medium and then transferred to plastic pots (5 cm in diameter) containing soil (soil: meal of coconut fruit 1:1) under controlled indoor conditions (temperature 25±2°C, 16 hours photoperiod and 2000 lux light intensity). After 1 week the plants were transferred to grow in outdoor condition.

Acclimatization and field establishment

After successful rooting, the rooted plantlets were transferred to pots containing sand for further development of root. Within two weeks, about 10 to 15 roots were developed per microshoots. After two weeks, the rooted plantlets were transferred to 6" arthen pots containing sand: soil: vermicompost in the ratio 1:1:1 and were kept in greenhouse for acclimatization. Watering was made at two day intervals. About 60% of the *in vitro* raised plantlets survived and flowering occurred within one month of transfer. Rooted plantlets with more than 4 roots longer than 10 mm were transplanted to soil. Using a soil mixture consisting of turf soil + garden soil + sand, more than 80% of the plants were successfully established in the greenhouse after 2-3 weeks. After 3 weeks interval 4-6 shoots were appeared in shoot induction medium. Selected shoots were subcultured to obtain multiple shoots. After 9 weeks roots were raised on rooting medium. After 2 weeks the plantlets were separated and transferred in to pot mixture in order to acclimatization in glass house.

Physical factors

Light Illuminance of 1.0 klux and higher illuminance (3.0 klux) inhibited rooting. However, found that 66 µmol m⁻² s⁻¹ for 12-14 hrs proved to be the best for rooting.^[41] Further, they observed better rooting in the microshoots by shading the lower portion of the culture vessels.

Temperature Roots emerged first at 25°C and approximately 2 and 4 days later, at 20°C and 15°C, respectively.^[11] Obtained best rooting response at 21°C and plants cultured at this temperature were also the most successful in the weaning stage. However,^[42] stated that a temperature of 28°C was optimum for *in vitro* rooting.

Plant regeneration

In vitro plant regeneration is often the most important step for successful implementation of various biotechnological techniques used for plant improvement programmes. The effect of subculture time on flower

induction *in vitro* was examined. After two consecutive subcultures, the 80% of the plants were flowering. The flowers were small, had normal petals and sepals, and proceeded to open. It seems that a period of 6 weeks in culture was appropriate for flowering in the present study. This is probably because differentiation to floral phase reached a peak after two consecutive subcultures.

Regenerated shoots were excised and transferred to 1/4 MS medium without growth regulators to induce roots. Rooted shoots were incubated for two weeks prior to transplanting to polystyrene pots containing soil mixture (1 sand: 1 manure: 1 decayed leaves). *In vitro*-derived plants did not display any phenotypic variation during subsequent vegetative development. Rooted plantlets with more than 4 roots longer than 10 mm were transplanted to soil. Using a soil mixture consisting of turf soil + garden soil + sand (2:2:1; v/v/v), more than 80% of the plants were successfully established in the greenhouse after 2-3 weeks.

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.

CONCLUSION

In vitro propagation of rose via somatic embryogenesis offers a great potential for rapid propagation and improvement, and direct regeneration protocols using leaf explants from *in vitro* raised shoots could be effectively used in maintaining the clonal fidelity of elites and in genetic transformation programmes. In conclusion, micropropagation of three commercial rose varieties was established by manipulating the culture condition and growth regulators. This review will help for conservation and commercial propagation of hybrid roses for horticulture as well as pharmaceutical industries. Auxin IBA, IAA and NAA were the most important hormones for the roots and shoots development although *in vitro* flowering was observed, more reliable culture regimes need to be elucidated.

ACKNOWLEDGEMENTS

The authors are express sincere thanks to Dr. V. Dhivaharan, Dean and Department of Life Science, S.T.E.T. Women's college Mannargudi for providing all the facilities to carry out work.

REFERENCES

- Gault, S.M., and P.M. Syngé, *The Dictionary of Roses in Color*. Ebury Press and Michael Joseph, Hague, 1971; 11.
- Bose, T.K., and L.P., Yadav, *Commercial Flowers*. First ed. Naya prokas, Calcutta, india, 1989; 15-20.
- Marchant, R., M.R. Davey, J.A. Lucas and B. Power, Somatic embryogenesis and plant regeneration in Floribunda rose (*Rosa hybrida* L.) *cv*s. Trumpeter and Gland Tidings. *Plant science*, 1996; 120:95-105.
- Norton, M.E. and A.A. Boe. *In vitro* propagation of ornamental rosaceous plants. *Horticulture science*, 1982; 17: 190-191.
- Khan, I.A. and Shaw J.J., *Biotechnology in Agriculture*. Punjab. Agric. Res. Coordination Board Faisalabad, Pakistan, 1988; 2.
- Debergh, P.C., and Read P.E., Micropropagation. In: *Micropropagation: Technology and application*. (Ed.): P.C. Debergh and R.H. Zimmerman. Kluwer Acad.Publishers, London, 1991; 1-13.
- Hsia C.N, Korban S.S, Organogenesis and somatic embryogenesis in callus cultures of *Rosa hybrida* and *Rosa chinesis minima*. *Plant Cell Tissue organ culture* 1996; 44: 1-6.
- Previati A, Benelli C. Da Re, F., Ozudogru, A, Lambardi, M., Micropropagation and *in vitro* conservation of virus-free rose germplasm. *Propagated Ornamental plant* 2008; 8: 93-98.
- Roberts, A.V.,and E.F., Smith, The preparation of *in vitro* rose for transplantation to soil. *Plant Cell Tiss. Org. Cult.*, 1990; 21: 129-132.
- Short KC, Roberts AV, Rosa species. (Roses): *in vitro* culture, micropropagation and the production of secondary products. In: Bajaj YPS (ed). *Biotechnology in agriculture in Forestry*. Medicinal and Aromatic Plants: III. Berlin: Springer-Verlag, 1991; 376-397.
- Horn W.A.H., Micropropagation of rose. In: Bajaj Y.P.S (ed)., *Agriculture and Forestry, Berlin, Springer-Verlag*, 1992; 4: 320-324.
- Pati P.K., Rath S.P., Sharma M., Sood A., Ahuja P.S., *In vitro* propagation of rose: a review. *Biotechnology Advances*, 2006; 24: 94-114.
- Pierik RLM. *Horticulture* new technologies and applications proceeding of the international seminar on new frontiers in horticulture. *Curr Plant Sci Biotechnology Agric* 1991; 12: 141-153.
- Nickel LG, Tulecke W. Responses of plant tissue cultures to gibberellins. *Bot Gaz* 1959; 120: 245-250.
- Hill GP. Morphogenesis of shoot primordia in cultured stem tissue of a garden rose. *Nature* 1962; 216: 596-597.
- Jacob G, Allan P, Bornman CN. Tissue culture studies on rose: use of shoot tip explants: I Auxin: cytokinin effects. *Agroplanta* 1969; 1: 179-188.
- Elliott, R.F., Axenic culture of meristem tips of *Rosa multiflora planta*, 1970; 95: 183-185.
- George EF, Plant propagation by tissue culture the technology. Exegetics Ltd, Basingstoke, UK, 1993; 112-118.
- Skirvin R.M., and Chu M.C., Young H.J., Rose. In: Ammirato P.V., Evans D.R., Sharp W.R., Bajaj Y.P.S. (eds), *Handbook of Plant Cell Culture*, vol. 5. New York, McGraw Hill: 1990; 716-743.
- Caboni E, Tonelli MG, Effect of 1.2-benzisoxazole - 3-acetic acid on adventitious shoot Regeneration and

- in vitro* rooting in apple. *Plant Cell Rep*, 1999; 18: 985-988.
21. Rani, V., Raina, S. N., Genetic fidelity of organized meristem-derived Micropropagated plants: A critical reappraisal. *In vitro* cellular and developmental biology-plant, 2000; 36(5): 319-330.
 22. Rajasekharan, P.E., *In vitro* conservation of horticultural crops. In: ICAR short course on *in vitro* conservation and cryopreservation new options to conserve horticultural genetic resources. Hessaraghatta Lake: *India Institute of Horticultural Research*, 2006; 66-73.
 23. Shikhamany, S.D. Horticultural genetic resources: role of *ex situ* conservation. In: ICAR short course on *in vitro* conservation and cryopreservation new options to conserve horticultural genetic resources. Hessaraghatta Lake: *Indian Institute of Horticultural Research*, 2006; 6-15.
 24. Engelmann, F., *in vitro* conservation of tropical plant germplasm-review. *Euphytica*, v. 1991; 227-243.
 25. Wissemann, V. classification. In: Roberts, A.V., Deberner, T., Gudin, S. *Encyclopedia of rose science*. Oxford: Elsevier Academic Press, 2003; 111-117.
 26. Ercisli, S.R., (*Rosa* spp.) germplasm resources of Turkey. *Genetic Research and Crop Evolution*, 2005; 15(6): 787-795.
 27. Roberts, A.V. Schum, A., Cell tissue and organ culture. In: Roberts, A.V. Debener, T. Gudin, S. (Ed). *Encyclopedia of rose science*. Oxford Elsevier Academic Press, 2003; 57-110.
 28. Murashige T., Skoog A., A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, 1962; 15: 473-497.
 29. Rout G.R., Samantaray S., Mottley J., Das P., *Biotechnology of the rose: a review of recent progress*. *Scientia Horticulture*, 1999; 81: 201-228.
 30. Pittet H, Moncousin C. Multiplication novella du rosier. *Rev Hortie Suisse* 1981; 54: 169-173.
 31. Curir P, Damiano C, Cosmi T., *in vitro* propagation of some rose cultivares. *Acta Hortie* 1986; 189: 221-224.
 32. Van der Salm, T.P.M., Van der Toorn, C.J.G., Hanisch ten Cate, C.H., Dubois, L.A.M., Devries, D.P., Dons, H.J.M., Importance of the iron chelate formula for micropropagation of *Rosa hybrid* L. Maney way T. *Plant Cell Tissue and organ culture*, 1994; 37: 73-77.
 33. Davies D R., Rabid propagation of roses *in vitro*. *Science horticulture*, 1980; 13: 385-389.
 34. Norton ME, Boe AA. *In vitro* propagation of ornamental rosaceous plants. *Horticultural science* 1982; 17: 190-191.
 35. Hyndman SF., Hasegawa PM., Bressan RA. Stimulation of root initiation from cultured rose shoots through the use of reduced concentrations of mineral salts. *Horticultural Science* 1982a; 17(1): 82-83.
 36. Badzian T, Hennen GR, Fotyma-kern J. *In vitro* rooting of clonal propagated miniature rose cultivars. *Acta Hortie* 1991; 289: 329-330.
 37. Khosh-khuim., sink K.C., rooting enhancement of *Rosa hybrid* for tissue culture Propagation. *Scientia horticultural*, 1982c; 17: 371-376.
 38. Sauer A, Walther F, Preil W. Different suitability for *in vitro* propagation of rose cultivars. *Gartenbauwissenschaft* 1985; 50: 133-138.
 39. Douglas GC, Rutledge CB, Casey AD, Richardson DHS. Micropropagation of Floribunda, ground cover and miniature roses. *Plant Cell Tissue Organ Cult* 1989; 19: 55-64.
 40. Maryam Jafarkhani Kermani, Gorban Ali Nematzadeh Mohammad Reza Bihamta *in vitro* propagation of *biotechnology*, 2007; 5: 2-5.
 41. Bressan PH, Kim YJ, Hyndman SE, Hasegawa PM, Bressan RA. Factors affecting *in vitro* propagation of rose. *J Am Social Horticultural Science* 1982; 107: 979-990.
 42. Rahman SM, Hossain M, Islm AKMR, Joarder OI. Effects of media composition and culture conditions on *in vitro* rooting of rose. *Science Horticulture* 1992; 52: 163-169.