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

Effect of Agar and Different Culture Media on the Micropropagation of *Rosa hybrida* cv.'Black Baccara'

Mina Bayanati [1], Dariush Davoodi [2] and Maryam Jafarkhani Kermani [3]
[1] Department of Horticultural Science, Ferdowsi University of Mashhad, Iran
[2] Department of Nanotechnology, Agricultural Biotechnology Research Institute of Iran, P.O. Box 31535-1897, Karaj, I.R. Iran
[3] Department of Tissue Culture and Gene Transformation, Agricultural Biotechnology Research Institute of Iran, P.O. Box 31535-1897, Karaj, I.R. Iran

Received: 03 April 2015 Accepted: 28 June 2015
*Corresponding author's email: mbayanati2014@gmail.com

Abstract

In vitro propagation of plant has played a very important role in rapid multiplication of cultivars with desirable traits and production of healthy and disease-free plants. In the present investigation, the objectives were to optimize the micropropagation of *Rose hybrid* 'Black Baccarat' cultivar. In proliferation step, the nodal segments (1.5 cm) was cultured on both liquid and solid media (MS, VS and WPM). The results showed that the highest shoot proliferation was obtained on VS medium. The highest amount of multiplication and the growth rate were obtained in the liquid medium. For rooting, three concentrations of VS mineral salts (full-, half-, and quarter-strength) containing NAA (0.5 µM) were tested in semi-solid and liquid media. Root initiation influenced by mineral concentration in the medium. The investigation showed that the highest number of roots was observed in semi-solid 1/4 VS medium. Variation in multiplication and growth rate of explants can be explained on the basis of water potential and mineral availability to the explants in the liquid medium.

Keywords: Agar, Growth, Media, Multiplication, *Rosa hybrida,* Tissue culture.

INTRODUCTION

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* (Khosravi *et al*., 2007). In the last few years, *in vitro* propagation has revolutionized commercial nursery business (Pierik, 1991). Tissue culture on the other hand is becoming increasingly popular as an alternative to the conventional plant propagation methods (Roberts and Schum, 2003). 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 (Kumar *et al.*, 2006). Micropropagation has five major advantages compared to the conventional methods of plant propagation: (i) it is an valuable 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 facts 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 (Jafarkhani Kermani *et al.*, 2011).

Horn (1992) marked a clear effect of genotypes on *in vitro* propagation in different cultivars of Floribunda and Hybrid Tea rose. He observed that it was easy to propagate cultivars Kardinal and Lilli Marleen, whereas it was very difficult to propagate Anthena, Mercedes, Pasadena and Golden Times.

By using liquid medium, it may be possible to reduce costs to a level lower than solid medium and liquid medium is better than solid medium in growth. Both the brand and concentration of agar also affect the chemical and physical characteristics of a culture medium (Debergh, 1983). Agar concentration and agar brand are known greatly influence the growth response of micropropagated plants (Signha *et al*., 1985; von Arnold and Eriksson, 1984). The effects of agar concentration are on the shoot elongation, and leaf and apex necrosis. Shoot elongation was shown to decrease with increasing agar concentration (von Arnold and Eriksson 1984), leaf mineral deficiency (necrosis of the apex) and their subsequent drying in vitrified plants (Debergh and Maene, 1984).The use of liquid medium for *in vitro* culture has many advantages and has been the subject of many studies over many years. It has also frequently been considered an ideal technique for mass production as it reduces manual labor and facilitates changing the medium composition (Berthouly and Etienne, 2002). Althorugh many positive results have been founded with liquid culture, vitrification, physiological disorder of tissue culturesd plants in which tissue, exhibit translucency, hyperhydric transformation, water loggying or glassiness, has been reported for many crops cultured directly in liquid media (Chu *et al*., 1993). It is well known that agar as a solidifying agent can have an effect on the growth and development of *in vitro* cultures (Pierik *et al*., 1997).Water relation and growth of plant *in vitro* are assumed to be closely related to the water status of the culture medium. However, only a few studies have concerned water status of plant *in vitro*. The objective of the study was to investigate the best media for micropropagation of *Rosa hybrid* cv. 'Black Baccara' *in vitro* condition.

MATERIALS AND METHODS

Plant material and general procedures

Nodal segments (1-1.5 cm) were taken from the stems of 'Black Baccara' plants in the rose garden of the Agricultural Biotechnology Research Institute of Iran (ABRII). They were washed thoroughly with running tap water for 30' and surface sterilized for 30 seconds in 70% (v/v) ethanol, followed by a 15 min soak in 2.5% (v/v) sodium hypochlorite solution with a few drops of Tween-20 as a wetting agent, and then rinsed three times with sterile distilled water. MS (Murashige and Skoog, 1962) 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 8 g/L plant agar. Media were

autoclaved for 15 min at 121°C and 1.2 kPa 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 ± 1°C.

Shoot proliferation step

Three culture media were employed; MS medium (Murashige and Skoog,1962), VS medium (van der Salm *et al*., 1994) and WPM medium (Mc Crown and Lioyd, 1980) macro and micro element (Duchefa). Axillary shoot of *Rosa* was cultured on both liquid and gelled (Fig. A). The pH medium was adjusted to 5.8 before autoclaving medium containing BAP_2 µM/l. Multiplication growth rate were recorded after 21 days for three subsequent subcultures and the averages were calculated.

Rooting stage and acclimatization

Shoots were cultured on shoot elongation medium (VS minerale salts and vitamin without hormones) for 27 days prior to rooting treatments. For rooting, three concentrations of VS mineral salts and vitamins (full-, half-, and quarter-strength) containing NAA (0.5 µM) were tested in semi-solid and liquid media. Each treatment involved 3 repeats with 3 explants. After 21 days, number of roots and their lengths were recorded and data for different concentrations of VS media (full, 1/2 and 1/4) and state of media (semi-sold and liquid) were recorded. Plantlets were acclimatized using a soil mixture consisting of peat moss and sand 1:1 (v/v) and successfully transferred to the greenhouse after 3 weeks.

Experimental design and statistical analyses

For the proliferation stage experiments were conducted as factorial experiments based on RCD with 5 replications and each replication included 3 explants in one glass baby food jar per treatment. Rooting experiment, *in vitro* conservation and recovery of shoots were carried out in a factorial based completely random design with 3 observations and 3 replications. In the rooting stage, the percentage of rooting, number of roots per plantlet and total root length per plantlet were recorded after four weeks. Analysis of variance was performed and comparisons of means were conducted using Duncan's Multiple Range Test.

RESULTS

Shoot proliferation

The results showed that there was significant difference between the effect of media type and vegetative traits of *R. hybrid* cv. 'Black Baccara' in proliferation stage ($p<0.05$). The lowest shoot multiplication was observed on WPM medium while the highest shoots were formed on VS medium and maximum number of leaves per explants (11.07) was production on the VS medium (Table 1). The observation indicates that there were significant differences between solid and liquid media and best result was achieved for proliferation by liquid medium (Table 2). Maximum number of shoot per explants (2.66) was produced on the liquid medium. Maximum number of shoot per explants (3.66) was produced on the VS liquid medium, whereas the maximum shoot length were obtained on the MS liquid medium (Fig. 1 and 2). The growth rate increased from four weeks and continued until the sixth week (Fig. 3).

Table 1. Effect of different media on some vegetative traits of *R. hybrid* cv. 'Black Baccara' in proliferation stage.

Medium Culture	Number of axillary shoots per explant	Number of new leaves produced per explant	Shoot length (cm)
Murashige & Skoog (MS)	2.1 [b]	9.37 [b]	2 [a]
Van der Salm (VS)	2.49 [a]	11.07 [a]	2.1 [a]
Woody Plant Medium(WPM)	1.47 [c]	3.49 [c]	1.78 [b]

Numbers followed by the same letter are not significantly differentns according Duncan Test ($P<0.05$).

Table 2. Effect of liquid and solid media on shoot number and length.

Medium	Number of shoot (per explants)	Length of shoot (cm)
Solid medium	1.66 [b]	1.65 [a]
Liquid medium	2.66 [a]	1.56 [a]

Numbers followed by the same letter are not significantly differentns according Duncan Test (P<0.05).

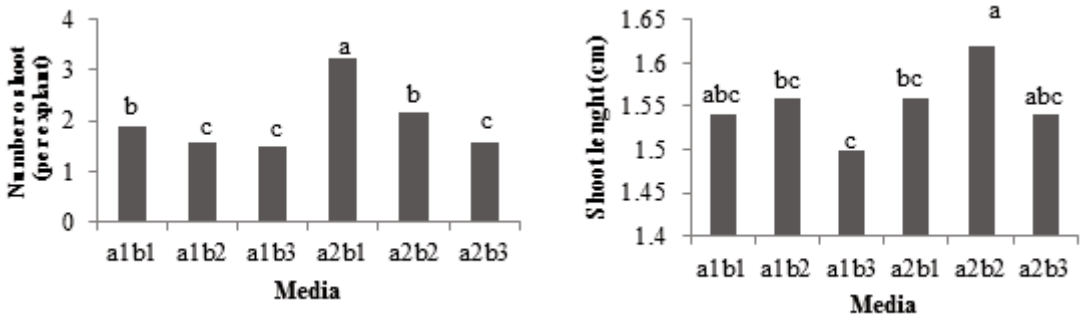

Fig.1. Effect of the basic medium (MS, VS, WPM) and the solid and liquid media on number of shoot and height shoot.

Fig. 2. Shoot proliferation; Left) liquid medium, Right) solid medium.

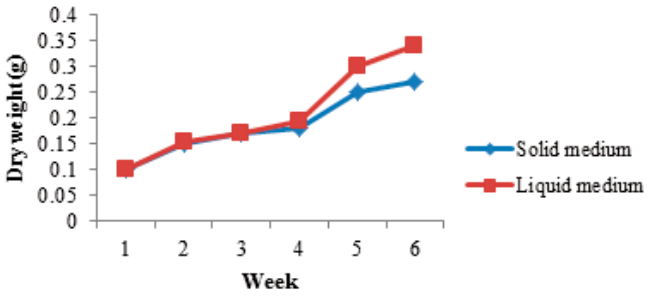

Fig. 3. The growth rate of axillary shoots grown on solid and liquid media within 6 weeks.

Root initiation

The average number of roots (4.6) and root length (4.5 cm) were significantly higher in 1/4 strength VS (Table 3). Table 3 compares the effect of semi-solid and liquid media. Statistical analysis indicates that there was a significant difference between the average root length in semi-solid and liquid media. The highest root length, root number was recorded in ¼ VS medium. The best results were obtained on VS medium containing 1/2 strength of VS macro- micro- salts and vitamins. The rooted plants were not difficult to acclimatization at ±24 °C and relative humidity of 80% during initial stages of development gradually reduced to 40% after 4 weeks of culture and was transferred to the greenhouse for flowering. Fig. 4 illustrates the morphogenetic responses of the shoots treated with three (full, 1/2 and1/4) strengths of VS salts and vitamins.

Table 3. Comparing average percentage of rooting, number of roots produced and root length in different concentrations of VS (full, ½ and 1/4) salts and vitamins and semi-solid and liquid media. Means in each column with different letters show significant differences according to Duncan's Multiple Range Test (P ≤ 0.05).

Concentration	Number of roots per explant	Root length (cm)
VS	1.5 [c]	2.8 [b]
½ VS	4.2 [a]	2.9 [b]
¼ VS	4.6 [a]	4.5 [a]
Semi solid medium	4.9 [a]	3.9 [a]
Liquid	2.7 [b]	1.8 [b]

Numbers followed by the same letter are not significantly differentnsaccording Duncan Test (P<0.05).

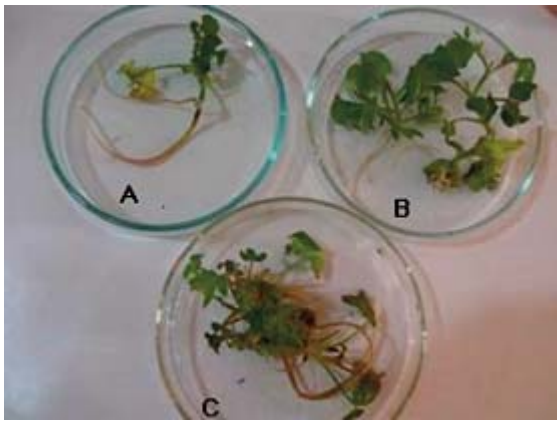

Fig.4. Shoot rooted in three strengths of VS (A: Full, B: 1/2 VS, C:1/4 VS).

DISSCUSION

Plant tissues from numerous species have performed better when cultured in liquid medium rather than on an agar medium (Berthouly and Etienne, 2005).Using liquid media in micropropagation processes is considered to be the ideal solution for reducing plantlet production costs and for considering automation (Debergh, 1983; Aitken-Christie *et al*., 1995). Indeed, liquid culture systems provide much more uniform culturing conditions, the media can easily be renewed without changing the container, sterilization is possible by ultrafiltration and container cleaning after a culture period is much easier (Berthouly and Etienne, 2005). Agar quality could affect, in principle, all developmental processes, the regeneration of adventitious shoots and roots being the most sensitive (Ahmadi *et al*., 2013). Pervios researches have also indicated that availability on cytokinins different a solid versus liquid medium(Chu *et al*., 1993). Perhaps, the higher multiplication rate of 'Black Baccara' on liquid compared to solid medium (Table 2) in our studies was due to greater availability of BAP or other compounds in liquid medium.

Decreasing agar concentration increased mineral availability and growth. Banana (*Musa* spp.) was micropropagated *in vitro* on agar at various concentrations: 0, 4, 6, 8, 10, 12 g l^{-1} (Amiri and Arzani, 2006).Growth is related to soluble mineral uptake. Mineral availability to the explants depends on its solubility and mobility through the gel, both depend on availability of water (free water). In other words, both solubility and transport of minerals decrease (possibly by precipitation and fixation in the gel matrix) with decreasing water availability. It can be mentioned that the availability of water within the system may be adequate for normal growth, but not sufficient for mineral solubility and mineral transport (water as carrier) (Amiri and Arzani, 2006). Variation in multiplication and growth rate of explants can be explained on the basis of water potential and mineral availability to the explants in the liquid medium.

For G N9, WPM and QL media were found to have a better effect on shoot proliferation rate than either MS or MS medium and the possible explanation given for this was the reduced nitrogen content in WPM (Arab *et al*., 2014). Hyndman *et al*. (1982) succeeded in enhancing root

number and length of *in vitro* grown shoots of *R. hybrida* cv. Improved Blaze by lowering total nitrogen concentration of MS salts (6.0 to 7.5 mM) in the culture medium keeping other salt concentrations constant.

The average number of roots and root length were significantly higher in 1/4 strength VS medium which is in accordance with Skirvin *et al*. (1990) who reported that the reduced salt concentration generally increased rooting in MS medium. Kumar *et al*. (2006) demonstrated that a decrease in KNO_3 and NH_4NO_3 concentration was the decisive factor for improving the rooting percentage. Enhanced root initiation and growth in 1/4 strength medium could be attributed to a more favorable nitrogen concentration availability and thus a higher rate of rhizogenesis than provided by full VS mineral salts (Khosravi *et al*., 2007). Rout *et al*. (1990) also reported that rooting of micro-shoots was better in solid medium than that in liquid medium too. Senapati and Rout (2008) reported rooting was readily achieved upon transferring the microshoots onto half-strength MS medium supplemented with 0.25 mg/l IBA and 2% (w/v) sucrose. Although rose shoots often proliferate readily in vitro, rooting of those shoots is proved to be more difficult. Kim *et al*. (2003) suggested that rooting is affected by genotype; MS medium salts concentration, cold dark treatment, and auxin type. The average number of roots and root length were significantly higher in 1/4 strength VS medium which is in accordance with Skirvin *et al*. (1990) who reported that the reduced salt concentration generally increased rooting in MS medium.

CONCLUSION

A micropropagation system for *Rosa hybrida* cv. 'Black Baccara' has been worked out utilizing nodal explants. Our investigation showed that the liquid VS medium with 2 µM/L BAP was the best for proliferation of *Rosa hybrida* cv. 'Black Baccara' and micropropagated plants were rooted and established in soil successfully. Also, the VS medium with additive Fe was better than MS medium in all stages of micropropagation of this plant.

Litrature Cited

Ahmadi, E., Lolaie, A., Mobasher, S. and Bemana, R. 2013. Investigation of importance parameter of plant tissue. International Journal of Agriculture and Crop Sciences. 5 (8): 900-905.

Aitken-Christie, J., Kozai, T. and Takayama, S.1995. Automation in plant tissue culture general introduction and overview. *In*: J. Aitken-Christie, T. Kozai, M. L. A. Smith (eds.) Automation and environment control in plant tissue culture. Kluwer Acad. Publ., Dordrecht, The Netherlands, pp 1-18.

Amiri, M.E. and Arzani, K. 2006. Mineral availability and growth of banana (*Musa acuminate* var. Dwarf Cavendish) explant under various relative matric potential *in vitro*. Journal of Food, Agriculture & Environment.4 (3 and 4): 84-88.

Arab, M., Yadollahi, A., Shojaeiyan, A., Shokri, S. and Shoresh Maleki, G. 2014. Effects of nutrient media, different cytokinin types and their concentrations on *in vitro* multiplication of G· N15 (hybrid of almond · peach) vegetative rootstock. Journal of Genetic Engineering and Biotechnology,12: 81–87.

Berthouly, M. and Etinne, H. 2002. Temporary immersion systems in plant micropropagation. Plant Cell, Tissue and Organ Culture, 69:215-231.

Chou, C.Y., Knight, S.L. and Smith, M.A.L. 1993. Effect of liquid culture on the growth and development of miniature rose (*Rosa chinensis*). Plant Cell, Tissue and Organ Culture, 212:55-249.

Debergh, P.C. 1983. Effects of agar brand and concentration on the tissue culture medium. Physiologiae Plantarum. 59: 270–276.

Debergh, P. and Maene, L. V. 1984. Pathological and physiological problems related to *in vivo* culture of plants. Parasitica, 40:69–75.

Horn, W.A.H. 1992. Micropropagation of rose. *In*: Bajaj, Y.P.S (ed). Biotechnology in agriculture

and forestry Vol 20 High-tech and micropropagation IV. Germany 7 Springer. pp. 320–402.
Hyndman, S.E., Hasegawa, P.M. and Bressan, R.A. 1982. Stimulation of root initiation from cultured rose shoots through the use of reduced concentrations of mineral salts. Horticultural Science, 17(1):82– 83.
Khosravi, P., Jafarkhani Kermani, M., Nematzadeh, G. and Bihamta, M. 2007. A protocol for mass production of *Rosa hybrida* cv. Iceberg through *in vitro* propagation. Iranian Journal of Biotechnology. 5(2): 100-104.
Kim, C.K., Chung, J.D., Jee, S.K. and Oh, J.Y. 2003. Somatic embryogenesis from *in vitro* grown leaf explants of *Rosa hybrida* L. Journal of Plant Biotechnolgy, 5:161–164.
Kumar, P., Siba, R., Sharma, M, Sood, A. and Singh, P. 2006. *In vitro* propagation of rose. Biotechnology Advances. 24: 94-114.
Mc Crown, B.H. and Lioyd, G .B. 1980. Commerically feasible micropropagation of mountain by use of shoot tip culture. Proc. Inter. Plant Propoter. Sco. 30: 421-437.
Murashige, T. and Skooge, F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue culture. Plant Physiology. 15: 473-497.
Pierik, R.L.M. 1991. Horticultural new technologies and applications proceeding of international seminar on new frontiers in horticulture. Current Plant Science and Biotechnology in Agriculture. 12: 141-530.
Pierik, R.L.M., Oosterkamp, J., Manschot, G.A.J., Barth, T. and Scholten, H.J. 1997. Agar brand ± A dominating factor for shoot growth of juvenile and adult *Quercus robur* L. `Fastigiata' *in vitro*. Plant Tissue Culture Biotechnology. 3(1): 27-31.
Roberts, A.V. and Schum, A. 2003. Cell, tissue and organ culture (micropropagation), *In*: Roberts, A.V., Debener, T. and Gudin, S. Encyclopedia of rose science. Elsevier Academic Press.
Rout, G.R., Debata, B.K. and Das, P. 1990. *In vitro* clonal multiplication of roses. Proc. Natl. Acata Sciences India. 60:8– 311.
Senapati, S.K. and Rout, G.R. 2008. Study of culture conditions for imporved micropropagation of hybrid rose. Hort Science, 35(1): 27-34.
Singha, S., Townsend, E.C. and Oberly, G.H. 1985. Mineral nutrient status of crab apple and pear shoots cultured *in vitro* on varying concentrations of three commercial agars. Journal of the American Society for Horticultural Science. 110: 407-411.
Skirvin, R. M., Chu, M.C. and Young, H.J. 1990. Rose. *In*: Amirato, P.V., Evans, D.A., Sharp, W.R. and Bajaj, Y.P.S. (eds). Handbook of Plant Cell Culture. Mc Graw Hill Publ. Co., Springer-Verlag, NewYork. 716-743.
Van Arnold, S. and Eriksson, T. 1981. *In vitro* studies of adventitious shoot formation in *Pinus contorta*. Canadian Journal of Botany, 59: 870–874.
Vander Salm, T.P.M., Van der Toorn, C.J.G.and Hanischten, Catech. 1994. Improtannce of the iron chelate formula for micropropagation of *Rosa hybrid* L. Moneyway. Plant Cell, Tissue Organ and Culture. 37: 73-77.