International Journal of Innovation in Agriculture Sciences and Rural Development
Vol.1, NO.2 , P : 132 - 139
Received: 25 Des 2018
Accepted: 22 Mar 2019

# Induction of callus in hybrid Rose (Rosa hybrida L.) in vitro culture medium

**Zahra Oghazi**[1]
Master student of Agricultural Engineering (Plant Breeding), Payame Noor University of Mashhad
zo1361@yahoo.com
**Mahmood Valizadeh**
Assistant Professor, Department of Agricultural Engineering, Payame Noor University
Valizadeh_mahmood@yahoo.com
**Ebrahim Mostafavi**
Assistant Professor, Department of Agricultural Engineering, Payame Noor University
Ammbot1991@yahoo.com

**Abstract**
Roses are one of the most beautiful flowers in the world and have a wide variety of uses. The number of rose cultivars in the world is increasing. With the scientific name Rosa hybrida L., hybrid Rose belongs to the Rosaceae family. This plant is an important ornamental and commercial plant in the flower industry, cut flowers, and medicine and perfumery industries. Due to the limitations of propagation by cuttings and other propagation methods of this species, determining the optimal conditions for hybrid rose propagation through tissue culture seems necessary to reduce its propagation costs. For this purpose, a small stem sample containing lateral buds was used for callus formation of hybrid roses. Micro-disinfected samples in MS culture medium containing growth regulators including 6-benzyl amino purine (BAP) with concentrations of 0, 1, 2 and 3 mg/L and naphthalene acetic acid (NAA) at concentrations of 0, 0.1, 0.5 and 1 mg/L were cultured. The experiment was performed as factorial in a completely randomized design with three replications, each of which consisted of 5 micro-samples. The results showed that the highest degree of callus formation was observed in treatments containing micro-samples of hybrid roses at a concentration of 0.5 mg/L NAA and 2 mg/L BAP.
**Keywords:** Hybrid Rose, Callus Formation, In Vitro Culture.

## Introduction

The genus of roses includes hundreds of species and thousands of cultivars, of which roses are undoubtedly one of the most important economic and ornamental plants. Billions of rose bushes are planted in gardens or vases and sold all over the world. A number of species are also used in the perfume and pharmaceutical industries (Olgunsoy et al., 2017). The hybrid rose with the scientific name of Rosa hybrida L. belongs to the Rosaceae family. There are Roses in many forms: bush, shrub, creeping, and deciduous or non-deciduous, and vary greatly in size, shape, aroma, and color of flower (Horn, 1992). These plants have been modified to produce flowering shrubs, garden roses, cut flowers, potted plants, and perfumes (Short and Roberts, 1991). In the world, the total area under cultivation of cut and potted roses is estimated at more than 16,000 hectares and in the outdoor is more than 3,000 hectares (Brichet, 2003). Propagation of rose species or cultivars is

1- Corresponding Author

traditionally done by seed, cuttings, bud or branch transplantation. In seed propagation, the plants produced may not be the same as the mother plant, and vegetative growth is very slow and time consuming. In the method of propagation by cuttings or grafting, there is a limitation of the mother base. In vitro cultivation of roses is one of the methods of producing new cultivars, the advantage of which is the elimination of the use of different rootstocks for different soil conditions, the use of cultivars compatible with different environmental conditions, rapid increase of cultivars and superior rose rootstocks, production of plants without disease and accelerate remedial programs (Olgunsoy et al. 2017). Today, the use of new tissue culture techniques for these plants is very important (Gremiaux et al., 2016). So far, this technique has been used to propagate a large number of ornamental plants (Nunes et al., 2018). Micropropagation seedlings are suitable for the production of cut flowers and more and better branching, and in some cases their flowering performance is higher. On the other hand, dwarf tissue culture roses, as potted plants, have higher growth rate, earlier flowering and shorter branches with more lateral branches than propagating plants obtained from traditional methods (Pati et al., 2006). Today, a large number of rose cultivars in the world have been improved and their number is increasing (Mostashar et al., 1398). In vitro culture of hybrid roses has been the subject of numerous studies such as lateral bud, stem tip, petiole, leaf, anther, petal and embryo culture. In 2006, a study was conducted on callus formation of rose hybrid petals. The best callus treatment was reported at 2 mg/L 2,4-D and 1 mg/L BAP (Khosh-Khui and Teixeira da Silva, 2006). In another study, callus production from hybrid rose petals was performed in MS medium containing 0.5 mg/L BAP and 1 mg/L NAA (Tarrahi and Rezanejad, 2013). In a study on callus formation of hybrid roses, the results showed that hybrid rose leaf explant on medium with low concentrations of NAA and 2,4-D (0.1 mg/L), induced low levels of induction of calluses and high concentrations of NAA + Kin hormone for producing bulky calluses (Kim et al., 2003). In another study on rose micropropagation (Rosa Hybrida var. Maurossia) through leaf microsamples, the results showed that the use of BAP hormone with NAA increased callus formation and indirect organogenesis. So that the highest percentage was at a concentration of 0.8 mg/L NAA and 2 mg/L BAP (Amin Salehi et al., 1398). In the study of regeneration of seven-color miniature roses in lateral bud culture, among 38 hormonal treatments in MS medium and 16 hormonal treatments in B5 medium containing 2 and 3 mg/L of 2,4-D hormone and 0.5 mg/L of NAA hormone in MS medium and medium containing 5 mg/L of 2,4-D hormone and also medium containing 0.5 mg/L of 2,4-D hormone, 2.5 mg/L of NAA hormone and 0.5 mg/L of Kinetin hormone in B5 medium were the best medium for callus production that had no statistically significant differences (Vishlaghi et al., 2010). Other studies have been performed on callus formation of other rose species. In a study, Mahdavi Mashki et al. (2014) cultivated Rosa damascena anthers. The results showed that in Kashan ecotype, replacement of calcium nitrate with ammonium nitrate increased callus formation. In rose, amino acids played an important role in the percentage of callus production of anther (Mahdavi Mashaki et al., 2014). In another study, callus production in Rosa gallica from vegetative explant including petioles, leaves and stems was examined. The results showed that concentrations of 2-3 mg/L 2,4-D and 1 mg/L BAP were optimal for stimulating callus production in different explants and GA3 reduced callus production (Reza Nejad and Tarrahi, 2013). Most reports have shown that the use of auxin has been effective in the production of calluses from in vitro leaves. These reports report the use of various concentrations of 2,4-D hormone in callus production (Hsia et al., 1996; Zakizadeh et al., 2008). The results of various experiments on embryo production show that the use of

BAP hormone along with NAA hormone has been effective in the production of embryogenic callus (Firoozabady et al., 1994). Despite commercial applications, these studies are very limited because very contradictory results and low propagation rates have been obtained in many important rose cultivars (Carelli and Echeverrigaray, 2002). Also, there are large differences in the type and concentration of plant growth regulators, culture medium and different concentrations of nutrients in rose species (Pati et al. 2006). For these reasons, despite studies performed, new studies are being conducted on the rose species. Accordingly, callus formation in hybrid roses was investigated using growth regulators in vitro medium.

**Research Methods**

**Selecting microsample and disinfection**

To achieve a suitable hormonal treatment for callus formation, first the hybrid rose branches with a length of 15-12 cm were separated from the mother plant and its extra leaves were removed. Then, from the middle part, the stems were cut into 2-3 cm pieces containing at least one lateral bud and washed under running water with a few drops of dishwashing liquid for 30 minutes. The microsample containing the lateral buds under the airflow laminar were first disinfected with 25% sodium hypochlorite solution for 20 minutes and then with 70% alcohol for 30 seconds and finally washed thoroughly with sterilized distilled water in three phases.

**Deployment phase**

Each of the disinfected microsamples in MS base medium containing growth regulators of NAA at concentrations of 0, 0.1, 0.5 and 1 mg/L in combination with BAP with concentrations of 0, 1, 2 and 3 mg/L were cultured. 20 days after planting, the number of callus formation in each treatment was evaluated based on the total number of cultured microsamples.

**Cultivation conditions**

To the MS medium was added 30 g/L sucrose as a carbon source, 7 g/L agar. The pH of the culture medium was adjusted to 5.8 before adding agar. The culture medium in the autoclave was disinfected at 121 ° C with a pressure of 1.2 kPa for 20 minutes. In each replication, 5 micro-hybrid rose specimens were cultured in 400 cc jars containing 25-30 ml of culture medium. All cultures were kept in the growth room under 25±2° C and in the photoperiod for 16 hours of light and 8 hours of darkness.

**Data analysis**

The data obtained in factorial experiment were analyzed in a completely randomized design with three replications in each treatment. Statistical analysis of the design was performed using SAS software. The averages were compared using Duncan's multiple range test with a probability level of 5%.

**Findings**

Comparison of averages based on Duncan test showed that there was a significant difference between different hormonal treatments at the level of 0.01 in the amount of callus formation (Table 1).

**Table 1- Analysis of variance of the effect of different hormonal treatments on callus induction in hybrid roses**

| The average of squares of MS | | |
|---|---|---|
| Callus formation | The degree of freedom of DF | Sources of changes |
| 15.9652** | 3 | BAP |
| 3.5763** | 3 | NAA |
| 5.4652** | 9 | BAP × NAA |

** and * indicate a statistically significant difference in the probability level of 1%, 5% respectively and ns is non-significant.

The presence of low concentrations and even the absence of NAA hormone along with high concentrations of BAP increased callus formation in the microsample (Table 2).

**Table 2- Comparison of the average number of callus formation in different hormonal treatments**

| Plant growth regulators (mg/L) | | The number of calluses |
|---|---|---|
| BAP | NAA | |
| 0 | 0 | 0i |
| 1 | 0 | 3c |
| 2 | 0 | 2.33d |
| 3 | 0 | 2.33d |
| 0 | 0.1 | 0i |
| 1 | 0.1 | 2.33d |
| 2 | 0.1 | 3.66b |
| 3 | 0.1 | 1.33f |
| 0 | 0.5 | 0.66h |
| 1 | 0.5 | 3.66b |
| 2 | 0.5 | 5a |
| 3 | 0.5 | 1g |
| 0 | 1 | 2e |
| 1 | 1 | 3c |
| 2 | 1 | 0i |
| 3 | 1 | 0i |

The same letters in each column have no significant difference at the 5% level based on Duncan's test.

The highest callus formation was observed in the treatments containing the concentration of 0.5 mg/L of NAA hormone (Diagram 1).

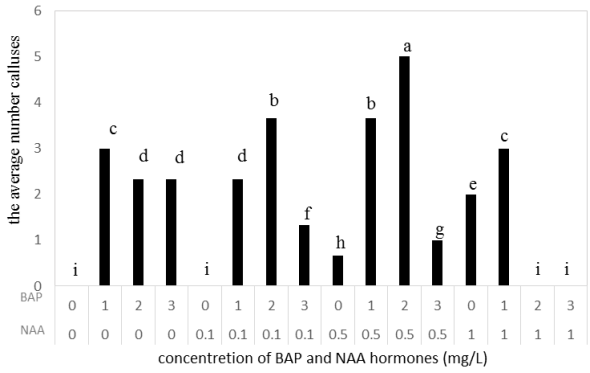


**Diagram 1- Effect of different concentrations of plant growth regulators on the average number of calluses**

Increasing the concentration of BAP hormone to 3 mg/L reduced callus formation in the microsamples (Figure 1). Lack of BAP hormone in low NAA treatments significantly

reduced callus formation, but the presence of BAP hormone concentration alone caused callus formation. Increasing the concentration of NAA and BAP hormones in the treatments caused a significant decrease in the amount of callus formation in the microsamples. The highest callus formation was observed in the treatments containing microsamples at a concentration of 0.5 mg/L NAA and 2 mg/L BAP (Figure 2). The results of this experiment showed that the absence of NAA hormone caused callus production on its own, but its small amount in the presence of BAP increased bulk of callus.

**Figure 1- Callus formed hybrid rose microsamples in treatments containing high concentrations of BAP and NAA**

**Figure 2 - Callus formation in treatments containing hybrid rose microsamples at a concentration of 0.5 mg/L of NAA and 2 mg/L of BAP**

**Discussion and conclusion**

In this experiment, callus formation in Rosa hybrida L. was investigated using BAP hormonal treatments in combination with NAA in MS culture medium. Studies have shown that in the Rosaceae family, genotype plays an important role in how surface disinfectants effects. In one study, the effect of disinfectants on hybrid rose microsamples for callus formation and regeneration was investigated. The results showed that the use of alcohol with sodium hypochlorite had a positive effect on the elimination of pathogens (Amin Salehi et al., 2016). For this reason, these materials were used to disinfect the microsamples in this experiment. In a number of studies on roses, such as the present study, MS base culture medium has been identified as a suitable base medium for propagation, which it depends on genotype (Asareh et al., 2006). In addition to the base medium, the effect of growth regulators on callus formation of microsample is undeniable. Growth regulator of benzyl adenine (BAP) in low concentrations in some rose cultivars has stimulated callus formation

of lateral buds and in others has been ineffective. Our results are consistent with the results of Amin Salehi et al. (1398) in the use of BAP hormone in callus formation of roses. In this study, the highest percentage of callus formation was related to the concentration of 2 mg/L BAP that its increasing has decreased the percentage of callus. It seems that the high use of BAP in this plant has inhibitory effects on callus formation of buds. The use of low concentrations of auxin hormones (2,4-D, NAA, IAA, and IBA) alone or in combination with cytokinin has been reported to be successful in callus production in many rose genotypes (Rout et al., 1991; Van der salm et al., 1996; Murali et al., 1996; Kintzios et al., 1999; Dohm et al., 2001; Hsia et al., 1996 and Noriega et al., 1991) which the results are exactly in line with the research conducted in this study. In this study, the highest percentage of callus formation was observed at a concentration of 0.5 mg/L of NAA. Other studies, such as the present study, have identified the effect of low levels of auxin (NAA) along with cytokinin (BAP) on callus formation in roses. Amin Salehi et al. (2017) in the study of hybrid roses of Mascara cultivar observed the highest amount of callus formation in the treatment of 2 mg/L of BAP and 0.4 mg/L of NAA. In another study, among the Rosa indica nodes on modified MS medium with different concentrations of IBA and NAA, they obtained callus (Memon et al., 2001). In this study, calluses were obtained in the same base medium but with different hormones (NAA with BAP). In another study, Rosa hybrida leaf extracts on callus with low concentration of NAA and 2,4-D (0.1 mg/L) induced callus in small amounts and high concentration of these hormones produced bulky calluses ( Kim et al., 2003). This study was contrary to the present results because in this study, NAA hormone was used alone, but in the present study, NAA hormone was used with BAP hormone to induce callus formation. Similar results were obtained in the study of somatic embryo production in hybrid roses (Pirniakan et al., 2014). During another study on the micro samples of Rose Galika, Reza Nejad and Tarrahi (2013) using concentrations of 2-3 mg/L of 2,4-D and 1 mg/L of BAP, stimulated callus formation on different extracts of this plant. In general, not much data is available on callus formation in hybrid roses, and due to inconsistent data on callus formation in this plant, this experiment was performed to optimize callus formation in hybrid roses. Based on the results, callus formation in hybrid roses is affected by growth regulators and the optimal use of this factor in callus formation to regenerate and use somaclone diversity is essential.

**References**


1- Amin Salehi, Mahsa; Jonoobi, Parisa; Razavi, Khadijeh; and Zeinipour, Masoumeh (1398). Micropropagation of rose (Rosa hybrid. Cv. Maurossia) and anatomical comparison of vegetative organs in regenerative and agricultural plants. Journal of Developmental Biology. Year 11 (4), 58-59.

2- Reza Nejad, Farkhondeh, and Tarrahi, Roshanak (2013). Effect of light and plant growth regulators on callus formation and anthocyanin accumulation in calluses from different explants (Rosa gallica). Journal of Plant Research. Volume 26 (2), 195-184.

3- Saeedi, Abbas; Irvani, Neda; and Zare Karizi, Amir Reza (1391). In vitro propagation of Rosa hybrida cv. City of leads through lateral bud culture. Journal of Crop Biotechnology, Year 2 (2), 87-95.

4- Asareh, Mohammad Hassan; Qamari Zare, Abbas; Ghorbanli, Mahlaqa; Allahverdi Mamqani, Bahareh; and Shahrzad, Shokoofeh (1385). Effect of culture medium and growth regulators on in vitro culture of Rosa damascena. Journal of Research and Construction in Agriculture and Horticulture, Volume 51 (4), 45-72.

5- Mostashar, Frank; Samiei, Leila; And Azadi, Pejman (1398). Increasing the fruiting of rose shoots of Rosa hybrida var. Boulevard grows in in vitro conditions using stimulants.

11th Iranian Congress of Horticultural Sciences. Urmia University.
6- Vishgholi, Neda; Jalali Javaran, Mokhtar; and Moeini, Ahmad (1389). Regeneration of a seven-color miniature rose (Rosa hybrida, CV 'Tanbakeda') from somatic embryos. Iranian Journal of Horticultural Sciences. Volume 41 (4), 375-34
7- Brichet, H. (2003). Distribution and Ecology. In: Roberts, A.V. Debener, T., and Gudin S. (eds), Encyclopedia of Rose Science. London, Elsevier Academic Press, 199–227.
8- Carelli, B. P., & Echeverrigaray, S. (2002). An improved system for the in vitro propagation of rose cultivars. ScientiaHorticulturae. 92, 69-74.
9- Dohm, A., Ludwig, C., Nehring, K. & Debener, T. (2001). Somatic embryogenesis in roses. Acta Horticultureae, 547, 341– 7.
10- Firoozabady, E., Moy, Y., Courtney-Gutterson, N. & Robinson, K. (1994). Regeneration of transgenic rose (Rosa hybrida) plants from embryogenic tissue. Biotechnology, 12, 609-613.
11- Grémiaux, A., Girard, S., Guérin, V., Lothier, J., Baluška, F., Davies, E., & Vian, A. (2016). Low amplitude, high-frequency electromagnetic field exposure causes delayed and reduced growth in Rosa hybrida. Journal of plant physiology, 190, 44-53.
12- Horn, W.A.H. (1992). Micro propagation of rose. In: Bajaj, Y.P.S (ed.), Agriculture and Forestry, Vol. 4. Berlin, Springer-Verlag . 320–324.
13- Hsia, C. & Korban, S. S. (1996). Organogenesis and somatic embryogenesis in callus cultures of Rosa hybrida and Rosa chinensis minima. Plant Cell Tissue Organ Cult, 44, 1-6.
14- Hsia, C. & Korban, S. S. (1996). Organogenesis and somatic embryogenesis in callus cultures of Rosa hybrida and Rosa chinensis minima. Plant Cell Tissue Organ Cult, 44, 1-6.
15- Khosh-Khui, M., & Teixeira da Silva. J. A. (2006). In Vitro Culture of the Rosa Species. Floriculture, Ornamental and Plant Biotechnology Volume II. Global Science Books, UK.
16- Kim, C. K., Chung, J. D., Jee, S. K., & Oh, J. Y. (2003). Somatic embryogenesis from in vitro grown leaf explants of Rosa hybrida L. Journal of Plant Biotechnology, 5, 161-4.
17- Kim, C. K., Chung, J. D., Jee, S. K., & Oh, J. Y., (2003). Somatic embryogenesis from in vitro grown leaf explants of Rosa hybrida L. Journal of Plant Biotechnology, 5, 161-4.
18- Kintzios, S., Manos, C. & Makri, O. (1999). Somatic embryogenesis from mature leaves of rose (Rosa sp.). Plant Cell Reports, 18, 467-472.
19- Mahdavi Mashaki, K., Moieni, A., & Jalali Javaran, M. (2014). Investigation on another culture in Damask rose (Rosa damascena Mill.) and miniature rose (Rosa chinesis). Iran. J. Med. Arom. Plant. 30 (1), 84-100. (In Persian)
20- Memon, N. A. E., Mckyes, M. S., Soomro, I. D., & Koondhar, M. (2001). Regeneration of roses via nodal segments in vitro. Sindy University. Research. Journal. (Sci. Ser.), 33, 31-34.
21- Murali, S., Sreedhar, D. & Lokeswari, T. S. (1996). Regeneration through somatic embryogenesis in Rosa hybrida L cv. Arizona I hybrid tea, Euphytica, 91, 271-5.
22- Noriega, C. & Sondahl, M. R. (1991). Somatic embryogenesis in hybrid tea roses. Biotechnology, 9, 991–993.
23- Nunes, S., Sousa, D., Pereira, V. T., Correia, S., Marum, L., Santos, C., & Dias, M. C. (2018). Efficient protocol for in vitro mass micro propagation of slash pine. In Vitro Cellular Developmental Biology Plant, 54 (2), 175-183.
24- Olgunsoy, P., Ulusoy, S., & Çelikkol Akçay, U. (2017). Metabolite Production and Antibacterial Activities of Callus Cultures from Rosa damascena Mill. Petals. Marmara Pharm J, 21(3), 590-597.
25- Pati, P. K., Rath, S. P., Sharma, M., Sood, A. & Ahuja, P. S. (2006). In vitro

propagation of rose a review. Biotechnology Advances, 24(1), 94-114.
26- Pirniakan, B., Kalantari, S., & Babalar, M. (2014). Somatic embryogenesis from leaf & petiole explants of some Rosa hybrida L. cultivars. International Journal of Biosciences, 5 (11), 1-7.
27- Rout, G. R., Debata, B. K. & Das, P. (1991). Somatic embryogenesis in callus cultures of Rosa hybrida L. cv. Landora. Plant Cell Tissue Organ Culture, 27, 65–69.
28- Short, K.C., & Roberts, A.V. (1991). Rosa spp. (roses): In vitro culture, micro propagation, and the production of secondary products. In medicinal and aromatic plants III. Springer- Verlag, Berlin Heidelberg. 377-397.
29- Tarrahi, R., & Rezanejad, F. (2013). Callogenesis and production of anthocyanin and chlorophyll in callus cultures of vegetative and floral explants in Rosa gallica and Rosa hybrida (Rosaceae). Turk J Bot. 37, 1145-1154.
30- Van der Salm, T. P. M., van der Toorn, C. J. G., Hanischten cate, C. H. & Dons, H. J. M. (1996). Somatic embryogenesis and shoot regeneration from excised adventitious roots of the rootstock Rosa hybrida cv. Money Way. Plant Cell Reports, 15, 522–526.
31- Zakizadeh, H., Debnea, T., Sriskandarajah, S., Frello, S. & Serek, M. (2008). Regeneration of Miniature Potted Rose (Rosa hybrida L.) via somatic Embryogenesis. European Journal of Horticultural Science, 73(3), 111-117.