Efficient callogenesis and Shoot organogenesis from nodal explants of *Rosa hybrida* L. (Lovely girl)

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**ABSTRACT:**

The callogenetic and shoot organogenic potential of ornamental plant, *Rosa hybrida* were investigated. Callus induction and shoot regeneration were induced from nodal explants of garden plants incubated on Murashige and Skoog (MS)-medium supplemented with different concentrations of Plant Growth Regulators (PGRs). The best callus induction (80-85%) was observed on explants incubated on MS-medium supplemented with 2.0 mg l⁻¹ 6-benzyladenine (BA) and 1.5 mg l⁻¹ BA along with 0.5 mg l⁻¹ α-Naphthaleneacetic acid (NAA) or 0.5 mg l⁻¹ 2,4 dichlorophenoxyacetic acid (2,4 D) after four weeks of culture. When MS-medium supplemented with 1.5 mg l⁻¹ BA alone induced 90% shoot organogenesis after 30-days following culture. 2.0 mg l⁻¹ BA in combination with 1.0 mg l⁻¹ Kin (Kinetin) also induced 90% shooting. Moreover, when shoots were transferred to an elongation medium, the longest shoots (3.9 cm) were observed with similar composition of PGRs. The regenerated shoots were inoculated on rooting medium supplemented with different concentrations of Indole Butyric Acid (IBA), NAA and Indole Acetic acid (IAA) but no rooting was observed.

**Keywords:**
*Rosa hybrida*; Callogenesis, shoot organogenesis, 6-Benzyladenine.

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INTRODUCTION

*Rosa hybrida* L. (Lovely girl) belongs to the family Rosaceae and is one of the popular ornamental plants worldwide. *Rosa hybrida* (*R. hybrida*) is also called queen of flowers due to its beauty and fragrance (Shabbir et al., 2009). More than 20,000 cultivars were produced from eight wild species in the genus *Rosa*. The cut flowers of *R. hybrida* are used in social events, religious rituals, in medicines and also used for the production of important secondary metabolites Vitamins C and essential oils (Kim et al., 2003; Shabbir et al., 2009).

According to the literature cited, *R. hybrida* is easily propagated through cutting, grafting and layering asexually but do not give true-to-type plants. Other limiting factors in the conventional propagation of *R. hybrida* are slow multiplication rate and susceptibility to bacterial and fungal diseases. Furthermore, germplasm conservation in seed bank is not pragmatic due to heterozygous nature induced through cuttings (Nair and Gupta, 2006; Khosravi et al., 2007; Razavizadeh and Ehsanpour, 2008). In order to facilitate multiplication rate and to get disease free plant, an efficient protocol is needed for *in vitro* regeneration of *R. hybrida*. *In vitro* regeneration is a potential source for mass propagation of ornamental and medicinally important plants (Ahmad et al., 2010; Abbasi et al., 2010). Razavizadeh and Ehsanpour (2008) reported that *in vitro* regeneration enhance multiplication rate, disease free plants, non seasonal production and germplasm conservation in *R. hybrida*.

The overall objective of current research was to develop an efficient regeneration protocol for *R. hybrida* from nodal explants to produce genetically pure, healthy and vigorous plants in relatively short span of time.

MATERIALS AND METHODS

Nodal explants were collected from 60-days-old garden plant of *R. hybrida*. Before use, the explants were given a quick rinse in 70% ethanol for 2 min, then immersion in 0.1% mercuric hypochloride for 1 min, followed by three rinses with sterile-distilled water. The aseptic explants were cut and cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with/without BAP, 2, 4-D, NAA, Kinetin or IBA, separately or in combination. The phases of callus induction, shooting, shoot elongation and shoot multiplication were sub-cultured/carried out in similar medium. Data on response of explants, number of shoots per explants were collected after day-15 and 40, respectively, and regenerated shoots were excised and sub-cultured on similar medium after day-40. All cultures were incubated in controlled environmental conditions with a 16-h photoperiod under cool fluorescent white light (~50 µmol m⁻² s⁻¹). The design of all experiments was a complete randomized block, and each experiment consisted of 2-3 explants per flask and eight replicate culture flasks per plant growth regulator treatment.

RESULTS AND DISCUSSION

Callogenesis is considered as a significant feature of indirect organogenesis and for research on biologically active molecules in ornamental and medicinal species (Abbasi et al., 2010). Investigations of *in vitro* regeneration were accomplished with callus induction, maintenance of calli, shoot organogenesis and shoot multiplication. The effects of various PGRs such as BA, 2, 4-D and NAA alone or BA in combination with 1 mg l⁻¹ 2, 4-D or 1 mg l⁻¹ NAA on indirect organogenesis were evaluated (Figure 1A-II). Nodal explants of *R. hybrida* used in present study responded to all PGRs used (Figure 3). Best callus induction was recorded on MS medium supplemented with 2.0 mg l⁻¹ BA (85%) and 1.5 mg l⁻¹ BA with 0.5 mg l⁻¹ 2, 4-D (80%; Figure 1A-II). Callus induction recorded for 2, 4-D (25%) and NAA (30%) in combination with BA was significantly lower than other PGRs, and no callus was observed on
MS0 medium. However, addition of 2, 4-D (0.5 to 1.0 mg l\(^{-1}\)) or NAA (0.5 to 1.0 mg l\(^{-1}\)) to the medium containing BA (0.5 to 2.0 mg l\(^{-1}\)) enhanced callus induction (70-85%) to comparative levels of 3.0 mg l\(^{-1}\) BA and 0.5 mg l\(^{-1}\) BA with 0.5 mg l\(^{-1}\) 2, 4-D or 0.1 NAA mg l\(^{-1}\) (Figure 3). Abbasi et al., (2010) reported in recent study that addition of NAA in medium containing BA/GA\(_3\) enhanced callus induction during in vitro regeneration of *Silybum marianum*. From the current experiment it was observed that the medium containing Cytokinins in combination with auxins enhanced callus induction. A similar report was also observed by Ahmad et al., (2010) that different concentration of BA promote callogenesis in important medicinal plant *Piper nigrum* L. There was distinct difference in appearance of callus medium supplemented with different phytohormones. Auxin and Cytokinins have chief effects on callus induction and regeneration, varying their concentration in the medium, cause differences in amount, rate and growth pattern of explants.

Data on shoot regeneration were recorded after 4-5 weeks of subculture (Figure 4). The highest shooting (90%) was recorded for leaf explants cultured on medium containing 1.5 mg l\(^{-1}\) BA alone and 2.0 mg l\(^{-1}\) BA along with 1.0 mg l\(^{-1}\) of Kinetin. Moderate concentrations of BA have shown highest shooting
response, however higher concentration has shown inhibitory action. In the current experiment it was also observed that the medium containing BA and Kinetin along with SA (Adenine Sulphate) inhibit shooting. For the combination containing 0.5 mg l⁻¹ of BA and Kinetin along with 0.5 mg l⁻¹ SA induced maximum 30% shooting in nodal explants. Also the addition of 2, 4-D and SA in medium already containing BA significantly inhibited % shoot induction. These findings were similar to the observations of Kanchanapoom et al., (2010). Nonetheless, BA was more effective than other PGRs used in current report. However, Razavizadeh and Ehsanpour (2008) find 70% shooting was induced in the medium containing combination of TDZ and BA. Shabbir et al., (2009) concluded from his work that combination of BA and Kin is effective in *in vitro* shoot formation from apical meristem of *R. hybrida*. Best shooting of 85% has been produced when the medium was supplemented with 3 mg l⁻¹ of BA. The findings of Tang et al., (2010) was also in agreement with our data that combination of BA and NAA induced 97% regeneration in *Lilium leucanthum*.
Kinetin produced three shoots/explant. Furthermore 8.4 shoots/culture were obtained on medium containing 2.0 mg l\(^{-1}\) BA and 0.5 mg l\(^{-1}\) Kinetin in rose plant (Shabir et al., 2009). The observation of Saglam (2010) are similar to the current study that combination of BA and NAA produced 6.79 cm shoot in Onobrychis sativa LAM. Combination of TDZ (0.05 mg l\(^{-1}\)), Kinetin (0.2 mg l\(^{-1}\)) and NAA (0.1 mg l\(^{-1}\)) induced six shoot/explant (Ozel and Arsalan, 2006). Addition of BA in shoot regeneration medium significantly enhanced number of shoots/explant (Murashige and Skoog, 1962) in different Rosa spp. (Hameed et al., 2006). Previously, the presence of 3.0 mg l\(^{-1}\) BA alone produced 2.6 shoots per explant (Nak-Udam et al., 2009). Surprisingly, shoot induction was recorded for all of PGRs tested in present study (Figure 2). The highest mean shoot length (3.9 cm) was observed in the presence of 2.0 mg l\(^{-1}\) BA in combination with 1.0 mg l\(^{-1}\) Kinetin (Figure 6). Significantly similar mean shoot length of 3.7 cm was recorded for 2.0 mg l\(^{-1}\) BA alone or 2.0 mg l\(^{-1}\) BA in combination with 0.5 mg l\(^{-1}\) Kinetin. However lower mean shoot length was observed with the addition of 2.0 mg l\(^{-1}\) SA in medium containing 0.5 mg l\(^{-1}\) of BA and Kinetin.

Highest number (7.6) of shoots/explant was recorded for 2.0 mg l\(^{-1}\) of BA along with 1.0 mg l\(^{-1}\) Kinetin and lowest number (1.0) of shoots/explant was recorded for 0.5 mg l\(^{-1}\) BA and 0.5 mg l\(^{-1}\) Kinetin and combination of 0.5 mg l\(^{-1}\) of BA and Kin along with 2.0 mg l\(^{-1}\) of SA (Figure 5). It was observed that addition of SA in medium incorporated with either BA or Kin significantly inhibited number of shoots/explant. Similar findings were previously reported for other plant species (Ahmad et al., 2010). Kanchanapoom et al., (2010) reported that the addition of 13.3 mM BA and 9.3 mM Kinetin produced three shoots/explant. Furthermore 8.4 shoots/culture were obtained on medium containing 2.0 mg l\(^{-1}\) BA and 0.5 mg l\(^{-1}\) Kinetin in rose plant (Shabir et al., 2009). The observation of Saglam (2010) are similar to the current study that combination of BA and NAA produced 6.79 cm shoot in Onobrychis sativa LAM. Combination of TDZ (0.05 mg l\(^{-1}\)), Kinetin (0.2 mg l\(^{-1}\)) and NAA (0.1 mg l\(^{-1}\)) induced six shoot/explant (Ozel and Arsalan, 2006). Addition of BA in shoot regeneration medium significantly enhanced number of shoots/explant (Murashige and Skoog, 1962) in different Rosa spp. (Hameed et al., 2006). Previously, the presence of 3.0 mg l\(^{-1}\) BA alone produced 2.6 shoots per explant (Nak-Udam et al., 2009). Surprisingly, shoot induction was recorded for all of PGRs tested in present study (Figure 2). The highest mean shoot length (3.9 cm) was observed in the presence of 2.0 mg l\(^{-1}\) BA in combination with 1.0 mg l\(^{-1}\) Kinetin (Figure 6). Significantly similar mean shoot length of 3.7 cm was recorded for 2.0 mg l\(^{-1}\) BA alone or 2.0 mg l\(^{-1}\) BA in combination with 0.5 mg l\(^{-1}\) Kinetin. However lower mean shoot length was observed with the addition of 2.0 mg l\(^{-1}\) SA in medium containing 0.5 mg l\(^{-1}\) of BA and Kinetin.

Fig. 3. Effects of various concentrations of BA with 2.4-D and NAA. Best % callus induction response in R. hybrida L was observed on MS medium containing 2.0 mg l\(^{-1}\) of BA in combination with 0.5 mg l\(^{-1}\) of NAA. Data were collected after 4 weeks of culture. Values are means of 3 replicates. Each columns with common letters are not significantly different at P<0.05.

Fig. 4. Effects of various concentrations of BA, Kin, SA, and BA with 1 mg l\(^{-1}\) Kin and BA with 2.0 mg l\(^{-1}\) SA on percent shooting in Rosa hybrida L. Data were collected after 5 weeks of sub-culture to MS media with similar composition of plant growth regulators. Values are means of 3 replicates. Columns with common letters are not significantly different at P<0.05.

Fig. 5. Effects of various concentrations of BA, Kin, BA with 1 mg l\(^{-1}\) Kin and BA with 0.5 mg l\(^{-1}\) of Kin and with 2.0 mg l\(^{-1}\) of SA on number of shoots per explant in Rosa hybrida L. Data were collected after 4 weeks of sub-culture to MS media with similar composition of plant growth regulators. Values are means of 3 replicates. Columns with common letters are not significantly different at P<0.05.
Regenerated shoots collected from shoot organogenesis medium were transferred to MS medium supplemented with different concentrations of IBA, NAA and IAA for root organogenesis but unfortunately no rooting was observed. However, Oo et al. (2008) reported that 1 mg l\(^{-1}\) of NAA induced 4-5 roots per culture. 1/4 MS-medium without plant growth regulators induced 75% rooting in \textit{Rosa hybrid} (9,5). While Ozel and Arsalan (2006) reported that ½ strength MS-medium without PGRs induced 50% rooting.

CONCLUSION

From the present investigation it was clearly demonstrated that the BAP alone or in combination with Kin induced shoot induction and multiplication and BA along with NAA and 2, 4-D enhance efficient callus formation.

REFERENCES


