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Research note

Study of embryo rescue in floribunda rose

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Abstract

In the past few decades, breeders have faced a lot of problems in rose improvement due to low sexual reproduction and poor germination because of embryo abortion. Immature embryos may be recovered *in vitro*. An efficient protocol for embryo rescue in two floribunda roses ('Arunima' and 'Shocking Blue') was developed. The germination of immature embryos was achieved by manipulating the growth media, growth hormones and culture conditions. The embryos (rescued) germinated and grew considerably on Murashige and Skoog basal medium supplemented with 2.5 mg l^{-1} BA (6-benzylaminopurine), 0.5 mg l^{-1} GA_3 (gibberellic acid) and 3% (w/v) sucrose under 16-h photoperiod. A higher rate of germination was observed in cultures incubated 2 weeks in dark and subsequently transferred to 2 weeks in light at 16-h photoperiod. The embryo derived plantlets were successfully transferred to greenhouse and produced flowers. Embryo rescue technique in floribunda roses has great potential in floriculture industry.

Abbreviations: BA – 6-benzylaminopurine; 2,4-D – 2,4-dichlorophenoxy acetic acid; GA_3 – gibberellic acid; IAA – indole-3-acetic acid; Kn – kinetin

Rose is one of the most popular flowering ornamentals in the World. Now-a-days, it is a favourite ornamental for landscapes, as well as the most important commercial cut flower. Cut and pot roses are cultivated worldwide over an estimated area of 16,000 ha in glasshouses or plastic tunnels and 3000 ha in the open field (Brichet, 2003). Every year, millions of plants are required to be planted. Further, the breeders are always looking for new and novel varieties to meet the ever-increasing demand of the consumers for new colour, disease-free flowers and enhanced shelf-life of flowers. However, many years are required to develop a new rose variety through conventional methods. Lammerts (1946) reported that the breeding of roses is occasionally hampered by premature abortion of the developing embryo resulting in few or no viable seeds. Because roses are highly heterozygous, this behaviour reduces the efficiency of breeding programs and genetic understandings (Gudin and Mouchotte, 1996). Lack of germination is due to a mechanical restriction of embryo expansion by the presence of a thick pericarp, or dormancy regulated by growth inhibitors within the achene (Von Abrams and Hand, 1956; Jackson and Blundell, 1963; Semeniuk et al., 1963). There are few reports on culture of mature embryos *in vitro* (Lammerts, 1946; Asen, 1948; Asen and Larsen, 1951) but the attempts were poorly defined. Burger et al. (1990) used immature embryos as explant source for *in vitro* organogenesis and plant regeneration. Subsequently, Gudin (1994) and Marchant et al. (1994) reported embryo rescue of *Rosa hybrida* and English roses. The present investigation was undertaken to establish an efficient protocol for

embryo rescue in floribunda roses by manipulating growth media and culture conditions.

Orange colour fruits (rose hips) were collected after 40 days of flowering from greenhouse-grown plants of Arunima (Frolic × seedling) and Shocking Blue (unnamed seedling × Silver Star). Hips were kept in polybags, levelled and incubated in the dark at 4 °C for 7 days. Intact rose hips were washed with 1% (v/v) detergent (Teepol) solution (Qualigen, India) and subsequently washed in running tap water for 30 min. Then, the hips were surface-sterilized by 0.1% (w/v) aqueous mercuric chloride solution for 30 min, followed by three rinses in autoclaved distilled water. After surface sterilization, the hips were opened under aseptic conditions using forceps and scalpel. The hips were placed in sterile water during opening of the achenes to prevent desiccation. After removal of the seed coat, the immature embryo (~0.25 mm long) and surrounding tissues were placed on semi-solid basal Murashige and Skoog (1962) (MS) medium supplemented with different concentrations and combinations of 6-benzylaminopurine (BA), kinetin (Kn), gibberellic acid (GA_3), indole-3-acetic acid (IAA) or 2,4-dichlorophenoxy acetic acid (2,4-D) and 3% (w/v) sucrose. The pH of the medium was adjusted to 5.8 before autoclaving using 0.1 N NaOH or 0.1 N HCl. Routinely, 20 ml of the molten medium gelled with 0.7% (w/v) agar (Qualigen, India) was dispensed into culture tubes (25 × 150 mm), plugged with non-absorbent cotton wrapped in one layer of cheese cloth and sterilized at 121 °C and 1.06 kg cm^{-2} pressure for 15 min. One excised embryo was cultured in each tube. The cultures were incubated in 16-h photoperiod (55 μmol $m^{-2}s^{-1}$) with cool, white fluorescent light at 25 ± 2 °C. Twenty cultures were taken in each treatment and the experiments were repeated twice.

Another experiment was conducted on the photoperiodic cycle. One set of cultures was incubate dinitially 2 weeks in dark and subsequently transferred to light (16-h photoperiod, 55 μmol $m^{-2}s^{-1}$) with cool, white fluorescent lamp at 25 ± 2 °C. In another set, the cultures were incubated in light (24-h photoperiod, 55 μmol $m^{-2}s^{-1}$) and in a third set, the cultures were incubated in light (16-h photoperiod, 55 μmol $m^{-2}s^{-1}$). Fifty cultures were made in each set of experiments and the experiments were repeated thrice. The subculture was made every four weeks on fresh medium of the same composition. The data pertaining to mean percentage of cultures showing responses/treatment and number of excised embryo germinated/treatment were statistically analysed by the post hoc multiple comparison test (Marascuilo and McSweeney, 1977). Between the treatments, the average figures followed by different letters were significantly different at $p < 0.05$ levels.

Seedlings derived from excised embryos after attaining a height of 2–3 cm were taken from the culture vessels. The medium adhering to the roots was removed by sterile water. Seedlings were planted in 6 cm high earthen pots containing equal volumes of sterile sand: soil: dry cow-dung and kept in the climate controlled greenhouse (20 ±2 °C; 50% relative humidity) for acclimatization. The seedlings were fed with ¼ strength of MS micro- and macro-salts for one month. After one month, the seedlings were removed from climate-controlled greenhouse and transferred to a glasshouse having a temperature of 28 ± 2 °C.

Out of the two cytokinins used (BA and kinetin), only BA resulted in embryo development. A combination of BA with GA_3 showed more positive effect than BA alone (Table 1). The immature embryos showed swelling after 12 days of culture on MS medium supplemented with 1.5–3.0 mg l^{-1} BA alone and 2.0–3.0 mg l^{-1} BA + 0.5 mg l^{-1} GA_3 (Figure 1). The immature embryos became greenish within 2 weeks of culture. There was no sign of development of immature embryos in Ms media without growth regulators. At higher concentrations of either BA or BA + GA_3, the immature embryos became vitrified (Table 1). Inclusion of either IAA or 2,4-D declined the growth response of immature embryos (data not shown). The maximum response was obtained in medium having 2.5 mg l^{-1} BA, 0.5 mg l^{-1} GA_3 and 3% sucrose. The embryos became vitrified with increasing the GA_3 concentration. After 3 weeks, the cotyledons enlarged considerably and the plumule and radicle elongated. The percentage of germination of excised embryos ranged from 28.7 to 82.5% in case of 'Arunima' and 32.5–77.5% in case of 'Shocking Blue' depending on the culture medium. The results are consistent with earlier reports on genus *Gossypium* and *Lupinus* (Kasten and Kunert, 1991; Liu et al., 1992). Our results contrast with findings of Marchant et al. (1994). We find

Table 1. Effect of BA, Kn, GA_3 on germination of immature embryos of 'Arunima' (A) and 'Shocking Blue' (B) after 4 weeks of culture under 16-h photoperiod (55 μmol $m^{-2}s^{-1}$)

MS + growth regulators (mg l^{-1})			Percent of immature embryos germinated (Mean (%) ± SE)	
BA	Kn	GA_3	A	B
0	0	0	0	0
0.5	0	0	0	0
1.0	0	0	0	0
1.5	0	0	28.7 ± 0.8 a	32.5 ± 0.6 a
2.0	0	0	46.2 ± 0.9 b	50.0 ± 0.4 d
2.5	0	0	67.5 ± 1.2 f	71.2 ± 1.0 g
3.0	0	0	55.0 ± 1.0 d	51.2 ± 0.8 d
0	0.5	0	0	0
0	1.0	0	0	0
0	2.0	0	0	0
0	2.5	0	0	0
0	3.0	0	0	0
2.0	0	0.5	62.5 ± 1.4 e	67.5 ± 1.2 f
2.5	0	0.5	82.5 ± 1.6 h	77.5 ± 1.0 h
3.0	0	0.5	71.2 ± 1.2 g	72.5 ± 0.8 g
2.0	0	1.0	46.2 ± 0.7 + b	42.5 ± 1.0 + b
2.5	0	1.0	60.0 ± 0.6 + e	55.0 ± 0.6 + e
3.0	0	1.0	51.2 ± 0.8 + c	47.5 ± 1.0 + c

The data include the development of heart-shaped and cotyledonary stage. Twenty immature embryos per treatment; repeated twice. + −embryos are vitrified. Mean having the same letter in a column were not significantly different by the *post hoc* multiple comparison test at $p < 0.05$ level.

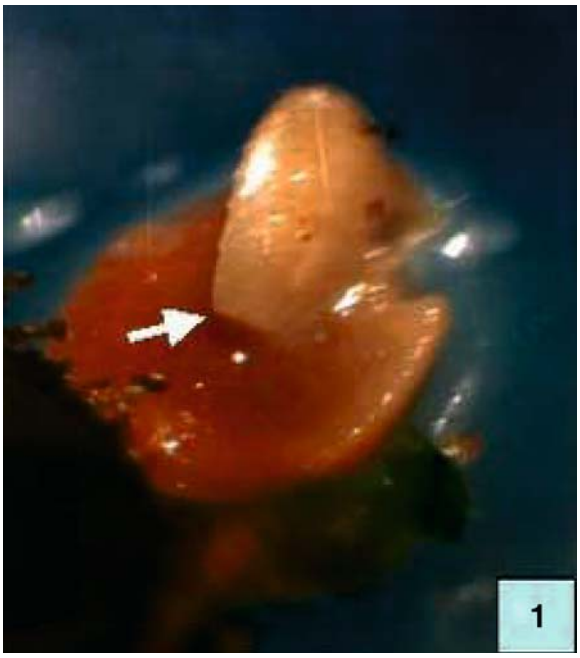

Figure 1. A immature embryo of 'Arunima' showing proliferation (arrow) on MS medium supplemented with 2.5 mg l^{-1} BA, 0.5 mg l^{-1} GA_3 and 3% sucrose after 10 days of culture.

that MS basal salts with 3% sucrose without growth regulator did not initiate the embryo germination.

Significant differences were observed in immature embryos growth response and germination on culture medium incubated in continuous light (24-h photoperiod) for 4 weeks, 16-h photoperiod for 4 weeks, and initially 2 weeks in dark and subsequently in light (16-h photoperiod). The study revealed that the cultures initially incubated in dark for 2 weeks and subsequently transferred to light at 16-h photoperiod germinated in higher percentage than cultures in continuous light (24-h photoperiod) and 16-h photoperiod (Table 2). The maximum percentage of germination was 86.0% and 87.3% in 'Arunima' and 'Shocking Blue', respectively. The immature embryos developed into complete seedlings within 4 weeks of culture on MS medium supplemented with 2.5 mg l^{-1} BA, 0.5 mg l^{-1} GA_3 and 3% sucrose (Figure 2). The effect of photoperiod on *in vitro* morphogenesis has been reported in other plants (Murashige,

Table 2. Effect of photoperiods on germination of immature embryos of 'Arunima' (A) and 'Shocking Blue' (B) after 4 weeks cultured on MS basal medium supplemented with 2.5 mg l^{-1} BA, 0.5 mg l^{-1} GA_3 and 3% (w/v) sucrose

Photoperiodic cycle	Percent of immature embryos germinated (mean(%) ± SE)	
	A	B
24-h photoperiod (55 μmol m^{-2} s^{-1})	62.5 ± 1.6 a	68.6 ± 1.4 a
16-h photoperiod (55 μmol m^{-2} s^{-1})	79.3 ± 1.2 b	77.3 ± 1.2 b
2 weeks dark + 2 weeks light (16-h photoperiod, 55 μ mol m^{-2} s^{-1})	86.0 ± 1.4 c	87.3 ± 0.9 c

The data include the development of heart-shaped and cotyledonary stage embryos. Fifty immature embryos per experiment and repeated thrice. Mean having the same letter in a column were not significantly different by the *post hoc* multiple comparison test at $p < 0.05$ level.

1974; Seibert and Kadkade, 1980). Interaction between photoperiods and growth regulators in shoot development *in vitro* were noted in Prunus species (Baraldi et al., 1988). The present findings indicate that a higher rate of germination was obtained when the culture was initially incubated in the dark and subsequently transferred to light. This might be due to low production of polyphenolic substances from the surrounding tissues of the excised embryos which help in better response (Rout et al., 1999). Marchant et al. (1994) reported that the reduction in germination rate of rose embryos was due to the presence of the excised pericarp or testa which contain diffusible substances suppressing embryo germination. Ninety four percent of seedlings derived from immature embryos survived under greenhouse condition. The growth of seedlings was faster and flowered within two months of transfer to the greenhouse.

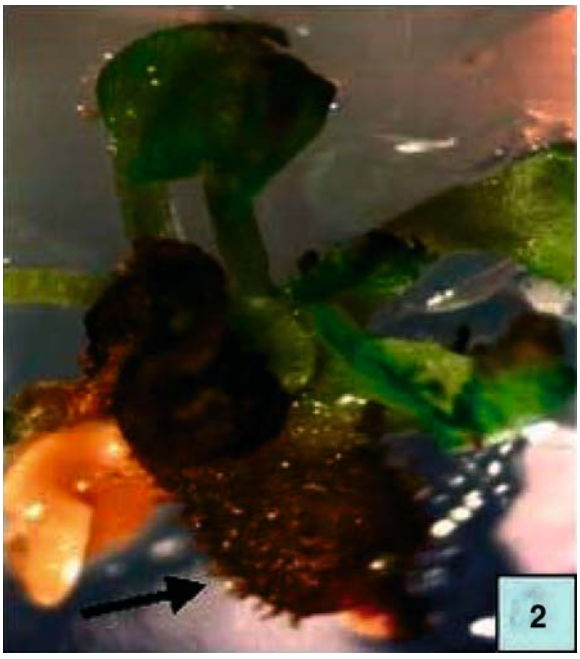

Figure 2. The immature embryo of 'Arunima' germinated on basal MS medium supplemented with 2.5 mg l^{-1} BA, 0.5 mg l^{-1} GA_3 and 3% sucrose after 4 weeks of culture. Arrow indicates the development of radicle.

In conclusion, this paper reports an efficient method of embryo rescue in floribunda roses which do not germinate under normal conventional practices. This investigation will help the breeder to develop new varieties by using this modern technique.

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