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SHORT COMMUNICATION

In vitro Callus Induction and Plantlet Regeneration in Roseapple (*Syzygium jambos* L.)

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***In vitro* callus induction from cotyledon and shoot morphogenesis from nodal explants were obtained in roseapple. Freshly extracted seeds were surface-sterilized best with 80 per cent ethyl alcohol for 10 s followed by 2.5 per cent sodium hypochlorite for 10 min. When inorganic salt concentration of Murashige and Skoog medium was reduced to quarter strength, the browning was reduced and survival percentage of explants was high. Both 2,4-D and NAA at 2 mg/l induced light green, compact callus on cotyledonary explants. Highest number of shoots (4.1) with maximum leaves (5.0) were proliferated from nodal explants on MS medium with BAP at 4 mg/l. Rooting of the induced shoots was obtained on medium with 6 mg/l IBA. *In vitro* morphogenesis strategies in roseapple along with parallel reports are discussed.**

Keywords: Media browning, micropropagation, rooting, tissue culture, roseapple.

Roseapple (*Syzygium jambos* L.) locally known as *Gulab jamun*, is an important subtropical fruit crop belonging to Myrtaceae. Fruits have a characteristic flavour and are consumed fresh as well as processed as preserves, jam, syrup and candy. Syrup is used for making refreshing drinks and also used for medicinal purposes. Rapid clonal propagation of elite types of roseapple through tissue culture would help to spread the cultivation of this otherwise minor fruit crop. *In vitro* studies in roseapple are rather limited. Previously, multiple shoot proliferation from the nodal and shoot tip explants of a related species *Syzygium cuminii* was reported (Yadav *et al.*, 1990). The only report on application of tissue culture in roseapple deals with germination of immature adventitious embryos on MS medium (Murashige and Skoog, 1962) with low concentration (1–2 mg/l) of BAP or on medium devoid of growth regulators (Litz, 1984). Cuttings of roseapple show no rooting response unless treated with high concentration (5000 ppm) of IBA (Litz, 1984) making seed as the only viable propagation agent. Seed propagation in *Syzygium* species leads to high genetic variability especially in fruit size, yield and seed weight due to cross pollination. Apart from variability, very low germination percentage and long juvenile period of seedlings limit the use of seeds for commercial propagation of roseapple. Hence the present study was undertaken as a first attempt to investigate the possibility of obtaining *in vitro* true to type seedlings with higher regeneration efficiency and to solve the problems associated with tissue culture of roseapple.

Ripe fruits collected from local cultivar of roseapple maintained at the farm of Division of Horticulture were used for the extraction of seeds. Seeds were surface sterilized using different agents (ethyl alcohol, sodium hypochlorite, mercuric chloride and cetrimide), among which a combination of 80% ethyl alcohol for 10 s followed by 2.5% sodium hypochlorite treatment for 10 min was best. This treatment was used in further surface-sterilization steps. Sterilized seeds were washed thoroughly for three times with sterile distilled water. Seedlings were raised by placing the surface-sterilized seeds on MS semisolid medium with 3% sucrose.

Polyphenols exuded from the cut plant parts undergo oxidation leading to browning of the medium. To overcome this problem, the following techniques were tried: (1) Pre-soaking of explants in polyvinyl pyrrolidone (PVP), (2) Pre-soaking of explants in ascorbic acid or (3) Reduction in nutrient strength (both macro and micronutrients) of MS medium to quarter or half.

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Observations on intensity of browning (Browning Intensity Scoring (BIS) method: 0 – no browning; 1 – very low; 2 – low; 3 – moderate; 4 – high) and explant establishment (%) were recorded.

For callus induction studies, cotyledons from 5 to 6 weeks old *in vitro* germinated seedlings were cultured on half strength semisolid MS medium supplemented with 2,4-D and NAA at different concentrations. Observations on percentage explant callusing response, amount of callus proliferated (scoring method: –, no callusing, +, poor callusing (0.5 to 1.0 cm width of callus), ++, good callusing (1.0 to 1.5 cm width of callus), +++, very good callusing (> 1.5 cm width of callus) and colour of callus were recorded.

Multiple shoot proliferation was obtained by culturing nodal segments of 2 cm length obtained from 5 to 6-week-old *in vitro* grown seedlings of roseapple on half strength MS medium. Media were supplemented with BAP or kinetin at 1, 2, 4, 6 or 8 mg/l for inducing shoot proliferation. Observations on mean number of shoots proliferated, mean length of the shoots and mean number of leaves induced were recorded.

For root induction studies, micro cuttings of 2.5–3.0 cm length from regeneration experiment were transferred to rooting media with IBA or NAA at 1, 2, 4 or 6 mg/l. Observations on percentage rooting, mean number of roots per shoot and mean length of roots were recorded 21 days after culture. The experiments were laid out using completely randomized design and the data were analysed statistically.

Among the different surface sterilants tried, a combination of 80 per cent ethyl alcohol for 10 s followed by 2.5 per cent sodium hypochlorite treatment for 10 min was the best for the decontamination of seeds.

When the inorganic salt concentration of MS medium was reduced to quarter strength, the browning was slightly reduced and survival percentage of explants was comparatively high (20.0) (Table 1). Reduced browning of medium with lowering of salt concentration in culture medium was observed in chestnut (Chevre *et al.*, 1983), *Pelargonium* × *hortorum* (Hildebrandt and Harney, 1988) and avocado (Biasi *et al.*, 1995). Pre-soaking the explants in ascorbic acid solution (0.1%) also ensured reduction in browning. Polyphenol production was comparatively lesser from nodal explants and the procedure standardized in this study holds good for nodes also.

At 2 mg/l 2,4-D and NAA, very good (callus diameter > 1.5 cm) and good (callus diameter 1.0–1.5 cm) responses of callus induction were observed with percentage of explants responded being 50 and 40 respectively (Table 2). It was observed that problems associated with browning of explants and media continued even after the initial stage of culture. The callus was light green initially and later turned brown to black. So, frequent change of callus to fresh media

Table 1. Effect of *in vitro* techniques for control of browning in roseapple cotyledonary explants.

Treatment	Browning intensity score (BIS)	Explant establishment (%)
MSB (control)	4	5
Reduction in MSB inorganic salts concentration to		
Quarter strength	1	20
Half strength	2	15
Pre-soaking of explants I 1% PVP		
1 h	3	10
2 h	2	10
Pre-soaking of explants in 0.1% ascrobic acid solution		
1 h	1	15
2 h	1	15

Number of explants per treatment = 20.
0, no browning; 1, very low; 2, low; 3, moderate; 4, high.
MSB, Murashige and Skoog (1962) basal medium.

Table 2. Efficacy of auxins 2,4-D and NAA on callus production in cotyledonary explants of roseapple.

Auxins and their concentrations (mg/l)		Percentage of explant response	Callus score	Colour callus
Control (MSB)		10	+	Light green
2,4-D	1	40	+	Light green
	2	50	+++	Light green
	5	30	+	Brown
NAA	1	20	+	Light green
	2	40	++	Light green
	5	30	+	Brown

Observations were recorded 18 days after transfer.
Number of replication per treatment = 10.
Scoring: –, No callusing; +, Poor callusing (0.5 to 1.0 cm width of callus); ++, Good callusing (1.0 to 1.5 cm width of callus); +++, Very good callusing (> 1.5 cm width of callus).

Table 3. Effect of BAP on responses of nodal explants to shoot proliferation in roseapple.

Concentrations of plant growth regulator (mg/l)	Percentage explant response		Mean number of shoots proliferated		Mean length of shoots (cm)		Mean number of leaves per shoot	
BAP/kinetin	BAP	Kinetin	BAP	Kinetin	BAP	Kinetin	BAP	Kinetin
Control (MSB)	40	60	0.8^{d}	0.8^{c}	0.5^{d}	0.5^{c}	1.5^{d}	1.5^{c}
1	80	70	2.0^{c}	0.9^{c}	0.8^{c}	0.6^{b}	3.0^{c}	2.0^{c}
2	80	90	2.2^{c}	2.2^{b}	1.0^{b}	0.8^{a}	4.0^{b}	2.1^{c}
4	100	100	4.1^{a}	3.6^{a}	1.2^{a}	0.8^{a}	5.0^{a}	4.0^{a}
6	100	100	3.2^{b}	2.0^{b}	1.0^{b}	0.8^{a}	4.0^{b}	3.2^{b}
8	70	90	2.0^{c}	0.9^{c}	0.9^{bc}	0.6^{b}	3.5^{bc}	2.2^{c}
Significance			*	*	*	*	*	*
SEm ±			0.11	0.10	0.03	0.05	0.23	0.26
CD at 5%			0.46	0.32	0.11	0.07	0.67	0.73

Observation was recorded 6 weeks after transfer.
Number of replication per treatment = 10.
MSB, Murashige and Skoog (1962) basal medium.

in every 5 days was necessary for further proliferation. Callusing was obtained even with hormone-free medium but the response was poor (10.0%). At all levels, 2,4-D gave a better callusing response than NAA. Superiority of 2,4-D over NAA in inducing callusing is reported in roseapple (Litz, 1984) and carambola (Litz and Conover, 1980). However, the calli induced using 2,4-D cultured on regeneration medium containing BAP/kinetin with or without auxins failed to respond.

Highest mean number of shoot (4.1) was produced with BAP at 4 mg/l (Table 3). Differences among different concentrations of BAP were significant. At 4 mg/l BAP, shoots produced were significantly longer (1.2 cm) over control (0.5 cm). The number of leaves produced was also significantly more at a concentration of 4 mg/l BAP (5.0) over control (1.5). The high concentration of BAP, that is at 6 and 8 mg/l was proved supraoptimal with respect to the number of shoots, length of shoots and number of leaves per shoots.

Number of shoots regenerated was significantly influenced by different concentrations of kinetin (Table 3). Mean number of shoots produced was highest (3.6) at 4 mg/l of kinetin, which also resulted in significantly higher number of leaves (4.0) on induced shoots. Benzyl amino purine appears to increase shoot proliferation with increase in concentration. This has been observed in other fruit plants such as mangosteen (Goh *et al.*, 1990) and carambola (Rashid *et al.*, 1992).

In vitro rooting was rather a difficult phenomenon in roseapple. Among the auxins tried (IBA and NAA at 1, 2, 4 or 6 mg/l), IBA at 6 mg/l only induced roots from the cut ends of the shoots. But the percentage of rooting was poor (30.0). On an average, 2.3 roots were induced and roots were 1.63 cm long and brownish in colour after 21 days of culture. In tissue culture system of difficult to root plants, weak auxins such as NAA and IAA often fail to induce rooting; whereas IBA is successful. Complete plantlets (10-week-old) obtained from *in vitro* cultures were washed in sterile water to remove the agar remains, transferred to glass bottles with autoclaved perlite and kept in incubation room for one week. Then the seedlings were transferred to earthen pots with a mixture of peat and perlite (2 : 1) and covered with 200 gauge polythene cover under greenhouse conditions where it was kept until completely hardened and ready for field planting. More than 85% plants were hardened successfully.

This study details the *in vitro* behaviour *Syzygium* species. Browning was identified as the major problem in the *in vitro* culture of roseapple, which could be limited by reducing the concentration of inorganic salts in the MS medium to one fourth. Best regenera-

tion results from nodal explants could be obtained with 4 mg/l BAP and rooting with 6 mg/l IBA.

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