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Micropropagation of Guava (*Psidium guajava* L.)

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Keywords: shoot bud culture, proliferation, MS media

Abstract

A foolproof method of micropropagation through in vitro shoot bud culture has been developed in guava cv. 'Allahabad Safeda'. The stock plant of guava was maintained at shade net house (75% shade) and pruned severely to encourage new growth. Around 3 cm long shoots were excised and defoliated. The explants were pretreated in solution containing 0.1% Carbendazime and 100 mg/L PVP for 1 hour and then washed with Tween-20 wetting agent. The pretreated explants were further treated with $HgCl_2$ 0.1% for 5 minutes aseptically followed by six washing in autoclaved distilled water. The sterilized explants were cultured on MS medium supplemented with BAP (3.0 mg/L) for shoot bud induction and proliferation. The proliferated shoots were then subcultured on MS medium containing 10 mg/L IBA for rhizogenesis. The rooted plants were finally shifted to autoclaved coconut husk fortified with ½ MS salt mixture for acclimatization. Acclimatized plantlets of guava cv. Allahabad Safeda were planted.

INTRODUCTION

Guava belongs to the Myrtaceae family. This is native to tropical America, but it is cultivated in every tropical and subtropical country of the world. In India, guava is well adapted in almost all the states. The major guava-growing states are Bihar, Uttar Pradesh, Karnataka, Gujarat and Andhra Pradesh. It is estimated that the area and production of guava in India is 150.9 thousand ha and 1710.6 M ton (Singh et al., 2003). Guava is an important tropical fruit tree and is normally propagated by layering, cuttings, stooling, and budding or recently by wedge grafting but the rate of multiplication by these methods is not very fast. Micropropagation method could assist in rapid and mass production of clonal stock of newly released improved cultivars of guava. Many workers have reported the protocols for micropropagation of guava (Amin, 1986; Jaiswal and Amin, 1986; Amin and Jaiswal, 1988, Papadatou et al., 1990; Prakash and Tiwari, 1993, 1996) using shoot buds. However, detailed reports are not available on acclimatization, field establishment and ex vitro growth of micropropagated plants. We report here a complete clonal micropropagation protocol of guava cv. Allahabad Safeda.

MATERIALS AND METHODS

The study was conducted at Biotechnology Laboratory of the Institute. About three years old tree of *Psidium guajava* cv. Allahabad Safeda was chosen for study. The tree was grown inside shade net house and sprayed with Carbendazime (0.5%) regularly. The long shoots were excised from the tree and brought to the laboratory. The defoliated shoots were cut in to 3-4 cm in size. The shoots were first washed under running tap water and than kept in a solution containing 0.1% Carbendazime (Bavestin) + 25 mg/L Rifampicin and two drops of Tween-20 for one hour. After thorough washing the explants were surface sterilized with 0.1% $HgCl_2$ for 6 minutes aseptically followed by 5-6 washing with sterile distilled water. The processed explants were inoculated on MS medium (Murashige and Skoog, 1962) fortified with two different cytokinins (BAP and Kinetin) with varying concentrations (0, 1, 2 and 3 mg/L) for in vitro shoot bud induction. The medium was gelled with 0.8% agar and supplemented with 20 g/L sucrose. The pH

was adjusted at 5.7 before autoclaving. The cultures were incubated at 4000 lux light illumination for 16 hours with 25±2ºC temperature and 55% RH. Twenty-five glass bottles (450 ml volume) formed one treatment and replicated thrice. The micro shoots obtained were further inoculated on MS medium supplemented with varying levels of cytokinins viz., BAP and Kinetin (0, 1, 2 and 3 mg/L) to examine the proliferating ability of microshoots. The established shoots were then inoculated on half and full strength MS medium fortified with different concentration of IBA (5, 10, 15, 20 and 25 mg/L) for in vitro rhizogenesis. The rooted plants were transferred to glass bottles (450 ml volume) containing autoclaved soil + sand + FYM, coconut husk and vermiculite. All the treatments were supplemented with ½ MS nutrient solution and 1.0 mg/L paclobutrazol. Initially, the bottles were incubated in the culture room under 4000 lux light regime and 25 ±2ºC temperature for about 15 days than they were shifted to plant growth chamber where illumination was increased to 10,000 lux with high humidity (70-80%). The plants were shifted to shade net house (50%) provided with misting. The caps of the bottles were loosened gradually and the plants were shifted to pots. The data on acclimatization was recorded after 45 days after root initiation. Once the plants showed stability, they were shifted to field during the months of July-August. The observations were recorded periodically and the data was subjected to statistical analysis using completely randomized design.

RESULTS AND DISCUSSION

The stock plant of guava was maintained in shade net house (75% shading) and was sprayed regularly with fungicide. Shoots were sterilized with fungicidal solution (0.1% Bavestine + Tween-20) followed by sterilization with $HgCl_2$ for 6 minutes aseptically. Data (Table 1) clearly revealed that around three cm long shoot explant inoculated on MS medium fortified with 3.0 mg/L BAP found to be optimum for quick bud induction (5 days) and in vitro shoot proliferation (2.67 shoots/culture). Frequent subculturing was prerequisite for removal of phenolocs. In vitro shoot proliferative efficiency of guava was observed to be very low (2.67 microshoots/culture). Amin and Jaiswal (1988) also reported that nodal segments taken from in vitro proliferated shoots of guava gave rise to 2-4 shoots by precocious axillary branching without an initial lag-period. By repeated sub-culture, a large number of shoots were built up with a shoot multiplication rate of 3 to 4 fold per sub-culture. Papadatou (1990) reported that proliferating shoot cultures were established from shoot tips excised from seedlings grown in a growth chamber and cultured on Rugini Olive medium (OM) supplemented with benzyladenine (BA). Maximum proliferation was achieved with 2 mg /L BA. The low multiplication rate may be due to low BA concentrations (1 mg/L). In vitro rhizogenesis was achieved when micro shoots were transferred to MS medium fortified with IBA. Maximum roots (3 roots/shoot) could be induced in approximately 17 days with 10 mg/L IBA. In vitro rooting was observed to be better in full strength MS than half strength. However, some workers have reported better rooting in half strength MS (Table 2). Shoots obtained from the proliferation stage were rooted on half-strength MS medium containing Indole butyric acid and alpha-naphthalene-acetic acid (Amin and Jaiswal, 1988). Rooting rate was about 33% in the initial cultures and 70-90% in shoots of the 5th and subsequent sub-cultures (Amin and Jaiswal, 1988). Maximum survival of micropropagated plants (84%) was noticed on autoclaved coconut husk fortified with ½ MS nutrient solution containing 1 mg/L paclobutrazol (Table 3). However, successful acclimatization of rooted shoots have been reported in peat-based compost and autoclaved FYM + Sand (1:1) by various workers (Amin and Jaiswal, 1988; Prakash ,1992; Prakash and Tiwari, 1993, 1996). Hardened plantlets were shifted in field where they are growing vigorously.

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<u>Tables</u>

Table 1. Effect of cytokinins on in vitro bud induction and shoot proliferation in guava.

Cytokinins (mg/L)	Days taken for shoot bud induction	Shoot length (cm)	Number of shoots	Number of leaves	Culture weight (gm)
0.0 BAP	15.67	0.17	1.0	0.0	0.0023
1.0 BAP	8.33	0.57	1.0	4.67	0.0208
2.0 BAP	6.67	0.73	1.0	5.67	0.0224
3.0 BAP	5.00	0.67	2.67	5.0	0.0208
1.0 Kinetin	4.67	0.57	2.0	4.0	0.0204
2.0 Kinetin	6.33	0.27	1.0	2.0	0.0046
3.0 Kinetin	7.33	0.33	1.0	2.67	0.0057
SEm±	0.98	0.23	0.50	0.75	0.0019
CD (0.05)	1.74	0.41	0.87	1.32	0.0034

Table 2. Effect of media concentration and IBA on in vitro rhizogenesis in guava.

IBA (mg/L)	Days taken for rooting	Number of root	Length of roots (cm)	Weight of roots (g)
Half MS + 2.5 IBA	31.0	1.33	1.967	0.0047
Half MS + 5.0 IBA	34.33	1.33	3.017	0.0067
Half MS + 10.0 IBA	29.67	1.33	2.283	0.0051
Half MS + 25.0 IBA	29.33	2.00	2.960	0.0058
Full MS + 2.5 IBA	22.33	2.33	2.950	0.0089
Full MS + 5.0 IBA	23.33	2.33	1.890	0.0077
Full MS + 10.0 IBA	17.67	3.0	6.750	0.0211
Full MS + 25.0 IBA	30.67	2.33	1.523	0.0080
Full MS+ 0.0 IBA	0.00	0.00	0.000	0.0000
SEm±	0.220	0.36	0.0	2.0
CD (0.05)	0.45	0.74	0.0	4.12

Table 3. Influence of carrier substrate on acclimatization of micropropagated plants after 45 days.

Carrier Substrate	Plant height (cm)	Number of leaves	Number of shoots	Number of roots	% survival of plants
Coconut husk	6.33	8.00	1.33	7.66	88.48
Vermiculite	4.06	5.00	1.00	4.33	69.25
Soil+Sand+FYM	3.30	3.33	1.00	2.33	50.48
SEm±	0.48	0.84	0.11	0.86	5.53
CD (0.05)	1.176	2.058	NS	2.107	13.549

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