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# *In vitro* micropropagation of guava (*Psidium guajava* L.) through nodal explant using different phytohormone combination

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**Abstract**

Guava is a popular fruit of subtropical area. Guava is one of the recalcitrant plant species for tissue culture. *In vitro* plant exudation and inborn contamination are two major bottle necks during *in vitro* culture of guava. Therefore the present study was undertaken to optimize the concentration of phytohormones for micropropagation of guava. Nodal explants were taken for the study and were excised from guava plants maintained in the germplasm block of the institute. Nodal buds (2.5cm length) were excised and brought in the lab in 50mg/l Ascorbic acid. For surface sterilization Bavestin (200mg/l) was used followed by 0.1% $HgCl_2$ for 5 minutes. Explants were inoculated vertically on MS medium supplemented with 50 mg/l PVP and BAP alone or in combination with Kinetin. The cultures were incubated at 25°C in the dark for two days then allowed to grow under a photoperiod of 16/8 hours light and dark period. Among the different combinations of BAP and Kinetin used, MS medium supplemented with 2 ppm BAP, 1 ppm Kinetin and 50 ppm PVP found best for shoot proliferation. MS medium supplemented with 1 ppm IAA found best for rhizogenesis.

**Key Words:** Guava, nodal explants, sterilization, micropropagation, phytohormones

## Introduction

Guava is a rich source of vitamins, minerals, organic acids, and pectins (Chan *et al.*, 1971; Rathore, 1976; and Loh and Roa, 1989). It belongs to the Mytraceae family with chromosome number 2n=22 and possesses 150 species. This is native to tropical America, but it is cultivated in every tropical and subtropical country of the world. In India, guava is well adopted 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 this method is not very fast. Micropropagation methods could assist in rapid and mass production of clonal stock of newly released improves 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, 1996) using shoot buds. However, we reported here effects of different phytohormonal combinations on micropropagation in guava (*Psidium guajava L.*)

## Materials and methods

The study was conducted in Tissue Culture Laboratory at Allahabad Agricultural Institute- Deemed University Allahabad. The tree was grown inside shade net house and sprayed with carbendanzime (5%) regularly. The long shoots were excised from the tree and brought to the laboratory in 50 mg/l ascorbic acid. The defoliated shoots were cut in to 2.5 cm in size. The shoots were first washed under running tap water and then kept in a solution containing 0.1% Bavestin + 0.1% Indofil + 0.1% 8 Hydroxyquinoline (8HQC) for one and half hours. After thorough washing the explants were surface sterilized with 0.1% $HgCl_2$ for 5 minutes aseptically followed by 56 washing with sterile distilled water. The processed explants were inoculated vertically on MS medium (Murashige and Skoog, 1962) supplemented with different concentration of BAP (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 ppm) + 50 ppm Polyvinilpyrrolidon (PVP) + 1 ppm kinetin. The cultures were incubated at 25°C in the dark for two days then allowed to grow under a photoperiod of 16/8 hours light and dark period. The medium was gelled with 8 g/l agar and supplemented with 30 g/l sucrose. The pH was adjusted at 5.8 before autoclaving. The cultures were incubated at 3000 lux light illumination for 16 hours with 25±2°C temperature. Visual observations were taken away every 3 days and effect of different treatments was quantified on the basis of percentage of explants showing response for regeneration. Data were collected after 3 weeks including shoot and shoot length. For rooting individual shoots were separated from shoot cluster and transferred to culture tube containing 20 ml of MS medium having different concentrations of IAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 ppm) and maintained under identical conditions. Observations

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were noted after 3 weeks including no. of roots.

**Results and discussion**

The present study was designed to standardize a protocol for micro propagation of guava from nodal explants. Guava is an important tropical fruit tree and is normally propagated by layering, cuttings and budding or recently by wedge grafting but the rate of multiplication by this method is not very fast. So, micropropagation is only devising method for propagation. The stock of guava was maintained in shade net house and was sprayed regularly with fungicide. Shoots were sterilized with fungicide solution (bavestin + 0.1% Indofil + 0.1% 8HQC) followed by sterilization with $HgCl_2$ for 5 minutes aseptically. Data (Table 1) clearly revealed that around 2.0 cm long shoot explants inoculated on MS medium supplemented with 2 ppm BAP and 1 ppm Kinetin found to be best for quick bud induction (6 days) and *in vitro* proliferation (3.0 shoot/culture). Frequent subculturing was prerequisite for removal of phenol. *In vitro* shoot proliferative efficiency of guava was observed to be very low (1-2 microshoots/culture). Maximum proliferation was achieved with 2 ppm BAP, 1 ppm Kinetin and 50 mg/l PVP (910 microshoots/culture). The low multiplication rate may be due to low BAP concentration (0.5 1.0 %). *In vitro* rhizogenesis was achieved when micro shoots were transferred to MS medium fortified with IAA. Maximum roots (7 roots/shoot) within 20 days supplemented with 2 ppm IAA.

Many combinations and concentrations of growth regulators were tried for shoot and root regeneration. The MS medium supplemented with 2 ppm BAP, 1.0 ppm Kinetin and 50 ppm PVP proved to be the best medium for shoot multiplication. It shows that multiplication increases with increase in BAP concentration up to 2 ppm but further increase in BAP concentration reduces the shoot multiplication (Table 1). The nodal segments taken from *in vitro* proliferated shoots were of guava rise to 2-4 shoots by precocious axillary branching without an initial lagperiod (Amin and Jaiswal, 1988). 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. The proliferated shoot cultures were established from shoot tips excised from seedlings grown in a growth chamber and culture on Rugini Olive medium (OM) supplemented with benzyladenine (Murashige and Skoog, 1962). For rooting from regenerated shoots different concentrations of IAA were tested (Table 2). Best rooting were observed on MS medium supplemented with IAA (2 ppm).

This report provides a simple protocol for *in vitro* micropropagation of guava by using nodal explants of field grown plant cultured on the MS medium supplemented with BAP (2 ppm), Kinetin (1.0 ppm) and 50 mg/l PVP after 5 to 6 week and maintained under identical conditions with frequent sub culturing. The regenerated shoots were rooted on different rooting media. Best rooting was observed on IAA (1 ppm) after 3-4 weeks.

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Table 1. Effect of different concentration of growth hormones on development of guava shoots when supplemented to MS medium.

| BAP (ppm) | Kinetin (ppm) | PVP (ppm) | Percentage of shoot responded after 4 weeks | Number of shoots/explants |
|---|---|---|---|---|
| 0.5 | 1 | 50 | 10 | 1-2 |
| 1.0 | 1 | 50 | 35 | 4-5 |
| 1.5 | 1 | 50 | 50 | 5-7 |
| 2.0 | 1 | 50 | 75 | 9-10 |
| 2.5 | 1 | 50 | 60 | 8-7 |
| 3.0 | 1 | 50 | 55 | 6-5 |
| 3.5 | 1 | 50 | 30 | 3-4 |
| 4.0 | 1 | 50 | 15 | 2-3 |

Table 2. Effect of different concentrations of IAA on rooting of *in vitro* proliferated shoots of guava on MS medium.

| IAA (ppm) | % age of root induction | No. of roots/ regenerated shoot |
|---|---|---|
| 0.5 | 10 | 1 |
| 1.0 | 25 | 2 |
| 1.5 | 40 | 4 |
| 2.0 | 75 | 7 |
| 2.5 | 45 | 5 |
| 3.0 | 20 | 2 |
| 3.5 | 9 | 1 |

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