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# *IN VITRO* RE-GENERATION OF GUAVA (*PSIDIUM GUAJAVA* L.) FROM SHOOT TIPS OF MATURE TREES

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**Abstract**

Micropropagation of guava through shoot tip culture from five years old bearing plants was carried out at Nuclear Institute for Food & Agriculture, Peshawar. Shoot tips after sterilization was cultured on modified MS medium supplemented with different concentrations and combinations of BAP and L-glutamin. Maximum number of shoots (72 %) was developed in to plantlets when MS was supplemented with BAP 1 mg/L and 500 mg/L L-glutamine. MS control and BAP 1mg/L combined with glutamine 250 mg/L gave minimum (22%) plantlets. Maximum (3.5) number of shoots per culture was found in 0.5 mg/L BAP alongwith 500 mg/L L-glutamine. MS supplemented with 2.5 mg/L IAA + 2.5 mg/L IBA gave maximum (54) plants rooted with average number of roots (3.8) per plantlet. The *In-vitro* produced plantlets were successfully established in soil.

## Introduction

Guava (*Psidium guajava* L.) belongs to the family Myrataceae with chromosome number 2n =22 and possesses 150 species. It is considered as the "poor man's fruit" or apple of the tropics" and is a popular tree fruit of the sub continent. It is native to the tropical America stretching from Mexico to Peru. Despite its origion in tropical America, guava is presently cultivated in every tropical and subtropical country around the world (Samsom, 1986).

Guava, an important tree crop in Pakistan is propagated sexually (Zamir *et al.*, 2003, Loh & Rao 1989). For being an open pollinated fruit species, there is an opportunity for the evolution of new genotypes of guava in existing plantings, which if propagated further by seed, will not usually breed true to type. In order to perpetuate selected breeding materials and varieties, therefore, one has to resort to vegetative means of propagation. Asexual propagation in guava has always been a great problem (Zamir *et al.*, 2003, Rehman *et al.*, 1991). Horticulturists have tried traditional methods such as cuttings, buddings, grafting, aerial layering and inarching. Aerial layering in general, is recommended by all the experts as a successful method in regeneration of this plant but such method is expensive and also cumbersome. Tissue culture is a technique, which offers an alternative procedure for the vegetative multiplication of various plant species. This technique has so far proved successful in many woody plants like eucalyptus (Burger, 1987) and fruits plants like Kiwi (Hassan *et al.*, 2000) and guava (Jaiswal & Amin, 1992; Amin & Jaiswal, 1987).

Micropropagation allows rapid multiplication of plantlets, production of pathogen-free plants, and maintenance of superior germplasm of elite cultivars and can also be practiced irrespective of the season. *In vitro* plant regeneration from nodal explants of mature trees of guava has been described by Amin & Jaiswal (1987) and Jaiswal & Amin (1992). Multiple shoot formation was induced using shoot tips and nodal segments of seedlings and grafted materials. In addition, adventitious shoot formation from hypocotyls and leaf segments was also achieved by Singh *et al.*, (2002). The present

investigation is an attempt to standardize cultural media for growth of guava explant *In vitro* and to find out the easy mass multiplication method for this species.

## Materials and Methods

Shoot tips of guava Cv. Safeda were obtained from five year old bearing plant in the orchard of NIFA Campus. The samples were brought to the laboratory and treated with 2 drops of Zip as a detergent, then washed by running tape water for 30 minutes. The material was agitated for 30-40 minutes in 0.5% Polyvenylepyrrolidone (PVP) an antioxidant for removal of phenolics. The explants were briefly rinsed with 70% ethanol. The surface sterilization of these shoots was carried out with 0.05% Mercuric chloride ($HgCl_2$) and a drop of surfactant (Tween 80) was added and agitated at 100 rpm on a rotary shaker for 5 minutes. Then the shoot tips were rinsed three times with sterile distilled water under laminar flow bench. Murashige & Skoog (1962) basal medium modified after Burger (1987) was supplemented with different concentrations of Benzyl Amino purine (BAP), glutamine and 3.0% sucrose, solidified with 0.8% plant agar was used for culturing the shoot tips. The pH of the medium was adjusted to 5.7 with 0.1N KOH or 0.1N HCL before autoclaving at 121$^{o}$C at 1.05 kg/cm$^2$ for 20 minutes.

Shoot tips 1.5-2 cm in size were excised asceptically from shoot and placed vertically on the medium in test tubes containing 10 ml medium. The test tubes were covered with autoclaved polypropylene sheet and tightened with rubber band. The cultures were kept in growth chamber at 25±2$^{o}$C under a 16-hrs photoperiod and 8 hours dark periods with a light intensity of 1500 Lux to initiate its growth.

After every 4 weeks, sub culture was transferred to 35 ml MS solid medium in autoclavable baby food jars. Twelve weeks plantlets were transferred to modified MS medium with different concentration of auxins for root induction. The experiment was arranged in a Completely Randomized Design with three replications per treatment, each with 20 explants.

Acclimatization of the plantlets was carried out by transferring well-developed rooted plantlets to medium in Jiffy pots containing clay, silt and sand. Before transplanting the plantlets were washed with distilled water to remove the medium from the roots. Initially the plantlets were covered with transparent plastic in order to maintain high humidity and then small holes were made in the plastic to acclimatize it gradually. The plantlets were watered with half Knop's solution and plastic cover was removed after a week. A regular irrigation to these plantlets were practiced at 15 days interval and later on shifted to natural environment.

## Results and Discussion

**i. Effect of growth hormones on number of shoots developed into plantlets:** The data regarding the effect of growth hormones on number of shoots developed into plantlets was significant. BAP 1mg/l along with glutamine 500 mg/l gave higher shoot development (72%) when cultured on MS medium followed by BAP 0.5mg/L + Glutamin 500mg/L (58%). MS with BAP 1mg/l gave (52%) and MS with BAP 0.5mg/L gave (42%) shoot development. MS (control) and MS along with BAP 1mg/L and glutamin 250 mg/L gave (22%) of shoot development. BAP 1mg/L alongwith glutamin 500mg/l gave maximum number of shoots (72%). When the concentration of BAP was reduced to 0.5mg/L, the number of shoots decreased to (58%), with the same concentration of glutamin. The observation is inconsistent with the results of Khattak *et al.*, (1993) who reported that BAP 2mg/L produced maximum number of axillary shoots per culture.

**Table1. Effect of different concentrations and combinations of growth hormones on development of guava shoots when supplemented to MS medium.**

| Media concentrations | No. of shoot failed | % shoot development | No. of shoots developed in to plantlets | Av. No. of shoots/ plantlet |
|---|---|---|---|---|
| MS (Control) | 47 | 22 | 13 d | 1.8 |
| MS + BAP 0.5 mg/l | 35 | 42 | 25 bcd | 2.0 |
| MS + BAP 1 mg/l | 29 | 52 | 31 abc | 2.7 |
| MS + BAP 0.5 mg/l + glutamin 250 mg/l | 38 | 37 | 22 cd | 2.9 |
| MS + BAP 1 mg/l + glutamin 250 mg/l | 47 | 22 | 13 d | 1.5 |
| MS + BAP 0.5 mg/l + glutamin 500 mg/l | 25 | 58 | 35 ab | 3.5 |
| MS + BAP 1 mg/l + glutamin 500 mg/l | 17 | 72 | 43 a | 3.0 |

Means of the same category followed by different letters are statistically different at 5% level of significance, using DMRT test.

**Table 2. Effect of various concentrations of auxins on rooting of guava culturing on MS medium.**

| Media concentrations | No. of plants failed to root | No. of plants rooted | Average No. of roots/plant |
|---|---|---|---|
| MS (control) | 60 | 0 d | - |
| MS + IBA 2 mg/l | 26 | 34 bc | 2.4 |
| MS + IBA 2.5 mg/l | 34 | 26 c | 3.4 |
| MS + IAA 2 mg/l | 32 | 28 c | 2.3 |
| MS + IAA 2.5 mg/l | 26 | 34 bc | 2.6 |
| MS + IBA 2 mg/l + IAA 2 mg/l | 15 | 45 ab | 3.13 |
| MS + IBA 2.5 mg/l + IAA 2.5 mg/l | 6 | 54 a | 3.8 |

Means of the same category followed by different letters are statistically different at 5% level of significance, using DMRT test.

Cytokinin like BAP is effective even at low concentration. Glutamin, which is a source of energy, enhanced growth process and produced maximum number of shoots alongwith BAP. In previous attempts glutamin was not used for micropropagation of guava.

Average number of shoots was maximum (3.5) when BAP 0.5mg/L along with glutamin 500mg/L was added to MS medium. Low concentration of BAP and high concentration of glutamin gave maximum number of shoots per culture. These results are inconsistent with those of Khattak *et al.,* (1993). Who found that 2mg/l of BAP gave maximum average number of shoots per plantlet. Results closely resemble to Loh & Rao (1989) who suggested using 0.5mg/L of BA to get an average of 3.2 shoots per segment.

**ii. Effect of auxins on rooting:** The data related to the effect of auxins on rooting of guava when cultured on MS medium showed that rooting percentage is high (54) when IAA 2.5mg/L combined with IBA 2.5mg/L followed by IAA 2mg/L and IBA 2mg/L with average number of roots 3.8 and 3.13 respectively. When IAA and IBA 2.5mg/L were used alone, it gave (34) and (26) rooted plantlets with average number of roots (2.6) and (3.4). IAA and IBA 2 mg /L alone gave (28) and (34) with average roots (2.3) and (2.4) respectively. The most inferior results were obtained when IBA 2.5mg/L was used alone (26), while no rooting was observed in auxin free medium. Jaiswal & Amin (1987) reported that 80% of shoots rooted well on a medium having both IBA and NAA (0.2 mg/L each). In the present studies both IBA and IAA 2.5mg /L gave highest percentage of rooting. The difference may be due to the reason that NAA 0.2mg /L was combined with IBA and the absence of dark period. They reported that higher temperatures and dark treatment have promoted *In vitro* rooting in micro cuttings of other tropical fruit trees like guava. No rooting of microcuttings cultured on auxin free medium confirms the essentiality of an

exogenous auxin for rooting of guava shoots. Variation in present studies may be the high concentration of auxin used and the absence of dark period. Amin *et al*., (1999) got 100% rooting in pomegranate by using half strength MS medium. Khattak *et al*., (1998) got best rooting on half strength MS medium containing 1mg/L of IBA.

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**References**

Amin, M.N. and V.S. Jaiswal. 1987. Rapid clonal propagation of guava through *in vitro* shoot proliferation on nodal explants of mature trees. *Plant Cell Tissue and Organ Culture*, 9: 235-243.

Amin, M.N., M.N. Islam and A.K. Azad. 1999. Regeneration of plantlets *In vitro* from the seedling explants of pomegranate (*Punica granatum*). *Plant Tissue Culture*, 9(1): 53-61.

Burger, D.W. 1987. *In vitro* micropropagation of *Eucalyptus sideroxylon*. *Hort. Sci*., 22(3): 496-497.

Hassan, S., R. Zamir and M. Tariq. 2000. Micropropagation of Kiwifruit (*Actinidia chinensis* ) through leaf callus culture. *Pakistan J. Agric. Res.*, 16(1): 31-34.

Jaiswal, V.S. and M. N. Amin. 1992. Guava and Jack fruit. *In Biotechnology of Perennial fruit crops* (End). F.A. Hammerselag and R.E. Litz. CAB international, Wallingford U.K. pp. 421-431.

Khattak, M.S., M.N. Malik and M.A. Khan. 1993. *In vitro* micro-propagation of guava Cv. sufeda. *Advances in plant tissue culture in Pakistan.* Department of Botany University of Peshawar.

Khattak, M.S. and R.M. Skirvin. 1998. Apple seed extract promotes organogenesis from stem section of guava (*Psidium guava* L.) *In vitro*. *Scientific Khaber*, 11(5): 55-60.

Loh, C.S. and A.N Rao. 1989. Clonal propagation of guava (*Psidium guajava* L.) from seedling and grafted plnats and adventitious shoot formation *In vitro*. *Scientia Hort*., 39: 31-39.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant*., 15: 72-497.

Rehman H.U., M.A. Khan, K.M. Khokhar and M.H. Lazhari. 1991. Effect of season on rooting ability of tip cuttings of guava treated with paclobutrazol. *Ind. J. Agri. Sci*., 61: 464-466.

Samson, J.A. 1986. *Tropical fruits*, 2nd ed., Tropical Agriculture Series, Longman Scientific & Technical, Longman Inc., New York.

Singh, S.K., P.R. Meghwal, H.C. Sharmand and S.P. Singh. 2002. Direct shoot organogenesis and hopocotyl explants from *In vitro* germinated seedlings of *Psidum guajava* L. Cv. Allahabad Safeda. *Scientia Horticulture*, 95: 213-221.

Zamir, R., G.S.S. Khattak, T. Mohammad, S.A. Shah, A.J. Khan and N. Ali. 2003. *In vitro* mutagenesis in guava (*Psidium guajava* L.). *Pak. J. Bot*., 35(5): 825-8228.

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