Pak. J. Bot., 40(3): 1195-1200, 2008.

IN VITRO REGENERATION OF PLANTLETS FROM SEEDLINGS EXPLANTS OF GUAVA (*PSIDIUM GUAJAVA* L.) CV. SAFEDA

S. TARIQ SHAH[1], ROSHAN ZAMIR[1], J. AHMAD[2], HAIDAR ALI[3] AND G. LUTFULLAH[2]

[1]*Nuclear Institute for Food and Agriculture Peshawar, Pakistan*
[2]*Centre of Biotechnology, University of Peshawar, Peshawar, Pakistan*
[3]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*

Abstract

Regeneration of plantlets from *In vitro* germinated seedling explants was carried out at NIFA, Peshawar**.** Multiple shoots were generated from *In vitro* germinated (50 days-old) seedlings of *Psidium guajava* L. Cv. Safeda. Modified Murashige & Skoog (MMS) medium supplemented with 6-benzylaminopurine (BAP), Zeatin and Gibberelic acid (GA_3), Zeatin 1.0 mg/L combined with GA_3 0.5 mg/L gave the highest response (47.6%) with the regeneration of (3.2) shoots per original explants. Each shoot gave rise to about 4.4 shoots per explant upon sub culture on MMS supplemented with BAP 1.0 mg/L combined with Kinetin 0.5 mg/L. The micro shoots were successfully rooted on ½ strength MMS medium supplemented with IBA 1.5 mg/L and NAA 0.5 mg/L, where 85% rooted plantlets were obtained. The *In vitro* plantlets after rooting were acclimatized in Jiffy pots and established in the soil.

Introduction

Guava (*Psidium guajava* L.) is one of the most common fruit crops of Pakistan, which ranks fourth production wise after citrus, mango and banana. Guava is grown throughout the country but the average yield, however, is much less than its potential, probably because of the out crossing. The importance of guava is increasing due to its nutritional value, bi-annual bearing and affordable price. It is widely distributed throughout the tropical and sub-tropical regions, where it is consumed fresh or processed into several products (Martin, 1984). Guava is usually propagated by seeds (Zamir *et al.,* 2003).

Clonal propagation of guava through micropropagation has been demonstrated using shoot tip and nodal segments (Amin & Jaiswal, 1987, 1988; Loh & Rao, 1989; Papdadatau *et al.,* 1990; Yasseen *et al.,* 1995). Guava propagation by layering, inarching, and budding is achievable. A unique technique of patch budding of guava scions demonstrated at the Horticultural Experiment Center, Basti, India, appears commercially feasible (Mortan, 1987). Sharma *et al*., (1992) reported positive influence of an aneuploid dwarfing guava rootstock on tree growth and productivity. Several clonal methods including rooting of succulent green wood, forkert or patch budding, air-layering (, layering, and inarching for propagating guava germplasm have been discussed by Chandra (1965) and Samson (1986). Micropropagation of guava has been achieved by single node cuttings from seedlings raised in green house, *in vitro* raised seedlings (Ali, *et al.,* 2003) and shoot tip explants from mature trees (Amin & Jaiswal 1987; Amin & Jaiswal, 1988; Loh & Rao, 1989; Papadatou *et al.,* 1990).

Tissue culture is an efficient technique combined with conventional methods, for clonal propagation of guava and applied with the objective of enhancing the rate of multiplication. Over a million plants can be obtained from a small portion of explant shoot tip within few months. Such a prolific rate of multiplication cannot be expected by

any of the *In vivo* methods of clonal propagation. Plant tissue culture technique could play pivotal role in improving the quality and production of this fruit. Micro-propagation is rapid, efficient and cost effective. Additionally it will help in germplasm preservation, genetic improvement and true to type plants can be produced year round for the market and growers by utility of this technique. The present studies were carried out to investigate rapid production of true-to-type plants *In vitro* from seedling explants of elite guava cv. Safeda.

Materials and Methods

Seeds were taken from mature fruits of guava cv. Safeda, thoroughly washed and surface sterilized with 0.05% $HgC1_2$ for 5 minutes followed by 4 rinsings with sterile distilled water (Zamir *et al.,* 2004). The surface sterilized seeds were cultured on sterilized filter paper in conical flasks, 500 ml containing cotton. Fifty days old seedling explants (0.5 - 0.05 cm) were aseptically excised and cultured on Modified Murashige & Skoog, (1962) (1/2 macro + full strength micro and organic salts) medium containing BAP, Zeatin and GA_3 alone and in combinations. The media was solidified with 0.8% agar then added 30 g/L sucrose and the pH was adjusted to 5.85 before autoclaving (102 kg/cm^2 for 15 min). The cultures were then incubated for 2 weeks in the dark (26 $\pm$ 2 °C) followed by shifting to light from cool white fluorescent lamps (2000-2500 Lux) maintained at 16h/8h photoperiod. The growth regulators 6-benzylaminopurine BAP and Kinetin individually and in combination were used for shoot regeneration. The proliferated microshoots were then transferred to rooting medium containing Indol butyric acid (IBA) and Naphthalene acetic acid (NAA).

After successful rooting *In vitro* microshoots were thoroughly washed with distilled water to remove any remains of the medium and transplanted in baby food glass jars filled with peat:perlite (1:1). The hardened plantlets (30-40 days) were then transferred to small plastic pots filled with soil: farmyard manure (well rotten): sand (1:1:1) and shifted to the glasshouse.

The experiments were laid out in Completely Randomized Block Design with 3 replications. The data was subjected to analysis of variance (MSTATC). Comparisons among treatments were made employing Duncan's Multiple Range Test.

Results and Discussions

Direct shoot organogenesis from seedling explants was noted after almost 4 weeks of culture (Table 1). The maximum number (3.5 shoots per explant) of adventitious shoot production was recorded on the medium having Zeatin 1.0 mg/L + GA_3 0.5 mg/L followed by 2.3 shoots per explant with Zeatin 2.0 mg/L + GA_3 0.5 mg/L (Fig. 1, A & B). The combination of BAP 1.0 mg/L + GA_3 0.5 mg/L gave raise to about 2.1 shoots per explant. The hormone-free medium (Control) did not show any response as the explants swelled and turned necrotic after 4 weeks. The maximum (47.6%) shoot organogenesis frequency was recorded with Zeatin 1.0 mg/L + GA_3 0.5 mg/L. Addition of GA_3 to the cytokinin-supplemented media reduced the days taken for shoot induction. BAP and Zeatin when applied individually resulted in callusing. The shoots obtained *via* direct organogenesis needed early transfer to shoot multiplication medium as prolonged exposure to high levels of cytokinin turned the leaves yellowish and thereafter necrotic. In the treatments with lower concentration of cytokinins, callusing was noted along with

shoot bud induction (Singh *et al*., 2002). The microshoots were further multiplied using shoot multiplication medium (Table 2). It is evident that shoot multiplication rate almost doubled when both the cytokinins (BAP & Kinetin) were employed in combination as compared to their individual treatments. The maximum (4.4) number of microshoots were obtained when BAP 1.5mg/L and Kinetin 1.0mg/L while the longest shoot of (2.3cm). BAP alone was better than Kinetin which produced plantlets of longer length.

Table 1. Effect of different cytokinins on shoot induction from seedling explants of guava Cv. Safeda.

Treatments (mg/L)	Percent explant showing shoot regeneration	No. of days to shoot induction	No. of shoots per explant
BAP			
1.0	20 c	48	1.4 c
2.0	14 d	51	1.0 c
Zeatin			
1.0	10.5 d	48	1.2 c
2.0	11d	52	1.1 c
BAP + GA3			
1.0 + 0.5	24 c	47	2.1b
2.0 + 0.5	10 d	50	1.3 c
Zeatin + GA3			
1.0 + 0.5	47.6 a	39	3.5 a
2.0 + 0.5	36.2 b	41	2.3 b
Control	-	-	-

Means followed by different letters are significantly different at p= 0.05 level by DMRT

Table 2. Effect of different cytokinins on regeneration of guava microshoot from seedling explants of guava Cv. Safeda

Treatments (mg/L)	No. of shoots per explant	Average length of microshoot (cm)
BAP		
1.0	3.4 b	1.4 c
1.5	2.6 c	1.0 d
Kinetin		
0.5	1.3 d	0.6 e
1.0	1.5 d	0.6 e
BAP + Kinetin		
BAP 1.0 + Kinetin 0.5	3.8 b	1.9 b
BAP 1.5 + Kinetin 1.0	4.4 a	2.3 a

Means followed by different letter are significantly different at P= 0.05 level by DMRT

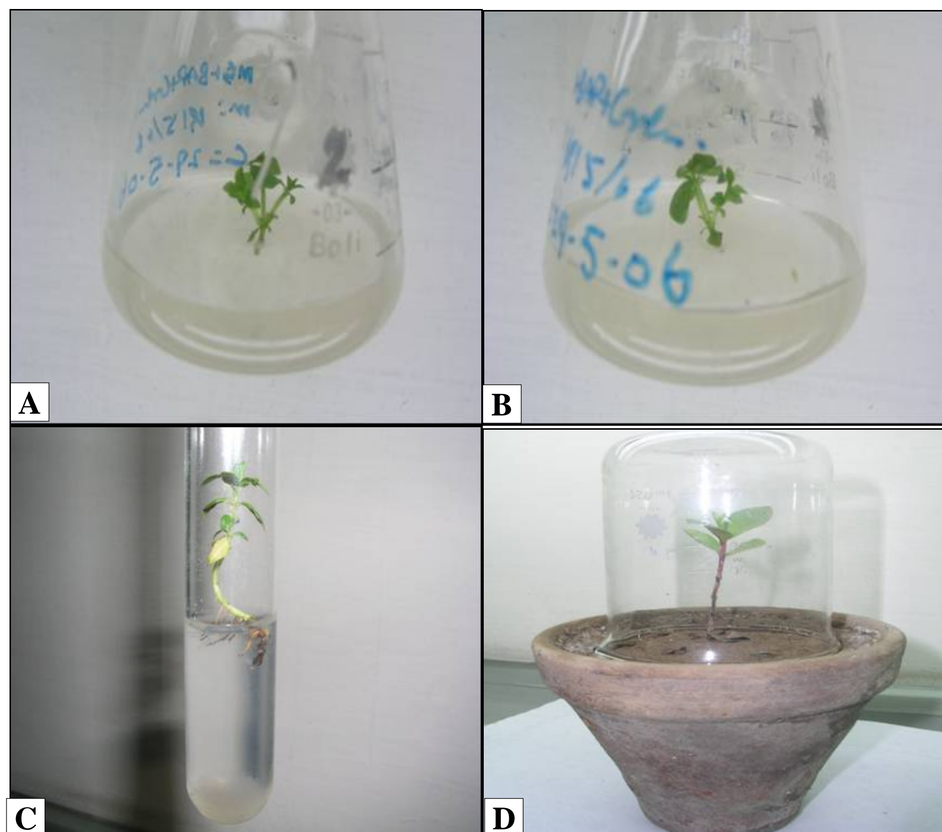

Fig. 1. Multiple microshoot formation from seedling explants (A & B), Root induction from microshoot (C) and acclimatization and weaning (D).

Table 3. Effect of different auxins on rooting of guava microshoot from seedling explants of guava Cv. Safeda

Treatments (mg/L)	Days to root induction	Rooting response %	No. of roots per shoot
IBA 1.0	39	64.8 c	2.0 b
IBA 1.5	34	73.5 b	2.3 b
IBA 1.0 + NAA 0.5	22	78 b	2.5 b
IBA 1.5 + NAA 0.5	25	85 a	3.6 a
Control	-	-	-

Means followed by different letter are significantly different at p= 0.05 level by DMRT

The data presented in Table 3 revealed that highest rooting (85%) was noted in the treatment having IBA 1.5 mg/L + NAA 0.5 mg/L (Fig. 1, C) while, IBA 1.0 mg/L + NAA 0.5 mg/L induced quick rooting (22 days). NAA supplementation to root induction medium not only decreased the days taken for root initiation but also enhanced the overall rooting percentage and root number. A maximum of 3.6 roots per shoot were recorded in the treatment having IBA 1.5 mg/L + NAA 0.5 mg/L while on the auxins free

medium (control) no root initiation was recorded. The rooted plantlets were successfully acclimatized in growth chamber and transferred to glasshouse with over 80% survival after 30 days (Fig. 1, D).

In vitro regeneration of woody perennial plant species is very difficult; hence success rate of micropropagation and genetic transformation success is very limited. Therefore, a successful regeneration protocol is required. Regeneration from seedling explants were achieved in guava cv. Safeda and the study clearly revealed that the synthetic cytokinin-like BAP, Kinetin and Zeatin along with low concentration of GA_3 could successfully be employed. These results are in conformity with results of Singh *et al*., (2002) in guava, Loh & Rao (1989) in guava and Amin & Razzaque (1993) in carambola.

Low concentration of BAP has been found effective in adventitious shoot proliferation in guava (Loh & Rao, 1989) and Rubus (Fiola *et al.,* 1990). Shoot multiplication in guava has been proposed with the application of a single cytokinin with varied success (Amin & Jaiswal, 1987; Loh & Rao, 1989; Papadatou *et al.,* 1990; Yasseen *et al.,* 1995; Fuenrnayor & Montero, 1997) and has been found to be dependent on genotypic factors. However, when cytokinins were used in combination as reported in the present study, the multiplication rate was almost doubled as compared to those obtained with individual cytokinins, i.e. BAP and Kinetin. Earlier, Loh & Rao (1989) had also observed similar results with the use of high cytokinin levels. A low level of GA_3 along with cytokinin has been found to give rise to proper growth of leaves and uniform growth in microshoots (Kouidar *et al.,* 1984). Similarly BAP has been found effective for shoot multiplication and elongation.

Rooting of guava microshoots has been found to be effective with the addition of low concentration of IBA (Amin & Jaiswal, 1987; Yasseen *et al.,* 1995) or on auxins free basal medium (Loh & Rao, 1989). In contrast to these reports, NAA supplementation at 0.5mg/L was found most responsive for cv. Safeda. The studies carried out have shown that microshoots can be successfully regenerated from guava seedling explants through direct organogenesis.

Acknowledgements

The authors are grateful to International Atomic Energy Agency (IAEA), Vienna, Austria for funding this research project through grant under IAEA Research Contract No.Pak-11289. The Administrative and Technical support provided by the Director, NIFA and other colleagues in the institute is also gratefully acknowledged.

References

Ali, N., R.M.S. Mulwa, M.A. Mortan and R.M. Skirvin. 2003. Micropropagation of guava (*Psidium guajava* L.) *Journal of Horticultural Science and Biotechnology.* 78(5): 739-741.

Amin, M.N. and V.S. Jaiswal. 1988. Micropropagation as an aid to rapid cloning of a guava cultivar. *Scientia Hort*., 36: 89-95.

Amin, M.N. and M.A. Razzaque. 1993. Regeneration of *Averhoa carambola* plants *In vitro* from cullus cultures of seedling explants. *Journal of Horticultural Sciences.* 68(4): 551-556.

Amin. M.N. and V.S. Jaiswal. 1987. Clonal propagation of guava through *In vitro* shoot proliferation on nodal experiments of mature trees. *Plant Cell Tissue Organ Cult.,* 9: 235-244.

Chandra, D. 1965. Recent advances in clonal propagation of guava. *Allahabad Farmer,* 39: 137-139.

Fiola, J.A., M.A. Hassan, H.J. Swain, R.H. Bors and R. McNicols. 1990. Effect of Thidiazuron, light fluence rates and Kanamycin on *In vitro* shoot organogenesis from excised *Rubus* cotyledons and leaves. *Plant Cell Tiss. Organ Cult*., 22: 223-228.

Fuenmayor, M.E.D. and N.J.M. Montero. 1997. *In vitro* clonal propagation of guava (*Psidium guajava* L.) from stem shoots of Cv. Mara-7. *Acta Hort*., 452: 47-51.

Kouidar, M., S.S. Korban, R.M. Skirvin and M.C. Chu. 1984. Influence of embryogenic dominance and polarity on adventitious shoot formation from apple cotyledons *In vitro. J. Am. Soc. Hort. Sci.,* 109: 38 1-385.

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

Martin, E.W. 1984. *Handbook of Tropical Food Crops.* CRC Press. *Boca Raton,* FL, pp. 31-39.

Mohamed-Yasseen, Y., S.A. Barrineger, R.J. Schnell and W.E. Splittoesser. 1995. *In vitro* shoot proliferation and propagation of guava from germinated seedling. *Plant Cell Rep*., 14: 525-528.

Mortan, J. F. 1987. Fruits of warm climates. *Julis F. Mortin* Publ., Miami, FL.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.,*15: 473-497.

Papadatou, P., C. Pontikis, E. Ephtimiadou and M. Lydaki. 1990. Rapid multiplication of guava seedlings by *In vitro* shoot culture. *Scientia Hort.,* 45: 99-103.

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

Sharma, Y.K., A.M. Goswami and R.R. Sharma. 1992. Effect of dwarfing aneuploid guava rootstock in high density orcharding. *Indian J. Hort.,* 49: 31-36.

Singh, S.K., P.R. Meghwal, H.C. Sharma and S.P. Singh. 2002. Direct shoot organogenesis on hypocotyls explants from *In vitro* germinated seedlings of *Psidium guajava* L. Cv. Allahabad Safeda. *Scientia Horticulturae*, 95: 213-221.

Zamir R., G.S.S. Khattak, T. Mohammad and N. Ali. 2003. *In vitro* mutagenesis in guava (*Psidium guajava* L.) *Pak. J. Bot*., 35(5): 825-828.

Zamir R., Syed Tariq Shah, Nawab Ali, G.S.S. Khattak and T. Mohammad. 2004. Studies on *In vitro* surface sterilization and antioxidants on guava shoot tips and nodal explants. *Pak. J. Biotch*., 1 (2): 12-16.

(Received for publication 6 March 2007)