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IN-VITRO MUTAGENESIS IN GUAVA (*PSIDIUM GUAJAVA* L.)

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Abstract

In-vitro mutagenesis followed by micro propagation via axillary bud proliferation in shoot tips of guava (*Psidium guajava* L.) cultivar Safeda was carried out. Shoot tips were irradiated with 15 to 90 Gy gamma rays using ^{60}Co gamma cell source and cultured on MS medium containing 3.0 % sucrose, 6-benzyleamino purine (BAP), and L-glutamine. The shoot proliferation was observed after 7 weeks of culturing. Best shoot proliferation was recorded on MS medium supplemented with 1.0 mg /L (BAP) and 250 mg/L L-glutamine. Rooting of the cultured shoots were observed on half strength MS medium supplemented with Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA). Radio sensitivity was assessed by determining the percentage shoot tips survival and shoot proliferation. The LD_{50} (the dose at which 50 % of the population killed) was observed on 45 Gy. The doses above 75 Gy were found lethal to explants.

Introduction

Guava (*Psidium guajava* L.) is one of the important fruit crops of the Indo-Pak subcontinent and its importance is increasing due to its nutritional value, bi-annual bearing and affordable prices. However, in Pakistan guava is usually propagated by seeds (Ahloowalia, 1995). Natural cross pollination, up to 35 % common in guava cultivars (Purseglove, 1968; Menzel, 1985) is responsible for the variability observed in seedlings. In order to grow guava as a commercial fruit crop, there is a dire need for evolution of improved and more specialized cultivars as well as for easy methods for its vegetative propagation. Guava is one of the few horticultural crops, which unfortunately does not lend itself to various asexual propagation methods (Rahman *et al.*, 1991).

Micro-propagation coupled with induced mutations is a suitable technique to improve vegetatively propagated crops (Donini, 1991; Osi-kofi *et al.*, 1996). However, no systematic work either *in vivo* or *in vitro* mutagenesis in guava has been carried out in Pakistan. The impact of mutation techniques for the improvement of fruit trees has been reported worldwide (Donini, 1991; Ahloowalia, 1995; Osi-Kofi *et al.*, 1996). In many vegetatively propagated plants, mutation induction in combination with *in vitro* culture may be the only effective method used for their improvement (Novak *et al.*, 1990; Ahloowalia, 1995; Osi-Kofi *et al.*, 1996).

The objective of the research was to induce *in vitro* mutations in shoot tips of guava for their rapid micro-propagation and to create genetic variability for selecting early bearing, short statured and less seeded guava mutants.

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 wrapped in a cloth and put in a brass jar and irradiated at 15 to 90 Gy of gamma rays with 15 Gy intervals, using ^{60}Co gamma source. Immediately after irradiation these shoot tips 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). 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 80 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 and Skoog (MS) basal medium as described by Murashige & Scoog (1962) supplemented with 1.0 mg/L (BAP), 250 mg/L L-glutamin and 3.0 % sucrose, solidified with 0.8 % plant agar was used for culturing the shoot tips.

The cultures were kept in growth chamber at 25±2 °C under a 16-h photoperiod with a light intensity of 2500 lux to initiate its growth. Experiment was arranged in a randomized complete block design with three replications per treatment, each with 20 explants. Radiation responses were evaluated in terms of explants survival and shoot proliferation after 7 weeks of culture. Surviving shoot tips were transferred to fresh medium and sub cultured to M_1V_1 and subsequently cultured upto M_1V_4. Rooting was induced on half strength MS medium supplemented with Indole-3-acetic acid (IAA) and Indole-3-butyric acid (IBA).

Results and Discussion

Shoot proliferation varied with the different concentrations of BAP and L-glutamine. (Table 1). Generally, the number of shoots developed into plantlets increased with increasing concentration of BAP combined with L-glutamine. These results are in conformity with the results reported by Osei-Kofi *et al.* (1996) who reported that up to a certain limit a high cytokinin favours buds and shoots formation. The highest number of shoots (43) was developed into plantlets when MS medium was supplemented with BAP 1.0 mg/L combined with 250 mg/L L-glutamine. Shoot tip cultured on medium without growth hormone produced few numbers of plantlets (13). The number of shoots development to plantlets increase with the increasing concentration of cytokinin (BAP and L-glutamine). From the foregoing observation it is evident that BAP and L-glutamine is a suitable cytokinin for proliferation of shoots on guava explant, which was optimum at 1.0 mg/L of BAP and 250 mg/L L-glutamine. The superiority of BAP over other cytokinin for multiple shoot formation in fruit plants has also been reported by other investigator (Jaiswal & Amin, 1987). Rooting of plantlets were achieved when half strength MS medium supplemented with Indole-3-acitic acid (IAA) and Indole-3-butyric acid (IBA). The highest numbers of plants were rooted (46) when half strength MS medium was supplemented with 2.5 mg/L IAEA and 2.5 mg/L IBA. No rooting was observed in hormone free medium. Radio-sensitivity showed that dose of 75 Gy gamma rays was lethal to explants. These results are in line with the studies of Osi-Kofi *et al.* (1996), in which it was found that doses above 80 Gy were lethal to shoot tips in two local pineapple cultivars. The LD_{50} found in the present experiment was 45 Gy (Fig. 1). The results of present investigation clearly show that shoot tip explant can be subjected to *in-vitro* induced mutations for creating genetic variability.

The *in-vitro* mutagenised plantlets were successfully established in small plastic pots with 1:1 garden soil and compost after washing the roots thoroughly to remove any remains of the medium. In the first week of transfer the potted plantlets were covered with glass beaker to maintain high humidity.

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

Media concentration	No. of shoot cultured	No. of shoot developed into plantlets	Av. No. of shoot/per culture
MS control	60	13 D	1.8
MS+ 2.22 µM /L BAP	60	25 BCD	2.0
MS+ 4.44 µM /L BAP	60	31 ABC	2.7
MS+ 2.22 µM /L BAP+ 1710.57 µM /L L-glutamine	60	35 AB	2.9
MS+ 4.44 µM /L BAP+ 1710.57µM /L L-glutamine	60	43 A	1.5

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

Table 2. Effect of different concentrations of auxins on rooting of guava cv. Safeda plantlets when cultured on MS medium.

Media concentrations	No. of plantlets cultured	No. of plants fail to root	No. of plants rooted	Av. No. of roots/ plantlet
½ MS (Control)	60	60	0 D	0
½ MS + IBA 2 mg/L	60	36	24 C	2.4
½ MS + IBA 2.5 mg/L	60	32	28 BC	3.4
½ MS + IAA 2 mg/L	60	39	21 C	2.3
½ MS + IAA 2.5 mg/L	60	26	34 B	2.6
½ MS + IAA 2 mg + IBA 2 mg/L	60	24	36 B	3.13
½ MS + IAA 2.5 mg/L + IBA 2.5mg/L	60	14	46 A	3.8

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

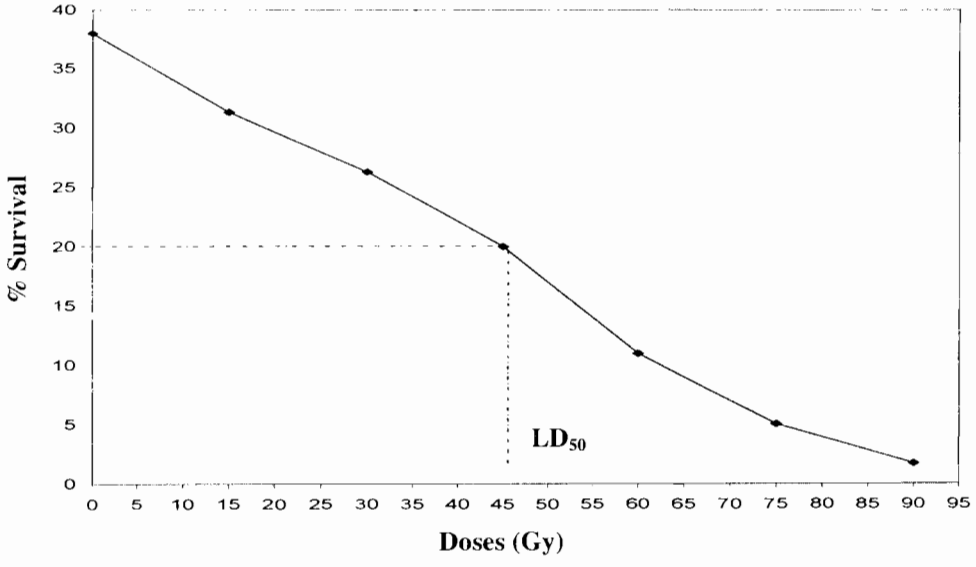

Fig. 1. Effect of different doses of gamma rays on survival percentage of the shoot tips in guava cv. Safeda

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