INDIAN JOURNAL OF ARID HORTICULTURE
2008. Vol. 3 (1): 7-10

Control of oxidative browning during *in vitro* culture initiation of guava genotypes

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Abstract

Micro propagation in guava is impaired by the problem of oxidative browning of explants and culture media during culture establishment phase. The study was conducted to see the efficiency of different techniques and antioxidant compounds on controlling phenolic browning and ultimate culture establishment for micropropagation. Significant differences were recorded between Allahabad Safeda and Aneuploid No. 82 in respect to quantum of exudated phenol and per cent sprouting. Agitation of explants in 0.5% PYPP and 2% sucrose for 40 minutes followed by a quick dip in a solution of citric acid and ascorbic acid @ 75 mg L^{-1} and 50 mg L^{-1} respectively before inoculation resulted in maximum sprouting and reduced exudation of phenol in both the genotypes. Mean phenol exudation in different seasons from nodal and shoot tip explant indicated lower phenol exudation and higher sprouting in Allahabad Safeda as compared to Aneuploid No. 82. Significantly higher sprouting was recorded when the explants were collected during spring or summer as compared to that of autumn season. The quantum of phenol leached into the medium and percent sprouting was found to be negatively correlated

Key words: *Oxidative browning, explant, polyphenol oxidase, antioxidants, in vitro culture initiation.*

Introduction

The poor response of explants from woody trees to *in vitro* manipulation is associated with leaching of phenolic compounds produced by the injured tissues of explants. The browning of explants and culture media is generally considered to be the results of oxidation of phenolic compounds by poly phenol oxidase (Mayer and Hasel, 1979), Peroxidases (Loomis and Battlaile, 1966) or by air (Robinson, 1983). The oxidized product quinones are known to be highly reactive and inhibit enzyme activity leading to the killing of explants due to necrosis (Hu and Wang, 1983).

In guava, tissue culture is essential for clonal propagation and genetic manipulation, since availability of planting material through conventional means is in short supply. Several previous studies have indicated the problem of oxidative browning during micropropagation from mature trees (Amin and Jaiswal, 1987; Prakash, 1992). Many techniques have been used in an attempt to limit detrimental effect of oxidized phenolics on explant survival. Vieitez and Vietez (1980) partially overcome the problem by soaking *Castanea* sativa explants in sterile distilled water for 2-3 hours prior to *in vitro* culture. Several antioxidants such as polyvinylpyrrolidone (Mc Comb, 1978), ascorbic acid and citric acid (Ziv and Havely, 1983) and glutathione (Rugini and Fontanazza,1981) have also been tried to overcome the problem of oxidative browning in different woody species. Partial etiolation of stock plants (Sharma *et al.*, 1997) and collection of explants in the beginning of spring (Chevre *et al.*, 1983) have also been found to minimize the problem. Various techniques have been employed to reduce the inhibitory effect of exudated phenols and its correlation with explants survival and bud sprouting is lacking. Hence, the study was aimed at quantifying the exudation of phenolics as affected by

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seasons of explanting, explants type and beneficial effect of media supplementation of different antioxidants and to establish correlation between exudated phenolics into the medium and successful culture establishment.

Material and methods

The study was conducted in the division of Fruits and Horticultural Technology and Central Tissue Culture Laboratory, NRC on Plant Biotechnology, IARI, New Delhi during 1997-99. Nodal and shoot tip explants were collected from coppiced growth of 12 -years old plants of variety Allahbad Safeda and Aneuploid No. 82 during three seasons i.e. Spring (March-April), Summer (May-June) and Autumn (September-October). Various techniques (Table 1) were adopted for controlling oxidative browning as pretreatment or medium supplement or both. The explants were surface sterilized using methods reported earlier (Meghwal et al., *2003)*. M S medium (Murashige and Skoog, 1962) supplemented with 1 mg L^{-1} BAP (6-Benzylaminopurine), 3 % sucrose and 0.8% agar was used. The cultures were initiated on semi solid medium in test tube 20 mm x 150mm size containing 15 ml of medium. They were incubated at 25 ± 2°C under a 16/8 hour photo period at photon flux intensity of 50-70 µ Em^{-2} S^{-1} provided from cool white florescent tubes.

The alcoholic extraction of total phenol exudated into the medium was prepared as per method followed in grape explants (Sharma, 1993), while total phenol was *estimated by* following improved procedure of Singleton and Rossi Jr. (1965). The total phenol leached into the medium was estimated 7 days after inoculation taking whole quantity 15 ml medium after removing the explants. The total phenol was expressed as µg gallic acid ml^{-1} medium. Before analysis, the explants were transferred on fresh media to see per cent sprouting till one-month of inoculation. The data were recorded on per cent sprouting and total phenol exudation into the media and a correlation coefficient was also worked out between total phenol exudation into the media and per cent bud sprouting. The experiment was laid out in CRD and repeated for three times. The data were analyzed statistically.

Results and discussions

The effect of different techniques for controlling phenolic browning on explants survival and phenol exudation into medium was found to be significant (Table 1). The genotypic differences were significant in respect to per cent sprouting and phenol exudation with Allahabad Safeda showing higher sprouting and lesser phenolic browning as compared to Aneuploid No. 82. Among different treatments, agitation of explants with 0.5% PVPP and 2% sucrose for 40 minutes followed by a quick dip in anti oxidant solution (T_6) before inoculation resulted in maximum sprouting and lower phenol exudation in both the genotypes. The lowest phenol exudation was however,

Table 1. Effect of different treatments on total phenol exudation and per cent bud sprouting during culture initiation of guava genotypes

Treatments	Total phenol exudation (µg ml^{-1} medium)		Bud-sprouting (%)	
	Allahabad Safeda	Aneuploid No.82	Allahabad Safeda	Aneuploid No.82
T_1 Control	9.66	41.00	72.00(58.18)	33.33(35.17)
T_2 Incubation in dark for 48 hrs	9.55	39.00	61.66(51.92)	28.33(32.01)
T_3 Addition of 0.4% PVP to the media	9.22	32.55	53.33 (47.96)	10.00 (18.43)
T_4 Agitation in 0.5% PVPP& 2 % Sucrose for 40 minutes at 100 rpm.	6.11	30.02	71.66 (59.54)	40.00 (39.15)
T_5 Rapid rinse in CA &AA (75&%50 mg L^{-1})	5.95	25.45	78.33 (62.47)	36.66 (37.26)
T_6, T_4&T_5	4.11	21.00	80.00 (63.92	50.66 (45.38)
T_7 Sealing basal ends of explants with wax	10.6	3.55	31.66 (34.02)	20.00 (26.45)
T_8 Media supplementation with 75&50mg L^{-1}CA&AA	10.11	36.78	55.00 (47.96)	15.58 (23.16)
Mean	6.97	28.66	69.44	34.20
CD(P=0.05)	4.30	8.64	14.11	14.95

CA-Citric Acid, AA-Ascorbic acid, PVP-Polyvinlyl pyrrolidone, PVPP-Poly vinyl polypyrrolidone; Figures in parenthesis- Angular transformed values

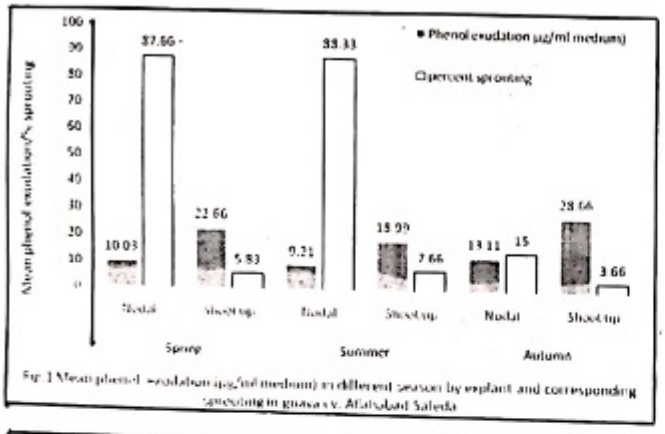

Fig. 1 Mean phenol exudation (µg/ml medium) in different season by explant and corresponding sprouting in guava cv. Allahabad Safeda

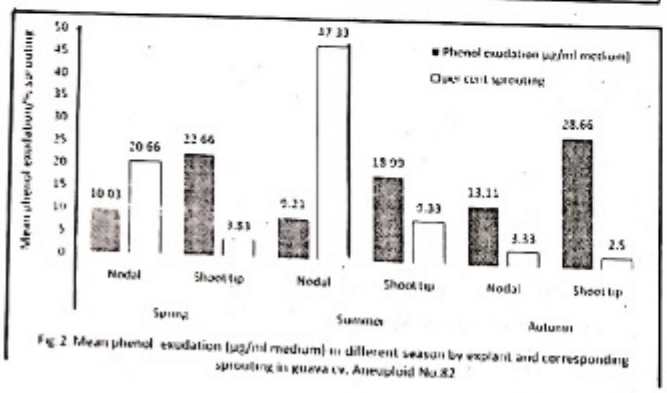

Fig. 2 Mean phenol exudation (µg/ml medium) in different season by explant and corresponding sprouting in guava cv. Aneuploid No.82

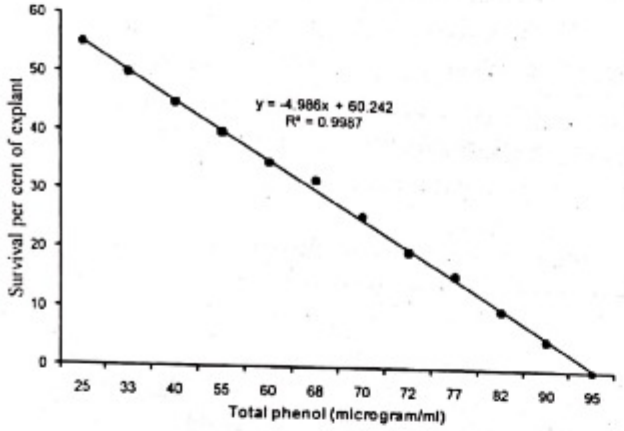

Fig. 3. Correlation between total phenol exudation into the medium and explant survival of guava

recorded by sealing basal cut end (T_7)with wax in both the genotypes but per cent sprouting was drastically reduced in that case. The positive effect of PVPP, ascorbic acid and citric acid on reduced phenolic browning have also been reported earlier in apple (Walkey, 1972), chestunt (Vieitez& Vieitez, 1980) and guava (Amin &Jaiswal, 1988). Media supplementation of PVP, citric acid and ascorbic acid resulted in reduced sprouting per cent as compared to control. This might be probably due to detrimental effect of these compounds. The toxic effect of citric acid and ascorbic acid were recorded by Broome and Zimmerman (1978) in black berry and in teak (Gupta *et al.*, 1980). Mean phenol exudation in different seasons from two types of explants and corresponding bud sprouting have been illustrated in Fig.1& 2 for Allahabad Safeda and Aneuploid No. 82 genotypes respectively. It was indicated that in general phenol exudation in the media was much less in Allahabad Safeda as compared to Aneuploid No. 82 in all the seasons and accordingly higher per cent bud sprouting was recorded in this variety. The difference in phenol exudation as well as per cent sprouting were non significant between spring and summer season but it was highly significant when compared with the values of these parameters recorded during autumn season in both the genotypes. The lesser phenol release from Allahabad Safeda explants might be due to lower tissue content of phenolic compound as compared to those in Aneuploid No. 82. This implied that phenol content of explants and phenolic browning of explants and media may be positively correlated. Although phenol content of explants was not estimated but the release of phenol in the medium and corresponding sprouting was found to be negatively correlated in Aneuploid No. 82 genotype (Fig. 3). In this context it is imperative to mention that many plants are naturally rich in polyphenolic compounds, which are commonly, regarded as inhibitory agents. Preece and Compton (1991) emphasized that these inhibitory agents have various phyto toxic effects on plants. It is possible that Aneuploid No. 82 being dwarfing in nature might have high level of inhibitory phenols, which would also have leached into the medium.

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