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STUDIES ON *IN VITRO* SURFACE STERILIZATION AND ANTIOXIDANTS ON GUAVA SHOOT TIPS AND NODAL EXPLANTS

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ABSTRACT:

In vitro studies were carried out to investigate the effect of different surface sterilization agents and antioxidants on the guava (*Psidium guajava* L.) Cv. Safeda shoots tips and nodal explants. The best sterilant observed for shoot tips was mercuric chloride ($HgCl_2$) at 0.05% for 5 minutes plus 70% ethanol which gave maximum survival percentage of (67%) while minimum were survival (22%) was observed with 2% Sodium hypochlorite for 10 minutes. In case of nodal explants the best sterilant was 4% Calcium Hypochlorite ($CaOCl_2$) for 10 minutes which gave 25% survival.

Blackening of media was reduced and gave 55% green shoot tips when 75:50mg/l citric acid and ascorbic acid was supplemented to the medium. While 75:50 mg/l citric acid and ascorbic acid, 100mg/l Polyvinyl pyrolidone (PVP) and 200mg/l Activated charcoal when added to the medium gave 13% green nodal 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 (Zamir et al 2003) and natural cross pollination (up to 35%) common in guava cultivars (Purseglove, 1968 & Menzel, 1985) is responsible for the variability observed in seedlings trees. Guava is difficult to propagate plant and *in vivo* propagation methods are very little successful. The application of root promoting hormones like IAA and IBA to stem cuttings can achieve little success. *In vitro* establishment of guava is very difficult, because the cut surface of the explants taken from the mother plant becomes brown and releases browning material into the media, which ultimately inhibits its morpho-genetic activity (Gasman *et al*; 1978).

The explant taken from field was grown very difficult in tissue culture because of infestation with microorganisms (Khattak *et al.* 1990). Various concentrations and combinations of sterilants has been reported and used in preliminary trials *in vitro* on tissue of juvenile and adult trees explant of guava to see the effect of free infestation explant and also on its growth.

Browning process of tissue is caused by the oxidation of tannin and poly phenols and the formation of guinoes, which are highly reactive and toxic to the tissue. Phenolic compounds contain at least one hydroxyl group on the benzene ring. Several oxidizes such as monophenolase (tryosinase), polyphenol (catecholoxidase) oxidize the hyroxy group resulting in the formation of

guinone and water (Loomis and Battaile, 1966). Plant tissue contains these substances in separate pools or compartments. During tissue wounding or senescence these pools are integrated and the oxidation process is initiated (Monaco *et al.* 1977).

It is possible to overcome this problem through the application of various antioxidants. These compounds were applied in apple (Jones *et al*. 1979), mango (Litz *et al.*1982). However, browning of guava and walnut tissue was not overcome (Gasman *et al*; 1978). Therefore these selective substances were applied on shoot tips and nodal explants from seedling and adult bearing trees of guava under sterilization agents to study their affects against browning.

MATERIALS AND METHODS:

Plant material was collected from field grown trees of guava cultivar Sufeda. Shoot tips of 2-3 cm and nodal explants of about 5-6 cm were taken from new growth flushes in April-May. After removing the unfolded leaves, the nodal explants and shoot tips were thoroughly washed under running tap water for ½ an hour with one drop of zip, as a detergent. Then the shoot tips and nodal explants were surface sterilized with 70% ethanol for 1 minute followed by surface sterilization with 0.05% $HgCl_2$ for 5 minutes with continuous shaking. Same procedure were followed with every sterilants i.e. 2% Sodium hypochlorite ($NaOCl_2$) for 5 and 10 minutes, 0.05 % $HgCl_2$ for 10 minutes and 4% $CaOCl_2$ for 5 minutes and 10 minutes followed by 3 washing with sterilized distilled water. All sterilization manipulation was carried out in Laminar Airflow Hood.

Shoot tips and nodal explants were culture on the agar gilled MS basal medium after an instant rinse in an antioxidant solution of Citric acid and Ascorbic acid (75:50mg/l distilled water, autoclaved), shaken for 30-45 minutes in 100mg/ l Polyvinyl pyrolidone (PVP) solution or PVP 100mg/l + 200mg/l Activated charcoal and 200mg/l Charcoal were used for the problem of oxidation of phenolic compounds in the culture medium. The results were compared to the MS (control) i.e. Murashige and Skoogs 1962 medium contained sucrose 30gm/l, gilled with 8gm/l agar and the pH was adjusted to 5.8.

Shoot apices and nodal explants both were cultured in 15 x 2.5 cm test tube containing 15 ml medium. The test tubes were covered after culturing with autoclaved polypropline sheet and airtight with rubber band. The cultures were incubated for 16 hours daily light of florescence Philips white tubes with light intensities of 2000 lux at $25^oC \pm 02^oC$ temperature. Cultures were maintained for 08 weeks and subculture regularly after 04 weeks. The experiment was arranged in Randomize Complete Block Design, repeated three times with 20 explants in each repeat. Comparison among treatment were made using LSD test.

RESULTS AND DISCUSSION:

1. Effect of sterilants on surface sterilization of shoot tips and nodal explants: The data in table-1 on the shoot tips treated with 0.05% Mercuric chloride ($HgCl_2$) for 5 minutes showed maximum survival (67%) while 0.05% Mercuric chloride for 10 minutes gave (37%) survival. Similarly 2% sodium hypochlorite for 5 minutes gave (33%) survival of shoot tips and 4% Calcium hydrochloride ($CaOCl_2$) for 5 and 10 minutes gave 25% and 28% survival respectively while minimum survival of 22 % was observed when they were

treated with 2 % Sodium hypochlorite for 10 minutes (Table 1).

The results shows that concentration of 0.05% of Mercuric chloride for minimum time of 5 minutes gives good results (67%) as compared to the same concentration for 10 minutes (37%). The results are in line with those of Khattak *et al.* (1990) who carried out surface sterilization with 0.05% solution of $HgCl_2$ for 5 minutes followed by 3 washing with sterilized distilled water. It was also observed that survival rate increases as dipping time of shoot tips in mercuric chloride decreases.

For nodal explants the effective sterilant was 4% Calcium hypochlorite for 10 minutes which gave 25% survival followed by 0.05% Mercuric chloride for 5 minutes (21%) while both 4% calcium hypochlorite for 5 minutes and 0.05% Mercuric chloride for 10 minutes gave the same (25%) survival. Similarly 2% $NaOCl_2$ for 5 minutes gave only 17% survival of nodal explants while 2% sodium hypochlorite for 10 minutes was inferior to all and gave only 10% survival (Table 1).

The above review depicts that the sterilants throw no significant effect in reducing contamination of nodal explants, while 0.05% $HgCl_2$ for 5 minutes proved to be effective in controlling the contamination of shoot tips. In nodal explants its shows inferior results. It might be due to the aged nature of the nodal explants, which may be heavily contaminated as compared to shoot tips.

2. Effect of Antioxidants on browning of shoot tips and nodal explants: Among all antioxidants 75:50mg/l citric acid and ascorbic acid was effective which gave 55% green shoot tips, followed by 100mg/l Polyvinyl pyrolidone (PVP) (45%), while MS (control) was inferior to all and gave only (17%) green shoots. Similarly 200mg/l Charcoal alone gave more percentage of green explants as compared to its interaction with 100mg/l Polyvinyl pyrolidone (PVP) (32%, 27%) respectively (Table 2). Fitchet (1990) studied Dimple guava and used antioxidants 75:50 mg/l Citric and Ascorbic acid. After giving a dip he allowed shoot tips to dry for 30 minutes then he cultured them on MT medium. He said that these solutions are very useful in preventing oxidative browning.

In present experiment the Citric acid and Ascorbic acid gave good results but leaving the explants to dry after dipping for any length of time leaves no extraordinary results. It was better to culture shoot tips immediately. The effect of 100 mg/l PVP (polyvinyl pyrolidone) is exactly the same as given (i.e. 45%) by Khattak *et. al.,* (1994).

The effect of antioxidants in reducing browning of nodal explants showed that 75:50mg/l Citric acid and Ascorbic acid, 100mg/l PVP and 200 mg/l activated charcoal gives 13% green nodal explants. The concentration of 100mg/l Polyvinyl pyrolidone (PVP) and 200mg/l Activated charcoal followed the above treatments gave 8% green nodal explants. While MS (control) proved to be inferior to all and gave only (6%) green nodal explants.

Gasman *et al* (1978) refers that *in vitro* establishment of guava tissue culture has been very difficult; because the cut surfaces of the explants taken from the mother plant becomes brown and releasing browning material into the medium which ultimately inhibits its morphogenetic activity. Present studies are in the line with the Gasman *et al.* (1978). He reported that browning of the guava and walnut tissue were not overcome by use of various antioxidants.

During experiment it was observed that 75:50 mg/l Citric acid and Ascorbic acid were somewhat effective in controlling browning of shoot tips as compared to nodal explants, which gave inferior results. It was noticed that browning of the material was more intense in case of nodal explants because the nodal explants contained more phenolic compounds as compared to shoot tips.

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Table –1: Effects of various sterilants on survival percentage of guava shoot tips and nodal explants

S. No.	Sterilants/ strength/ time	SHOOT TIPS			NODAL EXPLANTS		
		No. of shoot tips contaminated	No. of shoot tip survived	Survival %	No. of shoot tips contaminated	No. of shoot tip survived	Survival %
1.	$NaOCl_2$ 2% for 5 minutes	40	20 BC	36	52	8	17
2.	$NaOCl_2$ 2% for 10 minutes	47	13 C	22	54	6	10
3.	$HgCl_2$ 0.05% for 5 minutes	20	40 A	67	47	13	21
4.	$HgCl_2$ 0.05% for 10 minutes	38	22 B	37	51	9	15
5.	$CaOCl_2$ 4% for 5 minutes	45	15 BC	25	51	9	15
6.	$CaOCl_2$ 4% for 10 minutes	43	17 BC	28	45	15	25

Means of same category followed by different letters are statistically different from each other at 5% level of significance using LSD test. Total number of shoot tips cultured in each treatment = 60

Table 2: Effect of various antioxidants on the browning of MS media when cultured with guava shoot tips and nodal explants

S. No.	Antioxidants	SHOOT TIPS			NODAL EXPLANTS		
		No. of shoot tip (Brown)	No. of shoot tip (Green)	Survival %	No. of nodal explants (Brown)	No. of nodal explants (Green)	Survival %
1.	Dip in soln. Of Citric acid + Ascorbic acid (75-50 mg/l)	27	33	55	52	8	13
2.	Agitated for 30-45 minutes in PVP 100 mg/l	33	27	45	52	8	13
3.	PVP 100 mg/l + Activated Charcoal (200 mg/l)	44	16	27	55	5	8
4.	Charcoal 200 mg/l	41	19	32	52	8	13
5.	MS (control)	50	10	17	56	4	6

Total number of shoot tips cultured in each treatment =