Online ICV: 4.79; GIF: 0.287

www.hortflorajournal.com

HortFlora Research Spectrum, 3(3): 263-266 (September 2014) ISSN : 2250-2823

RESPONSE OF DIFFERENT STERILANTS, PHENOL BINDING AGENTS AND ANTIOXIDANTS ON *IN VITRO* ESTABLISHMENT OF GUAVA (*Psidium guajava* L.)

D.S. Mishra* and Rajesh Kumar

Department of Horticulture, G.B. Pant University of Agriculture and Technology, Pantnagar-263145 (Uttarakhand)
**E-mail: dsmhort@gmail.com*

ABSTRACT : Techniques were standardized for minimizing microbial contamination and leaching of phenols in the media for quick establishment of cultures in guava. The maximum number of aseptic explants with higher survival was obtained by sequential application of ethanol (70%) for 30 second, $HgCl_2$ (0.1%) for 5 minutes, KCl (1%) for one minute and NaOCl (1%) for 8 minutes. The problem of phenolic browning was successfully minimized to a great extent by supplementing MS media with various concentrations of antioxidant and phenol-binding agents. Citric acid at 500 mg/l in the culture medium reduced phenolic exudation the most and promoted the best survival of explants. Initial incubation of cultures under varying levels of light intensity showed escapement upto certain limit from phenolic browning of the media. The lowest frequency of browning of media was observed with cultures which were incubated in complete dark for 72 hours, however maximum survival was recorded with 24 hours of dark period.

Keywords : *Guava, in vitro establishment, phenolic browning, antioxidant, contamination.*

Guava is one of the most promising fruit crops of India and is considered to be exquisite, nutritionally valuable and remunerative. Guava fruits are used both for fresh consumption and processing purposes. It excels most other fruit crops in productivity, hardiness, adaptability and vitamin C content. Besides high nutritive value, it bears heavy crop every year and gives good economic return. In recent years, guava cultivation is getting popularity due to increasing international trade, nutritional contents and value added products. 'Pant Prabhat', a promising guava cultivar is becoming much popular among fruit growers of *Tarai* of Uttarakhand and adjoining areas of U.P. as it exceeds in yield and quality over established cultivars of guava *viz.,* Allahabad Safeda and Sardar. Therefore, there is need to develop the technique which could multiply it at faster rate to meet out the farmer's demand. *In vitro* propagation is now a days established technique for faster multiplication. But this technique is jeopardized by microbial contamination and leaching of phenolic compounds in the media which hamper growth. Disinfection requires the use of chemicals that are toxic to the microorganisms but relatively non-toxic to the plant material (Hartmann *et al.,* 6). For this purpose many sterilizing agents such as calcium hypochlorite, sodium hypochlorite, chlorine water, mercuric chloride, hydrogen-peroxide, ethylene oxide and ethanol are being used. The concentration of various sterilants and their exposure time varies with the plant parts and species (Haendre, 5). The use of tissue culture techniques for the propagation of woody plant species has invariably failed due to oxidative browning of the wounded explant. The brown exudate that diffuse into the medium was found to be detrimental to further development of the explants, which eventually become necrotic and die (Ziv and Halvey, 12). This paper describes in vitro methods for reducing contamination and oxidative browning which could enhance multiplication potential of guava cv. Pant Prabhat through *in vitro* techniques.

Received : 06-08-2014 ***Accepted : 29-08-2014***

MATERIALS AND METHODS

The explants of cultivar Pant Prabhat were collected from mature field grown stock plants from new growth in 200 ml of conical flask containing 100 ml distilled water, 2 ml Tween-20 and 1 mg refampicin and brought to the laboratory. The explants were washed gently with liquid detergent and kept under running tap water for two hours. These nodal segments were used for conducting three different experiments related with culture establishment. In the first experiment, surface sterilization of explants was done under laminar air flow cabinet using multiple disinfectants dip serially, i.e. 70% ethanol, 0.1% $HgCl_2$ and 1% NaOCl for different time periods. The explants were also treated with autoclaved 1% KCl for 1 minute in the cases which were receiving $HgCl_2$ treatment. The explants were washed with sterilized distilled water (5-6 times) after each treatment. The data were recorded on per cent aseptic culture and survival of explants.

In the second experiment, the sterilized nodal segments were cultured on MS medium supplemented with PVP (100, 500, 1000 mg/l), PVPP (1, 2, 3 g/l),

charcoal (250, 500, 1000 mg/l) as a phenol binding agents and citric acid (100, 500, 1000 mg/l), ascorbic acid (100, 250, 500 mg/l) as antioxidant agents. All constituents of MS medium were added before adjusting the pH to 5.7 with 1.0 N NaOH / HCl. After mixing it well, the boiled liquid medium was dispensed in 150 × 25 mm culture tube, plugged with non-absorbent cotton plugs and autoclaved at 1.06 kg/cm2 pressure (121°C) for 20 minutes. These cultures were incubated in culture room under 16/8 hours light and dark photoperiod at 25 ± 1°C. Data were recorded for percent browning and survival after 3 weeks of incubation.

In the third experiment, the sterilized explants were inoculated on MS medium and these cultures were initially exposed to various light intensities viz., no light, 1000 lux and 3200 lux light for different durations like 24, 48 and 72 hours. After this treatment, all cultures were given an illumination of 3200 lux with 16/8 hours light and dark photoperiod. The observations regarding percent browning and survival of explants were noted 3 weeks after inoculations.

RESULTS AND DISCUSSION

Effects of surface sterilants

The best sterilization treatment was obtained through sequential exposure of explants to 70% ethanol (30 second), 0.1% $HgCl_2$ (5 minute) and 1% NaOCl (8 minute) which resulted in 60.28% aseptic cultures with 77.48% survival of explants (Table 1). Further increase in exposure time to various sterilants resulted in significant reduction in survival of explants. One of the probable reason for the death of explants, when they are exposed to surface sterilants for longer duration may be due to contamination of mercury (Hg^{2+}) present in $HgCl_2$, causing phytotoxicity for survival of explants. Beneficial effects of $HgCl_2$ followed by NaOCl have also been reported by other workers (Amin and Jaiswal, 1; Kumar and Tiwari, 7; Parkash and Tiwari, 9) in guava. The best asceptic cultures as well as survival of explants of banana (Srivastava *et al.,* 10) were also obtained with 0.1% mercuric chloride.

Effect of phenol biding agents and antioxidants

The problem of browning due to leaching of phenols in the media was significantly reduced by inoculating explants on MS media fortified with various concentrations of antioxidants and phenol binding agents (Table 2). Observation after two weeks indicated significantly higher survival rate with PVP 500 mg/l (68.36%), PVPP 2 g/l (61.76%), activated charcoal 1000 mg/l (76.24%) and citric acid 500 mg/l (85.57%). So, out of various antioxidants and phenol binding agents at various concentrations, activated charcoal and citric acid significantly reduced the browning and improved the survival. The beneficial effect of activated charcoal was, however, associated with reduced vigour of surviving explants might be attributed to its absorption of organic molecules and plant growth regulators. Reduction in browning of media with higher survival rate might be due to the fact that phenolic compounds are involved in growth regulation process and a group of them are auxin protectors. The auxin protectors should be kept within the tissue to stimulate growth and their release to the culture medium should reduced to alleviate the

Table 1: Effect of surface sterilants and their exposure time on the aseptic culture and survival of explants.

Treatments	Surface sterilants with exposure time				Per cent aseptic culture (after one week)		Per cent survival of explant (after 3 weeks)	
	Ethanol (70%) (Second)	$HgCl_2$ (0.1%) (minute)	NaOCl (1%) (minute)	KCl (1%) (minute)				
T_0	Control	–	–	–	0.00	(0.00)	0.00	(0.00)
T_1	30	–	–	–	0.00	(0.00)	0.00	(0.00)
T_2	30	2	0	1	14.39	(22.29)	1.77	(7.61)
T_3	30	–	4	–	11.22	(19.55)	2.43	(8.93)
T_4	30	4	4	1	26.43	(30.93)	18.38	(25.38)
T_5	30	4	6	1	40.52	(39.53)	30.34	(33.42)
T_6	30	5	6	1	52.27	(46.30)	37.07	(37.50)
T_7	30	5	8	1	68.28	(55.72)	77.48	(61.68)
T_8	30	6	8	1	80.41	(63.74)	31.41	(38.04)
T_9	30	6	10	1	86.26	(68.24)	24.69	(29.79)
T_{10}	30	8	10	1	94.12	(76.01)	11.00	(19.36)
C.D. (P=0.05)						(1.40)		(1.25)

*Figures in parentheses indicate transformed value.

Table 2 : Effect of phenol binding and antioxidant additives on the degree of browning and survival of explants.

Treatment		Browning (%)		Survival (%)	
Control	_	100.00	(90.00)	20.07	(26.60)
PVP	100 mg/l	95.72	(78.19)	35.49	(36.55)
	500 mg/l	40.13	(39.30)	8.36	(55.77)
	1000 mg/l	56.72	(48.87)	30.37	(33.43)
PVPP	1 g/l	84.26	(66.63)	33.04	(35.08)
	2 g/l	66.15	(54.43)	61.76	(51.80)
	3 g/l	76.38	(60.93)	48.08	(43.89)
Citric Acid	100 mg/l	96.00	(78.53)	64.39	(53.36)
	500 mg/l	36.31	(37.65)	85.57	(67.69)
	1000 mg/l	63.76	(52.99)	58.66	(49.99)
Ascorbic Acid	100 mg/l	99.33	(87.28)	33.33	(35.24)
	250 mg/l	86.47	(68.44)	38.00	(38.05)
	500 mg/l	74.04	(59.37)	44.13	(41.63)
Activated charcoal	250 mg/l	67.97	(55.54)	63.33	(52.74)
	500 mg/l	60.15	(50.86)	70.75	(57.26)
	1000 mg/l	36.39	(37.09)	76.24	(60.38)

phytotoxic effect (Debergh and Read, 3). This observation is in agreement with those of Siddiquui and Farooq (11), Parkash and Tiwari (9) and Mishra *et al.* (8) in guava.

Light intensity and its exposure time

In the present investigation, the problem of browning was successfully reduced by incubating cultures at varying levels of light intensity and time (Table 3). The effect of light intensity was highly significant due to initial incubation of cultures under complete darkness for 72 hours which resulted in minimum browning (33.27%) of the explants with minimum survival rate (41.76%). However, maximum survival (79.20%) with significantly low browning (58.65%) were recorded with 24 hours dark period. Therefore, this treatment was found to be feasible for reducing leaching of phenolics in the media. This can be well explained by the fact that illumination have favourable effect on the synthesis and accumulation of phenolic compounds in in vitro cultures (Dalal *et al.,* 2) or it may be due to reduced enzymatic activity by dark incubation (George and Sherrington, 4). Therefore, reduced synthesis of phenolic compounds resulted in increased survival of explants. Similar results have also been experimentally substantiated by Kumar and Tiwari (7) in Chinese guava.

Table 3 : Effect of initial exposure to varying light intensities and time hours on the control of phenol leaching and survival of explants.

Light intensity	Exposure time (hours)							
	Per cent browning of explant (%)				Survival of explant (%)			
	24	48	72	Mean	24	48	72	Mean
No light	58.65 (49.98)	53.30 (46.89)	33.26 (35.20)	48.40 (44.02)	79.20 (62.92)	61.05 (51.38)	41.76 (40.25)	60.67 (51.52)
1000 Lux	47.16 (43.37)	44.31 (41.73)	38.73 (38.49)	43.40 (41.20)	60.60 (51.12)	64.19 (53.24)	69.29 (56.36)	64.69 (53.57)
3200 Lux	56.11 (48.51)	70.25 (56.96)	82.43 (65.25)	69.59 (56.91)	58.14 (49.69)	60.42 (51.02)	55.24 (48.01)	57.93 (49.57)
Mean	53.97 (47.29)	55.95 (48.53)	51.47 (46.31)	–	65.98 (54.58)	61.88 (51.88)	55.43 (48.21)	–
	Light	Time	Interaction		Light	Time	Interaction	
C.D.(P=0.05)	1.67	1.67	2.89		1.43	1.43	2.48	

*Figures in parentheses indicate transformed value.

REFERENCES

1. Amin, M.N. and Jaiswal, V.S. (1987). Rapid clonal propagation of guava through in vitro shoot proliferation on nodal explants of mature tree. *Plant Cell, Tissue and Organ Cult.,* **9** : 235-243.
2. Dalal, M.A., Sharma, B.B. and Sahini, C.K. (1992). Effect of incubation light intensity, antioxidants and phenol binding agents on *in vitro* oxidative browning on culture initiation of grape vine. *Indian J. Hort.,* **49** : 287-293.
3. Debergh, P.C. and Read, P.E. (1991). Micropropagation. In : *Micropropagation technology and application* (Debergh, P.C. and Zimmerman, H. Eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands, 1-13.
4. George, E.F. and Sherrington, P.D. (1984), Plant propagation by tissue culture – *Handbook and Directory of Commercial Laboratories*. Exegetics Limited, Edington, U.K.
5. Haendre, R.R. (1993), Nutritional requirement of plant tissue culture. In: Mascarenhas, A.F. ed. *Handbook of Plant Tissue Culture,* ICAR, New Delhi, pp. 25-32.
6. Hartmann, H.T., Kester, D.E. and Davies, F.T. Jr. (1993). *Plant Propagation : Principles and Practices,* 5 th ed. Prentice Hall of India Pvt. Ltd., New Delhi.
7. Kumar, R. and Tiwari, J.P. (2001). Effect of chemicals, light intensity and its duration on *in vitro* establishment of Chinese guava (*Psidium friedrichsthalianum*). *Prog. Hort.,* **33** (1) : 1-6.
8. Mishra, D.S., Tiwari, J. P., Kumar, R., Kumar, P. and Mishra, N.K. (2010). *In vitro* clonal propagation studies on the nodal explants of *Psidium cujavillis. Ann. Hort.,* **3**(1): 58-62.
9. Parkash, H. and Tiwari, J.P. (1996). Micropropagation of guava (*Psidium guajava* L.). J. *Appl. Hort.,* **2** (1-2) : 98-101.
10. Srivastava, V., Singh, A.K. and Singh, S.P. (2013). Explant surface sterilization technique for micropropagation of banana (*Musa sp.*) cv. Dwarf Cavendish. *HortFlora Res. Spectrum,* **2**(3) : 235-238
11. Siddiqui, Z.M. and Farooq, S.A. (1996). Role of antioxidants in the elimination of phenolic compounds from the *in vitro* cultures of *Psidium guajava* L. (guava). *Adv. Plant Sci.,* **9** (2) : 155-158.
12. Ziv, M. and Halvey, A.H. (1983). Control of oxidative browning and *in vitro* propagation of *Strelitzia reginae. HortSci.,* **18** (4) : 434-436.

❑

Citation : Mishra D.S. and Kumar R. (2014). Response of various sterilants, phenol binding agents and antioxidants on *invitro* establishment of guava (*Psidium guajava* L.). *HortFlora Res. Spectrum,* **3**(3) : 263-266.