

Effect of surface sterilizing agents on *in vitro* culture establishment of tamarind (*Tamarindus indica* L.)

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ABSTRACT

The experiment was conducted in the plant tissue culture laboratory of the Department of Horticulture, University of Agricultural Sciences, Dharwad during 2001-03. A method has been standardized for quick establishment of aseptic cultures in tamarind from mature field grown stock plants for micropropagation. The maximum aseptic culture was obtained by treating explants with mercuric chloride 0.1% for 10 minutes.

Key words : Tamarind, Mercuric chloride, Cutter

Tamarind (*Tamarindus indica* L.) is one of the arid fruits crops grown widely in the tropical and sub-tropical regions of the Indian sub-continent particularly in central and south India. Tamarind is popularly known as 'Indian date'. It is a multipurpose tree having high medicinal, industrial and nutritional values in addition to its main use as food, fodder and timber.

Surface sterilization is an obligatory step prior to *in vitro* culture of any plant tissue and can become a critical point in establishment of certain species especially when the explant is derived from field grown woody perennial plants. Surface disinfection of explant is difficult as it is very sensitive to disinfecting agents. There are conflicting reports regarding the nature of chemical, its concentration and duration of treatments for surface disinfection of guava (Prakash, 1992; Siddiqui and Farooq, 1997) and *Piper nigrum* (Fitchet-prunell, 1990) explants. In view of the above an experiment was designed to test the efficacy of surface sterilizing agents, their safest concentration and duration of treatment for maximum aseptic culture establishment of tamarind from field grown adult plants.

MATERIALS AND METHODS

The experiment was conducted in the plant tissue culture laboratory of the Department of Horticulture, University of Agricultural Sciences, Dharwad during 2001-03. The experiment material comprised of young shoots with shoot tips, leaves, axillary buds, stem segments were collected as explants from mature tree of DTS-1. The shoot tips of 1.0 to 1.5 cm, leaves of 0.5 cm and axillary buds of 1.0 to 1.5 cm length were used as explants. After taking the explants of optimum size from mature plant

source, they were washed with detergent (Tween-20) 0.1 per cent with bavistin (2 g/l) solution and later washed under running tap water for 4-5 times followed by 3-4 washings with double distilled water. All explants were surface sterilized using two chemicals *viz.*, Mercuric chloride (HgCl_2) and sodium hypochlorite (NaOCl) each at three concentrations HgCl_2 0.05, 0.10, 0.20 per cent and NaOCl 0.50, 1.00, 2.00 per cent. The duration of treatment was for 2, 4, 6, 8, 10 minutes.

After each treatment the explants were washed 2-3 times with sterile distilled water and finally they were surface dried before being inoculated into the culture media (MS media) vertically. The cultures were maintained at $25 \pm 2^\circ\text{C}$ and 16/8 hrs light dark period. Observations on contamination and per cent survival were recorded till 30 days after inoculation. The data generated from the experiment were statistically analysed by following completely randomized design (CRD) as described by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The response of explants for two chemicals each with three concentration and five durations on extent of contamination and per cent survival was recorded as mentioned in Table 1 and 2, respectively.

The highest number of aseptic culture and per cent survival of explants were obtained with HgCl_2 at 0.1 per cent for 10 minutes, which indicates optimal disinfecting action of HgCl_2 . The results are in conformity with those of Fitchet-prunell (1990) on *Piper nigrum* and Senevirantne and Wijesekara (1995) in *Hevea* species.

Eventhough contamination was not observed at higher concentrations of HgCl_2 (0.2%), there was no

Table 1 : Effect of surface sterilants on contamination rate of explants of tamarind						
Sterilants and concentration (%)	Duration (minutes)					Mean
	2	4	6	8	10	
HgCl ₂ – 0.05	100.00 (90.00)*	100.00 (90.00)	88.33 (70.09)	80.00 (63.44)	73.33 (58.89)	92.80 (74.48)
HgCl ₂ – 0.10	65.00 (53.73)	61.66 (51.77)	28.33 (32.14)	18.33 (25.33)	0.00 (0.00)	32.80 (34.96)
HgCl ₂ – 0.20	31.66 (34.27)	30.00 (33.21)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.70 (13.84)
NaOCl – 0.50	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	88.33 (70.00)	99.52 (86.00)
NaOCl – 1.00	100.00 (90.00)	98.33 (82.51)	91.66 (73.26)	73.33 (58.89)	78.33 (62.24)	91.80 (73.40)
NaOCl – 2.00	65.00 (53.73)	66.6 (54.76)	76.66 (61.44)	60.00 (50.77)	43.33 (41.45)	62.70 (52.36)
Mean	88.80 (70.50)	84.80 (67.04)	66.40 (54.60)	55.50 (48.16)	39.40 (38.90)	
	Chemical (C)		Duration (D)		Interaction (C x D)	
S.E.±	0.336		0.306		0.752	
C.D. (P=0.01)	1.916		1.992		3.025	

* Figures in parenthesis indicate arcsin values

Table 2 : Effect of surface sterilants on per cent survival of explants of tamarind						
Sterilants and concentration (%)	Duration (minutes)					Mean
	2	4	6	8	10	
HgCl ₂ – 0.05	0.00 (0.00)	0.00 (0.00)	11.66 (20.00)	21.66 (27.66)	25.00 (30.00)	7.30 (15.76)
HgCl ₂ – 0.10	31.66 (34.27)	43.33 (41.15)	71.66 (57.80)	80.00 (63.44)	100.00 (90.00)	70.90 (57.33)
HgCl ₂ – 0.20	66.67 (54.82)	70.00 (56.79)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	14.90 (22.67)
NaOCl – 0.50	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	11.66 (20.00)	0.60 (4.45)
NaOCl – 1.00	0.00 (0.00)	1.66 (7.49)	8.33 (16.74)	26.66 (31.11)	21.66 (27.76)	8.30 (16.73)
NaOCl – 2.00	33.34 (35.30)	30.00 (33.21)	21.66 (27.76)	38.33 (38.23)	53.33 (46.89)	34.90 (36.28)
Mean	12.90 (21.02)	15.60 (23.30)	12.30 (20.58)	20.50 (26.93)	34.33 (35.88)	
	Chemical (C)		Duration (D)		Interaction (C x D)	
S.E.±	0.277		0.253		0.619	
C.D. (P=0.01)	1.579		1.647		2.155	

*Figures in parenthesis indicate arcsin values

survival of explants due to high bleaching activity of chlorine which killed the cells thereby causing death of the explant. Similar observations were recorded in micropropagation studies on jack fruit with HgCl₂ 0.2 per cent where the explants failed to show any growth during proliferation stage (Chavan *et al.*, 1996).

The lowest aseptic cultures were obtained with lower

concentration of HgCl₂ at 0.05 per cent and NaOCl 0.5 per cent. This is because of reduced effectiveness of chemicals at lower concentrations. The lowest contamination rate was obtained in longer duration. It was noticed that as the duration of treatment increased, the contamination rate decreased, which indicate that higher duration results in more absorption of chemicals, lessens

the incidence of contamination as a many times source of contamination is internal.

Optimal concentration of chemical and duration of chemical in the present investigation was HgCl₂ at 0.1 per cent for 10 minutes with respect to least contamination rate and high per cent survival.

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