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SHORT COMMUNICATION

Effect of surface sterilizing agents on establishment of axenic culture in kinnow mandarin (*Citrus deliciosa*)

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The surface sterilization is the most important step prior to initiate tissue culture and can become a critical point in establishment of certain species particularly when the explants was derived from field grown woody perennial plants. Kinnow mandarin (*Citrus deliciosa*) is a cross, between the king sweet orange (*Citrus nobilis*) and Willow leaf mandarin (*C. deliciosa*) developed in California by H.B. Frost. Kinnow fruits have a great therapeutic value due to favourable ratio of K and Na. Kinnow fruits are not only delicious and refreshing but also possess great nutritive value. It contains 10-12% carbohydrate, 0.6-0.8% protein, 0.9-1.1% vitamin A, 0.45–0.55% vitamin C, 0.01-0.02% thiamine, 0.002 – 0.003% riboflavin, 0.25-0.35% calcium, 0.18-0.25% magnesium and 0.028-0.034% iron.

Tissue culture technology offers an advantage over conventional methods of propagation ('T'-budding) in producing large number of true to type plants from healthy plant with in a short period of time. In this perspective studies were taken out to standardize the safest concentration and duration of treatment for maximum aseptic culture establishment in kinnow from field grown adult plants.

Young, fresh growing branch from mature (4-5 years) plant of kinnow were harvested and cut in to segments, each segment having at least one bud. These nodal segment were washed under running tap water for two hours followed by 10 minutes in 0.5per cent Bavistin. These explants were treated with quickly once in 70 per cent ethanol followed by surface sterilized with 0.1per cent $HgCl_2$ for 4,6,8,10,12 and 14 minutes and 0.5 per cent sodium hypochlorite solution for 5,10,15,20,25 and 30 minutes and washed thoroughly with autoclaved double distilled water for 3-4 times. These surface sterilized segments were inoculated in screw cap Jam bottles of 200 ml. The bottles contained 30-40 ml of Murashige and Skoog (1962) medium containing 3 per cent sucrose and 0.8 per cent agar. All the surface sterilized explants were inoculated. The medium autoclaved at 121 °C (1.06kg cm-2) for 20 minutes. All the process was carried out under aseptic condition in laminar air flow cabinet.

The each treatment was replicated ten times. All the cultures were incubated in culture room at 27°C ± 0.5°C with 13/11 hrs photoperiod at photo lux intensity of 50-70 μE^2S^1 provided from cool white fluorescent tubes. The data were analyzed in CRD design.

The response of explants to various sterilizing agents in culture medium is presented in Table-1. The efficacy of sterilizing agents $HgCl_2$ and NaOCl with different exposure time was adjudged in terms of maximum aseptic explants produced, which responded to sprouting. In case of Mercuric chloride @ 0.1 per cent, the highest contamination free explants, 95.00 per cent were recorded when quick diped in Ethanol (70 per cent) followed by 12 minutes whereas with NaOCl @ 0.5 per cent at 30 minutes gave 85 per cent. The increase in the exposure period with mercuric chloride (0.1 per cent) upto 14 minutes resulted in death of explants whereas no such effect was observed with NaOCl. The decrease in exposure period with both the chemicals, the contamination of explants increased significantly. These results are in agreement with the results of Kour *et al.* (2007) who reported in rough lemon.

At the end of 30 days of inoculation, the maximum (80 per cent) survival of explants was recorded with mercuric chloride @ 0.1 per cent at 10 minutes with 80 per cent survival, whereas under NaOCl 0.5 per cent with 25 minutes exposure recorded 65 per cent survival of explants. Syamal *et al.* (2007) also reported that $HgCl_2$ was better surface sterilizing agents over other chemicals. The number of shoots (3.09) as well as shoot length (0.93 cm) per explants was maximum under sodium hypochlorite @ 0.5 per cent with 10 minutes exposure whereas in case of mercuric chloride @ 0.1 per cent maximum number of shoots(2.50) and shoot length (0.82 cm). It may be due to tissue injury by the chemical. These results are in concurrence with (Karwa, 2003, Meghwal *et al.*, 2001). The number of shoot and length of shoot decreased with increasing time exposure of treatment of both chemicals, may be due to presence of mercury in mercuric chloride and chlorite in sodium hypochlorite. Similar results have been reported by Amin and Jaiswal (1987)

Table 1. Effect of Surface sterilizing agents on sterilization on nodal segments of kinnow mandarin after 30 days of incubation.

	Mercuric chloride (HgCl@ 0.1%)					Sodium hypochlorite (NaOCl@0.5%)			
Duration (Min.)	Contamination free explant (%)	Final establishment of explants (%)	Number of shoots per explants	Length of shoot (cm)	Duration (Min.)	Contamination free explant (%)	Final establishment of explants (%)	Number of shoots per explants	Length of shoot (cm)
4	0.0 (0.0)	0.00 (0.00)	0.0	0.00	5	0.0 (0.0)	0.00 (0.00)	0.0	0.00
6	40.0 (39.23)	25.0 (30.00)	2.50	0.82	10	15.0 (22.79)	10.0 (18.43)	3.09	0.93
8	70.0 (56.79)	65.0 (53.73)	2.26	0.63	15	30.0 (33.21)	25.0 (30.00)	2.73	0.62
10	85.0 (67.21)	80.0 (63.43)	1.93	0.54	20	65.0 (53.73)	55.0 (47.87)	2.10	0.56
12	95.0 (77.08)	20.0 (26.57)	0.97	0.51	25	80.0 (63.43)	65.0 (53.73)	1.76	0.50
14	0.00 (0.00)	0.00 (0.00)	0.00	0.00	30	85.0 (67.21)	15.0 (22.79)	1.06	0.35
Mean	63.41 (52.77)	31.66 (34.20)	1.27	0.42	Mean	51.13 (45.63)	16.0 (22.92)	1.79	0.50
	0.33	0.037	0.035	0.08	SEm±	0.711	0.033	0.065	0.87
	0.95	0.158	0.157	0.22	CD at 5%	2.09	0.172	0.184	0.25

*Figures given in parentheses are angular transformed value

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