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SHORT COMMUNICATION

Development of contamination free culture in bael (*Aegle marmelos* Correa)

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Bael (*Aegle marmelos* Correa) is one of the important minor fruit crop with high nutritive and medicinal value. It is mainly propagated either by seed or "T" budding or soft wood grafting. True to type plants can be produced in latter two methods but the growth is very slow. Tissue culture technique offers an advantage over conventional methods of propagations in producing large number of true to type plants from healthy elite plant within a short period of time. The surface sterilization is the most important step in micro-propagation and become a critical step in establishment of certain species particularly when the explants are derived from field grown plants. In this perspective studies were undertaken to standardize the protocol for surface sterilization of nodal segment of field grown plant of bael.

The experiment was conducted at Central Institute for Arid Horticulture, Bikaner with a view to assess the efficacy of different surface sterilizing agents. Young, fresh growing branch from mature plant of bael (5-7 years) were harvested and cut in to segments, each segment having at least one bud. These nodal segments were washed under running tap water for two hours. These explants were given quick dip treatment once in Methanol followed by surface sterilization with mercuric chloride (0.1per cent) for 5, 7, 9 and 11 minute or sodium hypochlorite (0.75 per cent) solution for 6, 8, 10 and 12 minute. Subsequently, the explants were washed thoroughly with autoclaved double distilled water for 3-4 times. These surface sterilized segments were inoculated in 250 ml conical flask containing 30-40 ml of MS medium, Murashige and Skoog (1962), supplemented with 3.0 per cent sucrose and 0.8 per cent agar. All the surface sterilized explants were inoculated under aseptic condition using laminar air flow cabinet. The treatments were replicated ten times and maintained at 27°C ± 2.0°C and 13 hrs photoperiod.

The response of explants to various sterilizing agents in culture medium is presented in Table-1. The efficacy of sterilizing agent mercuric chloride ($HgCl_2$) and sodium hypochlorite (NaOCl) with different exposure time was assessed in terms of maximum aseptic explants produced, which responded to sprouting. In case of Mercuric chloride @ 0.1 per cent, the maximum 80 per cent contamination free explants were recorded when exposed to 7 minute. In case of different time exposer of NaOCl (0.75 per cent) the maximum 60 per cent contamination free culture was recorded at 5 and 7 minute. Thus, the present investigation revealed that application of $HgCl_2$ is better agent for surface sterilization. Syamal *et al.* (2007) had reported that in Kagzi lime the maximum (72.43 per cent) survival of explants was recorded when the explants were treated with mercuric chloride. Similar results have also been reported by Kour *et al.* (2007) in rough lemon and Karwa (2003) in *Citrus reticulata*.

In the present study, the data on number of shoot and length of shoot were also recorded on 30th day after inoculation. The results revealed that among mercuric chloride treatments, the maximum number of shoot (2.80), length of shoot (3.42 cm) was recorded at 5 minute after treatment. Although further increasing the time of exposure reduces number and length of shoot. However, the length of shoot and number of shoots produced at 5 and 7 minute exposures were statistically non significant.

At the end of 30 day maximum number of shoot (2.90) and length of shoot (3.71 cm) were recorded at 6 minute treatment of sodium hypochlorite (0.75 per cent) which declined on increasing the duration of exposure.

The data on time required for bud break reveals that when the explants were treated with $HgCl_2$, the minimum time required for bud break (6.20 days) was with 5 minute exposure which increased with increase in duration of exposure but was statistically non–significant. Similarly, in case of explants treated with sodium hypochlorite, the minimum time required for bud break was 6.50 days which increased with increase in duration of exposure.

Thus, based on all the four parameters studied, it was concluded that surface sterilization with Mercuric chloride was better with respect to production of aseptic culture and reducing time to bud break. The further increase in duration of exposure to either of sterilizing agents decreased the number and length of shoot, which may be because of toxic effect of these chemicals.

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Table 1. Effect of surface sterilization agents on establishment of axenic culture in Bael

Mercuric chloride (0.1 per cent)					Sodium hypo chlorite (0.75 per cent)				
Time Exposer (min)	Per cent survival explants	Time equired to bud break (days)	No. of shoot	Length of Shoot (cm)	Time Exposer	Per cent survival explants	Time required to bud break (days)	No. of shoot	Length of shoot (cm)
5	70	6.20	2.80	3.42	6	60	6.50	2.90	3.71
7	80	6.40	2.50	2.92	8	60	7	2.60	2.83
9	70	6.50	2.20	1.77	10	50	7.60	2.20	2.13
11	50	7.0	1.50	1.52	12	40	8.60	1.70	1.89
SEm +	0.37	0.30	0.18	0.12		0.27	0.57	0.16	0.06
CD (5%)	1.07	0.89	0.52	0.36		0.76	1.65	0.45	0.18

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