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Effect of Culture Media on Shoot Proliferation and Callus Induction of Bael (*Aegle marmelos* L.)

Pukh Raj, Mohan Lal Jakhar, Sarfraz Ahmad*, Kassim Yahya Mtilimbanya, Suman Chahar and Hari Ram Jat

Department of Plant Breeding and Genetics, S.K.N. Agriculture University, Jobner, Rajasthan-303029, India

**Corresponding author*

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A B S T R A C T

The present investigation was carried out at Tissue Culture Laboratory of S.K.N. Agriculture University, Jobner, India during 2018-19. Experiment was laid out using completely randomized design (CRD) with ten replications. Six culture media *viz.* Murashige and Skoog media, Nitsch & Nitsch media, woody plant medium, Schenk and Hildebrant medium, White's medium and Khudson solution–C were tested for direct shoot proliferation and callus induction at most responsive level of plant growth regulators in Bael (*Aegle marmelos* L.). Highest shoot bud induction (4.1) was observed using woody plant medium with 100 per cent morphogenetic response using nodal segment explant along with BAP at 2 mg/l concentration. Maximum callus induction on leaf explant was observed on Murashige and Skoog medium with 40 per cent morphogenetic response followed by woody plant medium (30 per cent) with 2,4-D at 2 mg/l concentration. Thus for large scale micropropagation, woody plant medium using nodal segment explant is recommended for shoot proliferation and for callus induction, MS medium taking leaf as explant will be rewarding for mass multiplication of Bael.

Introduction

Bael (*Aegle marmelos* L.) is a subtropical plant commonly known as Bengal quince, Bilva, Indian quince, Golden apple, Holy fruit, Bel, Belwa, Sriphal, Stone apple and Maredo (John and Stevenson, 1979). It have chromosome number 2n=18 in its genetic composition and believe to originated in India (Zeven and De Wet, 1982). Bael is one of the most important tree species used in various indigenous systems of medicine in India (Kritikar and Basu, 1994).

Out of more than 66 ethno-botanical uses of bael, 48 are exclusively for medicinal purposes. Almost all parts of bael plant are used in preparing medicine. Ayurveda practitioners commonly use the roots of bael as an ingredient of dasamula (ten

roots), which is useful in recovering the loss of appetite and fruits are uses in the preparation of chyavanprash. Ripe bael fruit is sweet, aromatic and nutritive, whereas fresh fruit is astringent and has laxative properties. Bael fruit powder exhibits anti-cancerous and anti-proliferative activities. Its wood is also suitable for making charcoal.

Bael is usually propagated by seeds and root suckers though many problems are associated with these methods. Due to all these drawbacks, micropropagation methods alone following tissue culture techniques can provide some hope for rapid mass multiplication and germplasm conservation of this rare endangered medicinal tree, though several limitations such as low shoot proliferation, excessive phenolic exudation, basal callusing, vitrification and shoot tip necrosis come in its way (Vennel Raj *et al*., 2012).

Propagation through tissue culture also eliminates the possibility of any interruption in the growing season because it can be carried out inside a carefully regulated, controlled environment (Lee *et al*., 2019). The composition of growth medium is an important factor affecting growth and morphogenesis of plant tissues. Plant tissue culture medium consists of macronutrients, micronutrients, vitamins, amino acids or other nitrogen supplements, carbon sources, organic supplements, solidifying agents and growth regulators.

Murashige and Skoog (MS) is the most commonly used medium in plant tissue culture. The B_5 (Gamborg *et al.,* 1968), N_6 (Chu, 1978) and Nitsch and Nitsch (Nitsch and Nitsch, 1969) have been widely used for many plant species. Moreover, for culture of woody species, the DKW (Driver and Kuniyuki, 1984) and the Woody Plant Medium (WPM) (Lloyd and McCown, 1980) are used. Thus the present investigation has been undertaken to see the effects of different culture media on propagation of bael under *in vitro* conditions to produce true to type and virus free plants.

Materials and Methods

The present investigation was carried out at Tissue Culture Laboratory of Department of Plant Breeding and Genetics, S.K.N. Agriculture University, Jobner, Rajasthan, India during 2018-19. Nodal segment with 2-3 nodes and leaves were used as explants which were collected from healthy tree planted in department of horticulture. Mercuric chloride (0.1 per cent) was used for surface sterilization. Different media like Murashige and Skoog, Nitsch & Nitsch, Woody Plant Medium, Schenk and Hildebrant Medium, White's Medium and Khudson Solution–C were tested for direct shoot proliferation and callus induction at most responsive level of plant growth regulators.

For shoot bud induction in nodal segment explant, BAP at 2 mg/l concentration and for callus induction in leaf explant 2,4-D at 2 mg/l concentration were used in different medium. All the cultures were maintained in culture room at temperature of 25 ± 2^0C under fluorescent light in a 14:10 hour's photoperiod. Cultures were thoroughly observed at a periodicity of 7 days till 45th day of each experiment.

The observations were recorded on days taken for shoot bud initiation, number of shoots per explant, morphogenetic response (per cent), days taken for callus initiation, callus growth, callus color and morphogenetic response of callus (per cent). The experiment was conducted in completely randomized design (CRD) comprising ten replications and data were analyzed for mean and standard error accordingly as described by Snedecor and

Cochran (1972). Test of significance was done according to Duncan's Multiple Range Test (DMRT) for different traits (Gomez and Gomez, 1984).

Results and Discussion

To see the effect of different culture media on shoot proliferation and induction of callus six types of culture media were studied. Significant differences were observed in different culture media for number of shoot bud induction and morphogenetic response. For shoot bud induction in nodal segment explants, different culture media were supplemented with most responsive level of BAP at 2.0 mg/l concentration. Highest shoot bud induction (4.1) was observed using WPM with 100 per cent morphogenetic response (Table 1 and Fig 1) followed by Murashige and Skoog medium (3.8) and Whites medium (2.1). These results were in close agreement with the observations of El-Agamy *et al*. (2009) in pomegranate and Kumar (2018) in pomegranate cv. Sindhuri cultivars propagation where WPM significantly produce higher average number of nodes followed by those grown on MS medium.

Table.1 Effects of different media on shoot bud induction using BAP (2.0 mg/l) in nodal segment explants of bael.

S. No.	Media	Days taken for shoot bud initiation	Number of shoot bud induced	Morphogenetic response (%)
1	Murashige and Skoog Medium	13.9	2.071* (3.8) **a**	100
2	Woody Plant Medium	13.5	2.121* (4.1) **a**	100
3	White's Medium	18.6.	1.532* (2.1) **b**	80
4	Schenk and Hildebrant Medium	-	0.707* (-) **c**	-
5	Nitsch and Nitsch Medium	20.3	1.496* (1.9) **b**	60
6	Khudson Solution-C	-	0.707* (-) **c**	-

(-) = No response;
(*) = Transformed value
() = Value in parenthesis represents mean number of shoot bud
Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

For callus induction in leaf explant different culture media were supplemented with most responsive level of 2,4-D at 2.0 mg/l concentration. Maximum callus induction was observed on Murashige and Skoog medium with 40 per cent morphogenetic response (Table 2 and Fig 2) followed by Woody Plant Medium (30 per cent) and Whites medium (20 per cent). MS medium and WPM were found ideal for induction of calli from leaf explants also reported by Vasantha and Shivanna (2005) in *Desmodium oojeinense*. Callus induction did not exhibit on the Nitsch and Nitsch, Schenk and Hildebrant medium and Khudson Solution-C medium even in the presence of plant growth regulators which were responsive on other media.Thus for large scale micropropagation of Bael (*Aegle marmelos* L.) it is recommended to use woody plant medium using nodal segment explant for profuse shoot proliferation and for high morphogenetic response to induce callus, use of MS medium taking leaf as explant will be rewarding**.**

Table.2 Effects of different media on callus induction using 2,4-D (2.0 mg/l) in leaf explant of bael

S. No.	Media	Leaf explant				
		Days taken for callus initiation	Visual growth	Colour	Texture	Morphogenetic response (%)
1	Murashige and Skoog Medium	16.2	+++	Light brown	Compact	40
2	Woody Plant Medium	16.6	+++	Creamish	Semi compact	30
3	White's Medium	17.1	++	Light green	Friable	20
4	Schenk and Hildebrant Medium	-	-	-	-	-
5	Nitsch and Nitsch Medium	-	-	-	-	-
6	Khudson Solution-C	-	-	-	-	-

+++ = Profuse callus, ++=Medium callus, +=Slight callus, (-) = No response

Figure.1 Shoot bud induction in nodal segment explant on WP medium supplemented with BAP @2.0 mg/l

Figure.2 Callus induction in leaf explant on MS medium supplemented with 2,4-D @2.0 mg/l

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