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Establishment of Aseptic Culture in Jamun (*Syzygium cuminii* L.)

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ABSTRACT: *Jamun (Syzygium cuminii L.) is an important minor fruit and conventionally propagated by vegetative methods of like cutting, grafting,* etc.*, which are cumbersome, time consuming, season bound with low multiplication rate. Hence, tissue culture propagation has advantages in ensuring extremely rapid rate of multiplication, year round production and requires limited space. However, it is very difficult to culture explants derived from woody trees due to their recalcitrant nature, high incidence of microbial contamination and high levels of polyphenol exudation. The present investigation was, therefore, carried out to standardize protocol for establishment of aseptic culture in Jamun* cv. *AJG-85. Effect of type of explants, surface sterilants and anti-oxidant on culture establishment was investigated. The observations on aseptic culture establishment, contamination, browning were recorded at 4 weeks after incubation. Studies revealed that successful establishment of aseptic culture in Jamun cv. 'AJG-85' is possible by surface sterilization of shoot tip explants with mercuric chloride at 0.75% for 5 minutes followed by incubation on half strength MS medium supplemented with activated charcoal at 0.50 %.*

INTRODUCTION

Jamun (*Syzygium cuminii* L.) is an important minor fruit belonging to the family Myrtaceae. It is native of India which is also known as Black Plum, Java Plum, Indian blackberry, Jambolan, *etc*. It is tall and evergreen tree distributed throughout India. It is hardy crop and suitable for marginal and wasteland. The fruit being highly nutritive and possess great medicinal value as such gained importance. They are good source of iron, minerals, sugars and proteins. Fruits are also used for making squashes, jellies, jam, wine, pickles and vinegar. Ripe fruit are highly relished and have a great demand in the season of availability. The fruits are tasty and pleasantly flavoured and are very much liked by the masses and mostly used as a dessert purpose (Ochse *et al*., 1961). The timber is used for making plywood and agricultural implements as it is durable.

Jamun is propagated by vegetative methods of like cutting, grafting, *etc*., which are cumbersome, time consuming, season bound with low multiplication rate. Further, it is a difficult-to-root species and cuttings fail to produce adventitious roots. Hence, tissue culture propagation has advantages in ensuring extremely rapid rate of multiplication, year round production and requires limited space. It also gives disease free propagules and the superior genetic characteristics are unaltered. However, it is very difficult to culture explants derived from woody trees due to their recalcitrant nature, high incidence of microbial contamination and high levels of polyphenol exudation. The present investigation was, therefore, carried out to standardize protocol for establishment of aseptic culture in Jamun *cv*. AJG-85.

MATERIAL AND METHODS

Preparation Explants

The twigs containing shoot tip as well as 3-4 nodes were taken from the field grown mother plant (Fig.1) at fruit orchard of KRC College of Horticulture, Arabhavi, Belgaum District, Karnataka. The plant materials were washed thoroughly in running water to remove debris. They were then washed 3-4 times with distilled water containing few drops of antiseptic solution. Plant materials were treated with a solution containing cetrimide (500 mg/l) + carbendazim (1000 mg/l) and then with streptocycline (500 mg/l) for 25 minutes. They were rinsed 4-5 times with distilled water and incubated in solution containing 0.30% sucrose and 0.01% ascorbic acid overnight. Different types of explants (Fig. 2) were excised and then

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washed repeatedly 4-5 times with sterile water under laminar air flow cabinet.

Explants isolated includes **Shoot tip (T_1):** explants from apical portion (1 cm) from the current season growth; **Single nodal segment, vertical position (T_2 & T_3):** nodal segment of 1 cm length of current and previous season shoot; **Double nodal segment (T_4 & T_5):** segment of double nodes of 2-5 cm length of current and previous season shoots; **Single nodal segment, horizontal position (T_6); Lateral shoot bud (T_7)** : buds arising out of the lateral shoots which are green but mature; **Leaf petiole (T_8):** leaf petiole of 1 cm length from green expanded leaves obtained from new flush. Later explants were surface sterilized with mercuric chloride 0.75% for 10 minutes and then washed repeatedly 4-5 times with sterile distilled water.

Initiation of Aseptic Culture

Effect of type of explants on establishment of aseptic cultures: Different explants were cultured on half strength MS media containing 3% sucrose, 100 mg/l ascorbic acid and 2 mg/l BAP. **Effect of surface sterilants on establishment of aseptic cultures:** Different surface sterilants were used for disinfecting the shoot tips explants and cultured on half-strength MS media (Sucrose 3%, Ascorbic acid 150 mg/l, BAP 2 mg/l). **Effect of anti-oxidant on culture**

Figure 1: Mother plant of Jamun *cv.* AJG-85

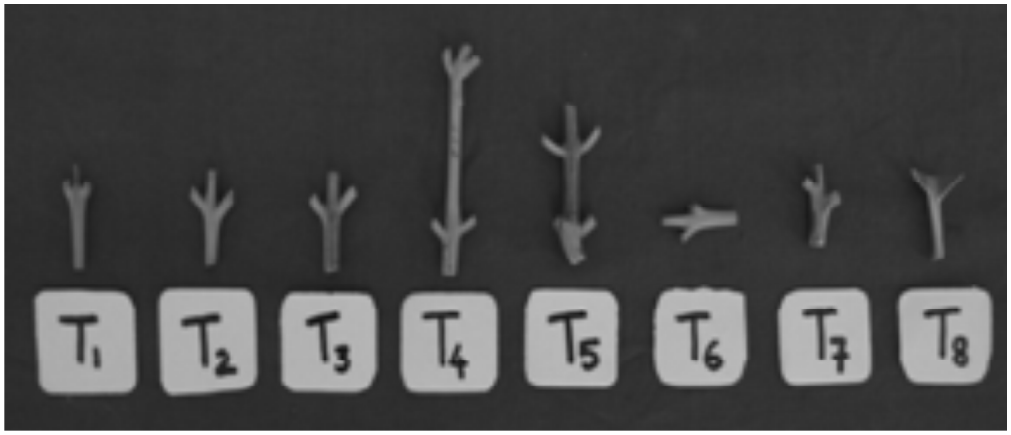

Figure 2: Explants of Jamun used for culture initiation

establishment: Anti-oxidants used includes ascorbic acid at 100 and 150 mg/l, activated charcoal 0.5% and 1%, polyvinylpyrrolidone (PVP) 2, 4 and 6 mg/l. In all the treatments, half-strength MS medium supplemented with 3% sucrose and 2 mg/l BAP.

Completely randomized design (CRD) was employed for the experiments. The observations on aseptic culture establishment, contamination, browning were recorded at 4 weeks after incubation. The data in percentages were transformed to arcsine values for statistical analysis. The data were subjected to ANOVA as suggested by Panse and Sukhatme (1967). Critical difference values were tabulated at one per cent probability where "F" test was significant.

RESULTS AND DISCUSSION

Response of Different Types of Explants

Shoot tip showed significantly maximum establishment (40.00 %) as compared to other explants (Table 1 & Fig. 3). This may attributed to presence of actively dividing meristematic cells and higher endogenous auxin level in shoot tips. These findings are in close agreement with the observations of Rahman and Blake (1988) in Jackfruit and Rai and Misra (2005) in Karonda.

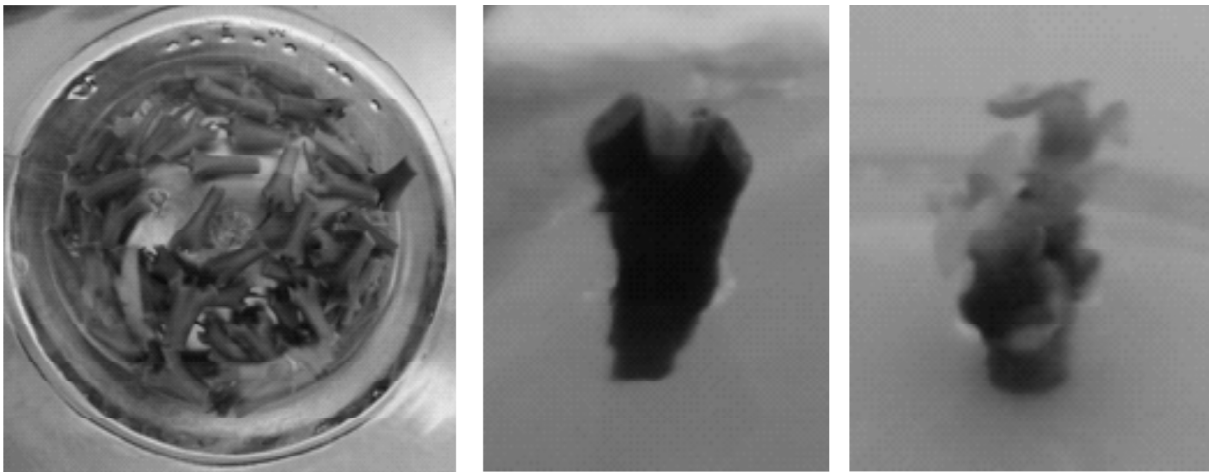

Figure 3: Establishment of aseptic culture by shoot tip culture

Surface Sterilization of Shoot Tip Explants

Mercuric chloride at 0.75 % for 5 minutes was found better compared to other treatments as recorded significantly lower contamination (20.00%) and maximum establishment of aseptic culture (53.33%) (Table 2). Pauling (1955) opined that mercuric chloride is extremely poisonous due to high bleaching action of two chloride atoms and also mercuric ions which combine strongly with protein causing death of the organism. Though there was lesser contamination at higher concentration of mercuric chloride, the survival percentage was less due to

Table 1
Effect of type of explants on establishment of aseptic culture in Jamun *cv.* 'AJG-85'

Treatments	*No. of explants inoculated*	*No. of explants established*	*Per cent aseptic culture establishment*	*Per cent contamination*	*Intensity of browning*
T_1 Shoot tip	15	06	40.00 (39.14)*	46.67 (43.08)*	+++
T_2 Single node (Current season shoot)	15	03	20.00 (26.54)	40.00 (38.86)	+++
T_3 Single node (Mature shoot)	15	00	0.00 (0.26)	80.00 (63.44)	++
T_4 Double node (Current season shoot)	15	01	6.67 (14.95)	40.00 (38.86)	++
T_5 Double node (Mature shoot)	15	00	0.00 (0.26)	93.33(80.97)	+
T_6 Single node (Horizontal)	15	00	0.00 (0.26)	86.67 (76.75)	+++
T_7 Lateral shoot bud	15	00	0.00 (0.26)	86.67 (76.75)	+++
T_8 Leaf petiole	15	00	0.00 (0.26)	66.67 (59.91)	++
S.Em±	-	-	1.25	9.14	-
CD at 1%	-	-	3.74	27.41	-

*The values given in parenthesis are arc sine transformed values ($Sin^{-1}\sqrt{X/100}$)

Table 2
Effect of surface sterilants on establishment of shoot tip explant in Jamun *cv.* 'AJG-85'

Treatments	*Per cent contamination*	*Per cent establishment*	*Days taken for shoot emergence*
T_1 $HgCl_2$ 0.1% for 5 minutes	40.00 (38.86)*	0.00 (0.26)*	0.00 (0.71)**
T_2 $HgCl_2$ 0.5% for 5 minutes	80.00 (63.44)	20.00 (26.54)	24.00 (4.95)
T_3 $HgCl_2$ 0.75% for 5 minutes	20.00 (22.02)	53.33 (46.93)	26.67 (5.22)
T_4 $AgNO_3$ 0.1% for 10 minutes	66.67 (55.00)	6.67 (14.95)	27.00 (5.24)
T_5 Ethanol 70% for 30 seconds	86.67 (72.20)	0.00 (0.26)	0.00 (0.71)
T_6 Sodium hypochloride 6% for 3 minutes	93.33 (80.97)	6.67 (14.95)	27.00 (5.24)
T_7 Carbendazim (1000 ppm)+ Streptocycline (100) for 30 minutes	100.00 (89.74)	0.00 (0.26)	0.00 (0.71)
T_8 Carbendazim (1000 ppm) + Streptocycline (100) for 1 hour	100.00 (89.74)	0.00 (0.26)	0.00 (0.71)
S.E m±	6.63	0.76	0.05
CD at 1%	19.89	2.30	0.15

* The value given in paranthesis are arc sine transformed values (Sin^{-1} J×1100)
** The value given in paranthesis are square root transformed values (√× + 0.5)

phytotoxicity. Similar observations were reported in coffee (Naidu *et al.*, 1993) and teak (Tiwari and Pandey, 1995).

Effect of Antioxidants on Browning of Medium

Culturing of shoot tip explants on half strength MS medium supplemented with activated charcoal at 1 and 0.50% resulted in minimum browning (0.00%) (Table 3). Charcoal probably retards the photo-oxidation of hydroxyl group of the polyphenols and hence prevent the formation of quinines thereby check the browning. These results are in accordance with earlier findings of Rajmohan and Mohan kumaran (1988) in jackfruit.

Table 3
Effect of anti-oxidants on browning in Jamun *cv.* 'AJG-85'

Treatments	*Browning (%)*	*Establishment (%)*
T_1 Control	93.33 (80.97)*	13.33 (21.42)*
T_2 Ascorbic acid 100 mg/l	100.00 (89.74)	6.67 (14.95)
T_3 Ascorbic acid 150 mg/l	80.00 (67.98)	12.00(16.06)
T_4 Activated Charcoal 0.5%	0.00 (26.57)	13.33 (21.40)
T_5 Activated Charcoal 1%	0.00 (26.57)	13.33 (21.40)
T_6 PVP 2 mg/l	100.00 (89.74)	0.00 (0.26)
T_7 PVP 4 mg/l	80.00 (67.98)	0.00 (0.26)
T_8 PVP 6 mg/l	66.67 (55.00)	0.00 (0.26)
S.Em±	6.69	0.36
CD at 1%	20.06	1.08

*The values given in parenthesis are arc sine transformed values (Sin^{-1} √X/100)

In conclusion, successful establishment of aseptic culture in Jamun cv. 'AJG-85' is possible by surface sterilization of shoot tip explants with mercuric chloride at 0.75% for 5 minutes followed by incubation on half strength MS medium supplemented with activated charcoal at 0.50%.

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