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# *IN VITRO* REGENERATION OF JAMUN (*SYZYGIUM CUMINII* L.) *CV.* AJG-85

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**ABSTRACT**
Studies on in vitro regeneration of jamun (Syzygium cuminii L.) *cv*. AJG-85 was carried out to optimize a mass multiplication protocol. Among the different explants tried, shoot tip was found best for establishment of aseptic culture. Surface sterilization of the explants was effectively achieved when 0.75% mercuric chloride was used for 5 minutes as it resulted in lower contamination and better culture establishment. Activated charcoal at 0.50% proved to be best in controlling phenolic browning but recorded lower establishment as compared to ascorbic acid at 150 mg/l which resulted in moderate browning and higher establishment. Among the different basal media studied half strength WPM showed best response with higher number of shoots per explants, length of shoots and number of leaves per shoot. Among the different growth regulators used for shoot proliferation, 2 mg/l BAP + 0.1 mg/l NAA recorded better shoot length, number of leaves and number of adventitious buds per explants. Due to short shoot length, elongation study was carried out using $GA_3$, where $GA_3$ at 2 mg/l showed better performance in terms of shoot length and number of leaves per shoot. Highest extent of in vitro rooting with significantly more number of roots, root length and number of roots were recorded with 2 mg/l IBA.

**KEYWORDS:** Jamun, Aseptic culture, Browning of medium, Shoot proliferation, *In vitro* rooting

## INTRODUCTION

Jamun (*Syzygium cuminii* L.) is an important minor fruit belonging to the family Myrtaceae. It is tall and evergreen hardy tree 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. Ripe fruit are highly relished and have a great demand in the season of availability. The fruits are tasty, pleasantly flavoured and are very much liked by the masses.[7] Jamun is propagated by vegetative methods of like cutting and grafting 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. It also gives disease free propagules and the superior genetic characteristics are unaltered. However, it is very difficult to culture explants derived from mature trees due to their recalcitrant nature, high incidence of microbial contamination, high levels of polyphenol exudation and difficulty in root induction. The present investigation was, therefore, carried out to optimize protocol for mass multiplication of Jamun *cv*. AJG-85 through tissue culture.

## MATERIALS & METHODS

Different explants were collected from field grown mother plant (Fig.1 and 2) grown at fruit orchard of KRC College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkot, Karnataka. Explants tried includes shoot tip: explants from apical portion (1 cm) from the current season growth; single nodal segment: nodal segment of 1 cm length of current and previous season shoot; double nodal segment: segment of double nodes of 2-5 cm length of current and previous season shoots; lateral shoot bud: bud arising out of the lateral shoots which are green but mature and leaf petiole: leaf petiole of 1 cm length from green expanded leaves obtained from new flush.

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

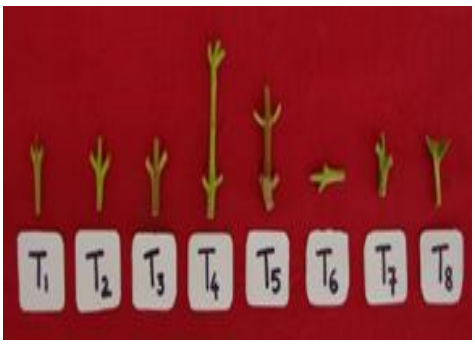


**FIGURE 2:** Different explants of Jamun

The twigs containing shoot tip as well as 3-4 nodes were taken from the mature tree and 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. Explants were treated with a solution containing 500 mg/l cetrimide + 1000 mg/l carbendazim + 500 mg/l streptocycline 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. The different types of explants were excised and then washed repeatedly 4-5 times with sterile water under laminar air flow cabinet. Later explants were surface sterilized with 0.75 % mercuric chloride ($HgCl_2$) for 10 minutes and then washed repeatedly 4-5 times with sterile distilled water. These explants were used to carry out following experiments.

**Effect of explants type on establishment of aseptic cultures:**

Different **e**xplants viz., shoot tip, single nodal segment, double nodal segment, lateral shoot bud and leaf petiole were cultured on half strength MS media containing 3% sucrose, 100 mg/l ascorbic acid and 2 mg/l benzyl aminopurine (BAP). **Effect of surface sterilants on establishment of aseptic cultures:** Different surface sterilants such mercuric chloride, silver nitrate, ethanol, sodium hypochloride and carbendazim were used for disinfecting the shoot tips explants and cultured on half-strength MS medium containing 30 g/l sucrose, 150 mg/l ascorbic acid and 2 mg/l BAP. **Effect of anti-oxidant on culture establishment:** Anti-oxidants used includes 100 and 150 mg/l ascorbic acid, 0.50 % activated charcoal and 1% polyvinylpyrrolidone (PVP). In all the treatments, half-strength MS medium supplemented with 3% sucrose and 2 mg/l BAP. Intensity of browning was recorded as per scale: 0 = no browning; + = low browning; + + = moderate browning and + + + = high browning. **Effect of different media strength on initiation and shoot proliferation:** Different media strength used were ¼ MS, ½ MS, full MS, ¼ WPM, ½ WPM and full WPM. In all the treatments, media were supplemented with 3% sucrose and 2 mg/l BAP. **Effect of cytokinins on shoot proliferation:** Cultures were incubated on MS medium supplied with 2 mg/l BAP, 3 mg/l BAP, 2 mg/l BAP + 0.1 mg/l NAA, 2 mg/l BAP + 0.5 mg/l NAA, 3 mg/l BAP + 0.1 mg/l NAA, 3 mg/ l BAP + 0.5 mg/l NAA, 2 mg/l BAP + 0.5 mg/l Kinetin, 2mg/l BAP + 1 mg/l Kinetin, 3 mg/l BAP + 0.5 mg/l Kinetin and 3 mg/l BAP + 1 mg/l Kinetin. Medium was supplemented with full strength MS media + 3 % sucrose + 5 g/l agar + 150 mg/l ascorbic acid. **Effect of $GA_3$ on shoot elongation:** $GA_3$ was used at 1, 2, 4, 6 and 8 mg/l. Full strength MS medium containing 2 mg/l BAP + 35 mg/l adenine sulphate + 3 % sucrose and 4.5g/l agar was used. **Effect of auxins on *in vitro* rooting:** 1, 2, 5 and10 mg/l IBA, 0.5, 1.5, 2 and 2.5 mg/l NAA, 1 mg/l IBA + 1 mg/l NAA and 5 mg/l IBA + 5 mg/l NAA were tried. 1/4$^{th}$ strength MS media + 3g/l activated charcoal, 4.5 g/l agar and 3% sucrose was used.

Completely randomized design (CRD) was employed for the experiments. The data in percentages were transformed to arc sine values for statistical analysis. The data were subjected to ANOVA as suggested by Panse and Sukhatme [8]. Critical difference values were tabulated at one per cent probability where "F" test was significant.

## RESULTS & DISCUSSION

**Response of different types of explants**

Shoot tip showed better establishment 40.00 % than any other explants (Table 1) (Fig. 3). This may be probably due to presence of actively dividing meristematic cells and higher endogenous auxin level in shoot tips. Similar results were reported in Jackfruit (Rahman and Blake[10]) and Karonda (Rai and Misra[11]).

**FIGURE 3:** Establishment of aseptic culture by shoot tip culture

**Surface sterilization of shoot tip explants**

$HgCl_2$ at 0.75 % for 5 minutes proved better compared to all other treatments as it recorded significantly minimum contamination 20.00 % and maximum establishment 53.33 % (Table 2). Pauling [9] opined that $HgCl_2$ 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 $HgCl_2$, the survival percentage was less due to phytotoxicity. Similar observations were reported in coffee (Naidu et al[6]) and teak (Tiwari and Pandey[13]).

**Effect of antioxidants on browning of medium:**

Culturing of shoot tip explants on half strength MS medium supplemented with activated charcoal at 0.50 % resulted in minimum browning 0.00 % (Table 3) but recorded lower establishment 13.33 % as compared to ascorbic acid at 150 mg/l which exhibited moderate browning 40.00 % and higher establishment 40.00%. Charcoal 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 Mohankumaran[12] in jackfruit.

**Role of media strength on initiation and shoot proliferation:**

Half strength WPM found better in terms of the number of shoots/explants 11.80, length of shoot 2.22 cm and number of leaves/shoot 15.60 (Table 4) (Fig. 4). However, shoot thickness in quarter strength WPM (1.34 mm) and number of adventitious buds/explants was significantly higher in half MS medium (23.20). Similar results were also reported by Feng et al[3] in ber and Lemos and Blake[5] in *Annona* species.

**Effect of growth regulators on shoot proliferation:**

2 mg/l BAP + 0.1 mg/l NAA found better for increasing length of shoot 1.74 cm, number of leaves/shoot 14 and number of adventitious buds/ explant 16.5 (Table 5) (Figure 5). But 3 mg/l BAP + 0.5 mg/l Kinetin was found to be better in terms of number of shoots/explant (10.90) which was statistically on par with 2 mg/l BAP + 0.5 mg/l NAA (10.70), 2 mg/l BAP (9.30) and 2 mg/l BAP + 1 mg/l Kinetin (8.80). BAP as a source of cytokinin was found best as compared to kinetin in jackfruit (Adiga[1]; Rahman and Blake[10]) and neem (Upadhyaya[14]).

**Effect of $GA_3$ on shoot elongation:**

The shoots were significantly longer 2.22 cm when $GA_3$ was used at 2 mg/l, with increase in number of shoots (10.3) and number of leaves (21.5) (Table 6) (Fig. 6**)**. Belaizi et al[2] observed elongation of apple shoots with $GA_3$ at 1.50 µM. Even though $GA_3$ had a significant influence on the number leaves produced, the leaves formed were small. This may be due to the reason that $GA_3$ brings about shoot elongation and thereby the leaves formed are extremely disturbed with no proper development.

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

| Treatments | | % aseptic culture | % ontamination | Intensity of browning |
|---|---|---|---|---|
| $T_1$ | Shoot tip | 40.00 (39.14)* | 46.67 (43.08)* | +++ |
| $T_2$ | Single node (Current season shoot) | 20.00 (26.54) | 40.00 (38.86) | +++ |
| $T_3$ | Single node (Mature shoot) | 0.00 (0.26) | 80.00 (63.44) | ++ |
| $T_4$ | Double node (Current season shoot) | 6.67 (14.95) | 40.00 (38.86) | ++ |
| $T_5$ | Double node (Mature shoot) | 0.00 (0.26) | 93.33(80.97) | + |
| $T_6$ | Single node (Horizontal) | 0.00 (0.26) | 86.67 (76.75) | +++ |
| $T_7$ | Lateral shoot bud | 0.00 (0.26) | 86.67 (76.75) | +++ |
| $T_8$ | Leaf petiole | 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 | | % contamination | % establishment |
|---|---|---|---|
| $T_1$ | $HgCl_2$ 0.10 % for 5 minutes | 40.00 (38.86)* | 0.00 (0.26)* |
| $T_2$ | $HgCl_2$ 0.50 % for 5 minutes | 80.00 (63.44) | 20.00 (26.54) |
| $T_3$ | $HgCl_2$ 0.75 % for 5 minutes | 20.00 (22.02) | 53.33 (46.93) |
| $T_4$ | $AgNO_3$ 0.10 % for10 minutes | 66.67 (55.00) | 6.67 (14.95) |
| $T_5$ | Ethanol 70 % for 30 seconds | 86.67 (72.20) | 0.00 (0.26) |
| $T_6$ | Sodium hypochloride 6 % for 3 minutes | 93.33 (80.97) | 6.67 (14.95) |
| $T_7$ | Carbendazim 1000 ppm + Streptocycline 100 for 30 minutes | 100.00 (89.74) | 0.00 (0.26) |
| $T_8$ | Carbendazim 1000 ppm +Streptocycline 100 ppm for 1 hour | 100.00 (89.74) | 0.00 (0.26) |
| | S.E m± | 6.63 | 0.76 |
| | CD at 1% | 19.89 | 2.30 |

**TABLE 3:** Effect of anti-oxidants on browning of the medium

| Treatments | | % browning | Intensity of 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 | 40.00 (39.24) | ++ | 40.00 (39.24) |
| $T_4$ | Activated Charcoal 0.50 % | 0.00 (0.26) | ++ | 13.33 (21.40) |
| $T_5$ | Activated Charcoal 1 % | 0.00 (0.26) | ++ | 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 ($Sin^{-1}\sqrt{X/100}$)

**TABLE 4:** Effect of different media strength on initiation and shoot proliferation
Observations are taken at 6th week of transfer to the culture media

| Treatments | | Number of adventitious buds/explant | Number of shoots/explant | Length of shoot (cm) | Number of leaves/ shoot | Shoot thickness (mm) |
|---|---|---|---|---|---|---|
| $T_1$ | ¼ MS | 22.15 | 6.95 | 1.42 | 10.60 | 1.28 |
| $T_2$ | ½ MS | 23.20 | 9.45 | 1.42 | 10.90 | 1.31 |
| $T_3$ | Full MS | 13.75 | 10.90 | 1.66 | 13.50 | 1.10 |
| $T_4$ | ¼ WPM | 11.60 | 9.95 | 1.72 | 12.55 | 1.34 |
| $T_5$ | ½ WPM | 11.90 | 11.80 | 2.22 | 15.60 | 1.31 |
| $T_6$ | Full WPM | 7.15 | 7.80 | 1.80 | 10.40 | 1.24 |
| | S.Em± | 1.08 | 0.76 | 0.04 | 0.66 | 0.03 |
| | CD at 1% | 3.22 | 2.25 | 0.13 | 1.96 | 0.08 |

MS- Murashige and Skoog medium; WPM- Woody Plant Medium

**FIGURE 4:** Shoot proliferation in half strength WPM media

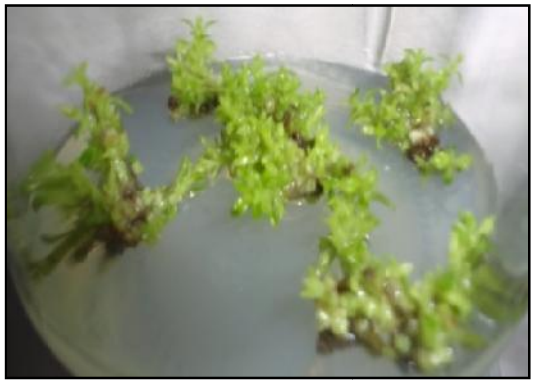

**FIGURE 5:** Shoot proliferation on 3 mg/l BAP + 0.5mg/l Kinetin medium

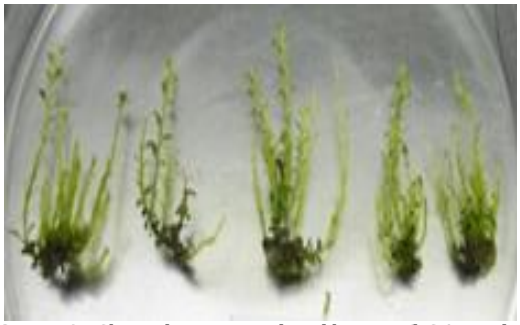

**FIGURE 6:** Shoot elongation induced by 2 mg/l $GA_3$ medium

**FIGURE 7:** *In vitro* rooting of microshoots on 2 mg/l IBA medim

**TABLE 5:** Effect of cytokinins on shoot proliferation

| Treatments | | Number of adventitious buds/explant | Number of shoots/ explant | Length of shoot (cm) | Number of leaves/ shoot | Shoot thickness (mm) |
|---|---|---|---|---|---|---|
| $T_1$ | BAP 2 mg/l | 10.60 | 9.30 | 1.43 | 12.30 | 1.12 |
| $T_2$ | BAP 3 mg/l | 12.80 | 8.50 | 1.59 | 13.50 | 1.21 |
| $T_3$ | BAP 2 mg/l + NAA 0.1 mg/l | 16.50 | 7.30 | 1.74 | 14.00 | 1.27 |
| $T_4$ | BAP 2 mg/l + NAA 0.5 mg/l | 14.30 | 10.70 | 1.20 | 10.80 | 1.27 |
| $T_5$ | BAP 3 mg/l + NAA 0.1 mg/l | 13.10 | 5.30 | 1.01 | 11.20 | 1.27 |
| $T_6$ | BAP 3 mg/l + NAA 0.5 mg/l | 18.40 | 8.30 | 1.66 | 12.90 | 1.22 |
| $T_7$ | BAP 2 mg/l + Kinetin 0.5 mg/l | 11.30 | 6.90 | 1.33 | 11.00 | 1.25 |
| $T_8$ | BAP 2 mg/l + Kinetin 1 mg/l | 7.90 | 8.80 | 1.09 | 11.50 | 1.26 |
| $T_9$ | BAP 3 mg/l + Kinetin 0.5 mg/l | 8.98 | 10.90 | 1.16 | 9.86 | 1.29 |
| $T_{10}$ | BAP 3 mg/l + Kinetin 1 mg/l | 15.39 | 7.81 | 1.58 | 14.21 | 1.28 |
| | S.Em± | 0.58 | 0.58 | 0.05 | 0.58 | 0.16 |
| | CD at 1% | 1.70 | 1.70 | 0.16 | 1.70 | NS |

Observations are taken at 6th week of transfer to the culture media
NS- Non significant

**TABLE 6:** Effect of $GA_3$ on shoot elongation

| Treatments | | Mean shoot length (cm) | Mean number of shoot | Mean thickness of shoots (mm) | Mean no of leaves/ shoot |
|---|---|---|---|---|---|
| $T_1$ | $GA_3$@ 1 mg/l | 1.94 | 12.60 | 1.11 | 22.50 |
| $T_2$ | $GA_3$@ 2 mg/l | 2.22 | 10.30 | 1.11 | 21.50 |
| $T_3$ | $GA_3$@ 4 mg/l | 1.64 | 3.85 | 1.34 | 14.00 |
| $T_4$ | $GA_3$@ 6 mg/l | 1.55 | 7.10 | 1.33 | 9.50 |
| $T_5$ | $GA_3$@ 8 mg/l | 1.77 | 8.95 | 0.97 | 16.00 |
| | S.Em± | 0.11 | 1.32 | 0.05 | 0.70 |
| | CD at 1% | 0.32 | 3.97 | 0.15 | 2.10 |

Observations are taken at 120 days of transfer to the media.

**TABLE 7:** Effect of auxins on *in vitro* rooting in Jamun *cv.* 'AJG-85'

| Treatment | | Per cent rooting | Number of roots per shoots | Root length (cm) | Number of primary roots | Number of secondary roots |
|---|---|---|---|---|---|---|
| $T_1$ | IBA 1mg/l | 73.33 (59.21)* | 1.28 | 1.47 | 1.28 | 0.87 |
| $T_2$ | IBA 2 mg/l | 86.67 (72.21) | 1.49 | 1.67 | 1.51 | 0.83 |
| $T_3$ | IBA 5mg/l | 53.33 (46.92) | 1.13 | 1.38 | 1.23 | 0.87 |
| $T_4$ | IBA 10 mg/l | 46.67 (43.08) | 1.11 | 1.29 | 1.11 | 1.08 |
| $T_5$ | NAA 0.5 mg/l | 20.00 (22.01) | 0.86 | 0.98 | 0.90 | 0.71 |
| $T_6$ | NAA 1.5 mg/l | 66.67 (59.92) | 1.42 | 1.33 | 1.28 | 0.90 |
| $T_7$ | NAA 2 mg/l | 66.67 (59.92) | 1.35 | 1.30 | 1.35 | 0.75 |
| $T_8$ | NAA 2.5mg/l | 26.67 (30.79) | 0.97 | 0.98 | 0.97 | 0.71 |
| $T_9$ | IBA 1mg/l + NAA 1 mg/l | 26.67 (30.79) | 0.91 | 1.01 | 0.91 | 0.71 |
| $T_{10}$ | IBA 5mg/l + NAA 5 mg/l | 66.67 (55.00) | 1.30 | 1.07 | 1.22 | 0.71 |
| | S.Em± | 7.80 | 0.08 | 0.12 | 0.09 | 0.08 |
| | CD at 1% | 23.02 | 0.24 | 0.36 | 0.26 | NS |

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

**Effect of auxin on *in vitro* rooting:**

Significantly maximum rooting 86.67 %, number of roots/shoot 1.49, root length 1.67 cm and number of primary roots 1.51 were observed with IBA at 2 mg/l (Table 7) (Fig. 7). These results are similar to the report of Kopp and Nataraja[4] in tamarind. Among the two auxins used, IBA performed better than NAA.

## CONCLUSION

In the present study a simple protocol for *in vitro* regeneration is standardized which could be used for rapid mass multiplication of healthy and disease free plantlets of Jamun cv. AJG-85.

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