

Enhanced Release of Axillary Buds- A Promising In Vitro Propagation Method for Jack (*Artocarpus Heterophyllus Lam.*) Variety Sindoor

*Shily C., Unnikrishnan B.S., Soni K.B., Swapna Alex

Department of Plant Biotechnology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram

*Corresponding author

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Abstract: Jack (*Artocarpusheterophyllus* Lam.) is a gifted tree with many medicinal and nutritious qualities. It is the largest edible fruit in the world. Sindoor is a variety developed by Kerala Agricultural University which has preferable consumer qualities such as short stature of the tree, small-sized fruits, sunset orange colored flakes, sweetness of the flakes, bearing twice-yearly, etc. In the current study, micropropagation of the variety experimented with different surface sterilization treatments and media with varied concentrations of plant growth regulators. Media supplemented with different concentrations of cytokinins were tried, of which MS media with 3 ppm BAP showed better response. Thus, the present study gives information on the role of different plant growth regulators in the release of axillary buds during micropropagation.

Keywords: Jackfruit (*Artocarpusheterophyllus* L.), *In vitro*, Micro-Propagation, Shoot Tip

I. Background

*Artocarpusheterophyllus*Lam., which is commonly known as jackfruit, is a tropical climacteric fruit belonging to the Moraceae family. It is native to the Western Ghats of India and is common in Asia, Africa, and some regions of South America. It is known to be the largest edible fruit in the world, reaching up to 50 kg in weight and 60-90 cm in length. Jackfruit is rich in nutrients, including carbohydrates, proteins, vitamins, minerals, and phytochemicals.[1&2] Both the seeds and the flesh of jackfruit are consumed in boiled forms and the flesh in the fully ripened stage directly as a fruit. Jackfruit contains functional compounds that can reduce various diseases such as high blood pressure, heart disease, strokes, and bone loss. [3] In 2018, the Kerala state government declared the jackfruit as the official fruit. Jack fruit is cauliflorous and produced all over the tree trunk. Harvesting is very difficult in jack since the tree is generally tall and the fruit is large. So for cultivation, farmers prefer jack trees having short stature. Soft-fleshed and firm-fleshed varieties are there. Consumers prefer firm-fleshed variety.[4] Consumer preference is changing over time. Small families prefer small-sized and sweet jackfruit. Considering the difficulty in harvesting, most farmers prefer high-yielding and short-statured jack varieties. One such variety is “Sindoor,” a highly sweet variety of jack with attractive sunset orange-colored flakes. The flesh size of this variety is big, and it bears twice a year. Sindoor produces small-sized fruits, and the tree is short in stature. It can be grown in small homesteads. Due to its short stature, harvesting is very easy. So, it is most favored by farmers. There is high demand for the planting material of the Sindoor variety.

Commercial cultivation of jackfruit is still at a primitive stage in India, primarily because of the difficulty in procuring elite planting materials. Jack is easily propagated through seeds. The seedlings take 8-10 years to bear fruits. Due to the highly cross-pollinated nature of the crop, vegetative propagation is essential to get true-to-type plants. Clonal propagation through the grafting of selected genotypes is highly desirable but the number of plants produced by these conventional methods is relatively low. Rootstock is a problem in jackfruit grafting. Delayed incompatibility may occur when some seedlings are used as rootstock. It is reported that when *Artocarpus hirsute* is used as rootstock for grafting poor growth and dying of plants within two years of planting occurred.[1]

Micropropagation is a method of choice for the multiplication of certified, disease-free plants at a much faster rate than using conventional propagation techniques[6]. Tissue culture techniques have been developed for faster multiplication of improved genotypes using nodal segments and terminal and axillary buds as explants. Successful micropropagation protocols have been developed in Egypt and Bangladesh. Micropropagation of jackfruit in India has been initiated during the nineties [5]. Reports on the development of reliable methods for large-scale multiplication of jackfruit are limited. So far, no protocol has been developed for the popular jackfruit varieties in Kerala. Success in the micro-propagation of jackfruit has been very limited due to problems such as contamination, phenolic exudation, vitrification, delayed induction of rooting, acclimatization, etc. So greater thrust needs to be given to developing reproducible *in-vitro* methods of propagation to multiply promising planting material of Sindoor variety jack. In this study, we have tried getting sterile cultures with different combinations of surface sterilants and then succeeded to get the enhanced release of axillary buds.

II. Methods

Materials:

The chemicals used for conducting this study were purchased from Merck India Ltd and Himedia. The glassware and plasticware were purchased from Borosil and Tarson.

Plant material

Explants for micropropagation were collected from the grafts of the Sindoor variety brought from the Farming Systems Research Station, Sadanandapuram. Shoot tips were the major explants used for inoculation. Single-noded cuttings were also used. A bavistin treatment was given to the plants one day before inoculation.

Sterilization of equipment

All the glassware was soaked in detergent water for 2 hours followed by repeated washing in tap water to remove components of detergent. After that, they were rinsed two to three times with distilled water. Plant tissue culture bottle jar, beakers, etc. were autoclaved at 121°C, 15 lb, for 20 minutes. All the other equipment was also sterilized under the above condition.

Surface sterilization of explants:

Since jack is hardy plant contamination is the major problem that occurs during micropropagation. Different combinations of surface sterilants were used in this experiment. Fungal contamination was prevented using bavistin and taxim treatment. Tween 20 was also used to avoid microbial contamination. The major surface sterilant used in this experiment were mercuric chloride and sodium hypochlorite. The effect of these chemicals at different concentrations for different intervals of time was analyzed.

Establishment of jackfruit explants:

In this experiment, media were prepared in a 250 ml plant tissue culture bottle jar. Each bottle contains 50ml media. The sterilized explants were cultured individually into the plant tissue culture bottle jar containing the following media:

- MS medium (control)
- MS medium supplemented with 2ppm BAP
- MS medium supplemented with 3ppm BAP
- MS medium supplemented with 2ppm BAP +1ppm kinetin
- MS medium supplemented with 1ppm BAP+ 2ppm kinetin
- MS medium supplemented with 2ppm 2iP
- MS medium supplemented with 1ppm 2iP
- MS medium supplemented with 0.5ppm 2iP

Cultures were incubated at $27 \pm 2^\circ \text{C}$ under light provided by white fluorescent tubes giving the the intensity of about 2000 lux for 16 hours/day. The explants were subcultured with the same media every three weeks.

III. Results

Effect of explants:

Both shoot tips and single-noded cuttings from grafts were used as explants for this experiment. An early response was observed to the shoot tips. Axillary buds from the single-noded cuttings were also developed, but the bud emergence took more time than the tips.

Effect of surface sterilants:

Different types of surface sterilants were used in this experiment at different concentrations. Mercuric chloride and sodium hypochlorite are used at different concentrations. The most successful sterilization method noted was as follows.

Spray the branches with bavistin 2g/L one day before inoculation. Safely cut the shoot tips and wash them with tap water. Treat the explants with 0.1% bavistin, 1 drop of laboline&taxim 10mg/L for 15 minutes. Wash the explants with sterile distilled water. Treat it with ascorbic acid 0.08% for 30 minutes and wash it with sterile distilled water. Treat it with 0.2% mercuric chloride & one drop of tween20 for 5 minutes. Wash the explants with sterile distilled water 3 to 4 times. Treat it with 0.4% sodium hypochlorite for 20 minutes. Wash the explants with sterile distilled water 3 to 4 times. Dry it with sterile tissue paper. The effect of surface sterilants is described in the **table1**.

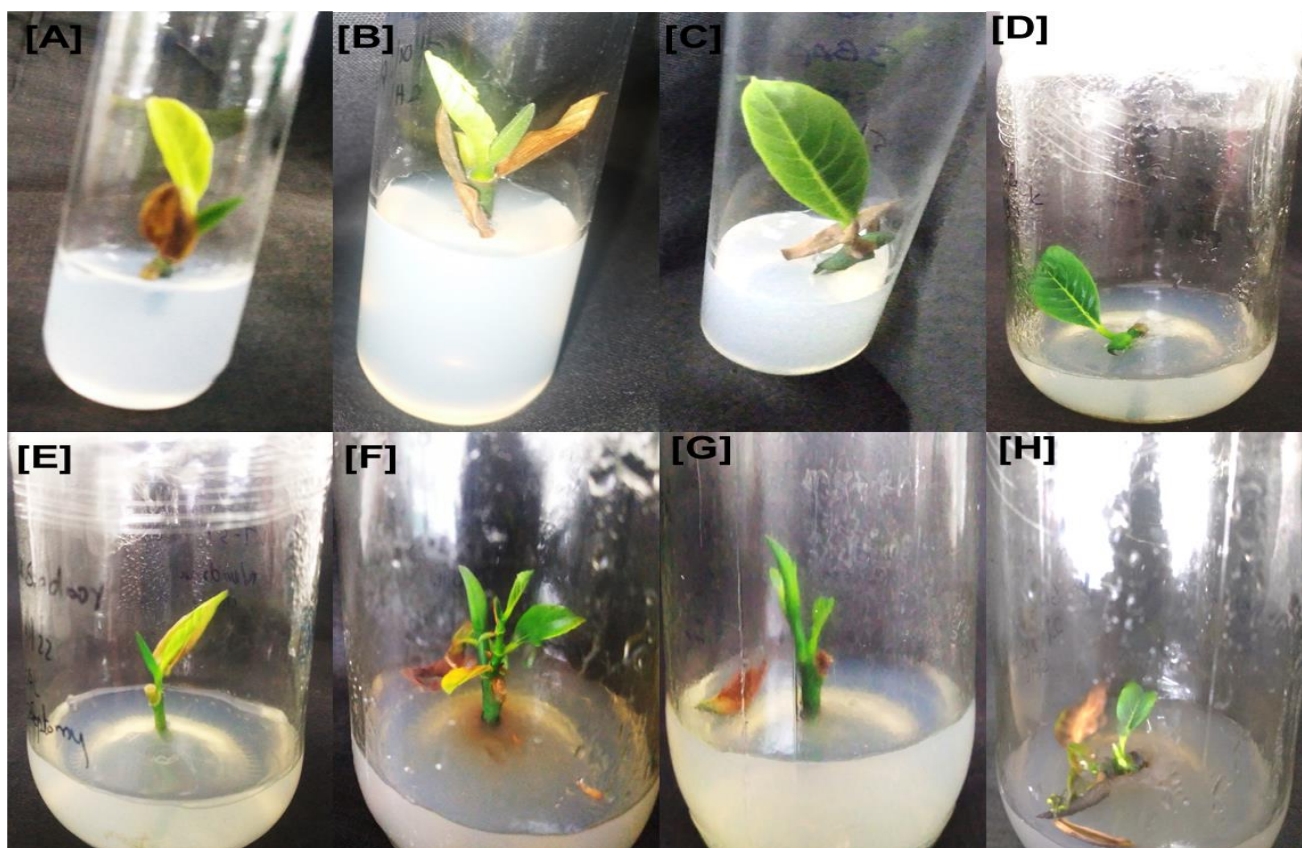


Figure 1: [A]Shoot emergence with MS + 2 BAP' [B] Shoot emergence with MS + 2 BAP + 1 Kinetin, [C] Shoot emergence with MS + 3BAP, [D] Shoot emergence with MS+1BAP +2kinetin, [E] Shoot emergence with MS+ 2 ppm 2iP[F] Shoot emergence with MS + 3BAP [G] Shoot emergence with MS + 3BAP [H] Shoot emergence with MS + 2BAP

Table 1: Effect of surface sterilants

Surface sterilization			
Treatments	% of contamination	Survival %	Mortality%
Wipe with 70% alcohol+ Treatment with 0.1% bavistin+ Ascorbic acid 0.08%+ 0.3% mercuric chloride + 0.4% sodium hypochlorite	0	0	100
0.1% bavistin+ 1 drop laboline + Ascorbic acid 0.08%+ 0.1% mercuric chloride + 0.05% tween 20	100	0	0
0.15% bavistin+ 1 drop laboline + Ascorbic acid 0.08%+ 0.2% mercuric chloride + 0.05% tween 20	75%	25	0
0.1% bavistin+ 1 drop laboline + Taxim (10mg/1000ml)+Ascorbic acid 0.08%+ 0.1% mercuric chloride + 0.4% sodium hypochlorite	100	0	0
0.1% bavistin+ 1 drop laboline + Taxim (10mg/1000ml)+Ascorbic acid 0.08%+ 0.2% mercuric chloride + tween 20+ 0.4% sodium hypochlorite	35	75	0

Effect of Plant Growth Regulators:

Sindoor variety jack showed positive responses towards cytokines like 6-Benzylaminopurine, 6-(g.g-dimethylallylamino)purine (2 iP), and kinetin. In this experiment, I used 1mg/L, 2mg/L, and 3mg/L 6-Benzylaminopurine in full-strength MS medium along with control. Out of the different combinations tried, full-strength MS media supplemented with 6-Benzylaminopurine at 3ppm

concentration gave the maximum number of shoots and faster response. The following figures show the effect of different growth hormones on shoot emergence.

IV. Discussion

Effect of explants:

Micropropagation in hard wood trees is difficult due to various factors like juvenility vs maturity inherent slow growing habit , exogenous and endogenous infection , presence of phenolic compounds, long complex life cycle etc.[23,26&27]. Shoot tips always show apical dominance. When we remove the apical meristem the lateral buds start to grow. Cytokinins are hormones that help to overcome apical dominance. In this experiment, axillary bud development was enhanced by the increase in the number of cytokinins.

Effect of surface sterilants:

Bavistin is a common fungicide used in agriculture. For reducing the fungal load in the explants used we used to spray the shoots with bavistin one day before inoculation. Bavistin wash is also given to the explants. Labolene is a cleaning agent which helps to clean the surface adhering particles. Cefotaxime is an effective anti-bacterial agent that helps to reduce the bacterial count in the explants[19]. Phenolic exudation during the excision of plants is a major problem in the micropropagation of woody perennials. This may stimulate the browning of the media and may become poisonous to the explant. Ascorbic acid is an effective anti-oxidant that helps reduce this browning effect. [7,20,23,24] . Mercuric chloride is an effective antifungal and antibacterial agent. It is showing a good effect at a concentration of 0.2% for 5 minutes. Low concentrations of mercuric chloride will not give a sterilization effect and a high concentration of this chemical may be lethal to the tissues of the explant. [5]. Tween 20 is a wetting agent which helps the explant to be wet and repel air. Using this along with a surface sterilant makes it more effective. Since this is an ester molecule, it is inert and non-toxic. Sodium hypochlorite is an anti-microbial agent. Hypochlorous acid and hypochlorite ions present in this chemical lead to amino acid degradation and hydrolysis. It leads to interference with cellular metabolism and helps in disinfection.[16]

Effect of Plant Growth Regulators:

Adenine-based cytokinins like 6-Benzylaminopurine, 6-(g,g-dimethylallylamino)purine (2 iP), and kinetin help in reducing the apical shoot dominance and induce both axillary and adventitious shoot formation in the explants used.[21]. Cytokinins in lower concentrations lead to callus formation which later dries off. When we used 3mg/L BAP or 2mg/LBAP+1mg/L kinetin etc we could prevent callus formation and get axillary shoot development.[8,22&25]

V. Conclusion:

From the above findings, it is concluded that MS medium fortified with 3.00 mg/l BAP is the best for *in vitro* shoot multiplication of Sindoor variety jackfruit. This is the first micropropagation study in the Sindoor variety jack. In this study, we could achieve successful culture establishment and enhanced release of axillary buds. This experiment gives the base for further establishment of *in vitro* raised plantlets from the Sindoor variety jack.

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Declaration of Competing Interest

The authors declare no competing interest.

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