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# *In Vitro* Conservation of *Artocarpus heterophyllus* Lam.

**Srinivasan Ganeshan • Punathil Ellath Rajasekharan* • Sunitha Bhaskaran**

*In Vitro* Conservation and Cryopreservation Facility, Division of Plant Genetic Resources, Indian Institute of Horticultural Research
Hessaraghatta Lake-PO Bangalore-560 089, India
*Corresponding author*: * **pers@iihr.ernet.in**

## ABSTRACT

Jackfruit (*Artocarpus heterophyllus* Lam.) is a tropical fruit tree indigenous to rainforests of Western Ghats and distributed throughout India, Burma, Sri Lanka, Southern China, Malaya and the East Indies. Though cultivated to a certain extent in India and the East, this species has been reported to be regionally endangered in South India. Tissue culture techniques for propagating identified elite scion varieties would help to retain genotypic characters and produce a large number of plant material. *In vitro* methods for propagation of jackfruit are in a developmental stage in 8 South Asian countries. There is no report on *in vitro* conservation of *A. heterophyllus* to date. Thus, in order to overcome recalcitrance, retain genetic purity of elite varieties and prevent escalation of this species in the hierarchy of the Red Data Book, this study on *in vitro* conservation was taken up, where micropropagation formed a pre-requisite for conservation studies. Protocols have been optimized to conserve germplasm *in vitro* at 10°C without an intervening subculture for 4 years, which could support jackfruit conservation programs *ex situ*. The present paper highlights the use of *in vitro* conservation methods for jackfruit under reduced culture conditions for establishment of *In vitro* Active Genebank (IVAG).

**Keywords:** *ex situ* conservation, *in vitro* propagation, jackfruit

## INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.), a member of Moraceae family, is a tropical fruit tree, indigenous to rainforests of Indian Western Ghats and is distributed throughout India, Burma, Sri Lanka, Southern China, Malaya and East Indies. Jackfruit is highly popular and ranked next to mango and banana, in South India. The jackfruit tree is a multipurpose tree and all parts of the plant are equally important; its pulp and fruit are used in a variety of cuisines. The properties of jackfruit also transcend beyond 'table fruit' tag to a healing plant. Clinically, all parts of jackfruit are used for treating an array of health problems. Leaves are used in skin diseases and as an antidote to snake-bite, roots are used in diarrhea, juice of the plant is applied to glandular swellings and abscesses to promote suppuration, and the ripe fruit is used as a laxative (Anonymous 1985). Though cultivated to a certain extent in India and the East, this species has been reported to be regionally endangered in South India (Rajasekharan and Warren 1994). This can be attributed to the recalcitrant nature of the seeds, wherein 70% germination is obtained within 15 days of extraction, which is reduced to 40% if the seeds are dried for germination 30 days after having been extracted (Samaddar 1990). In Tropical Asia the plant is mainly grown in homestead farms and sometimes in orchards. Jackfruit has been recommended as a number one priority fruit crop for commercialization in Asia.

*A. heterophyllus* is an out-breeding species. Therefore, germination of seeds may result in seedlings with vast genetic diversity. Clonal propagation of selected genotypes is very useful in maintaining selected characters. The prevailing practice currently followed by cultivators for propagation of this species is mainly clonal selection in the form of scion grafts. Propagation through seed is practiced mainly to generate variability and select desirable clones. Seed-derived progenies of *A. heterophyllus*, being highly heterozygous and cross-pollinated, are not true to type. The fruits from trees differ widely in density of spike in the rind, bearing habit, size, shape, quality and period of maturity (Mitra and Mani 2000). Tissue culture is the best means of propagating identified elite scion varieties wherein genotypic characters can be retained while obtaining a large number plant material.

An *in vitro* method for Jackfruit which is the priority crop under the UTFANET (Underutilized Tropical Fruits in Asia Network) program (Anonymous 2003) is in a developmental stage in 8 South Asian countries. Among these countries, *in vitro* methods for propagation of jackfruit have been reflected as one of the ongoing activities. Rao *et al.* (1981) have reported *in vitro* propagation of Jackfruit via callus induction, wherein they encountered problems with *in vitro* rooting of the regenerated plants. There is no report on *in vitro* conservation of *A. heterophyllus* to date. Hence, in order to overcome recalcitrance, to retain genetic purity of elite varieties and to prevent escalation of this species in the hierarchy of the Red Data Book, this study on *in vitro* conservation was taken up, where micropropagation formed a pre-requisite for conservation studies. The protocols optimized could support Jackfruit conservation programs under the UTFANET. The present paper reports a protocol for *in vitro* conservation of jackfruit under reduced culture conditions optimized, with a reduced subculture frequency.

## MATERIALS AND METHODS

### Sterilization of explants

Actively growing terminal and auxiliary shoot buds derived from healthy branches of mature jackfruit trees and 3 week old seedlings were excised and used as explants for initiating aseptic cultures. Explants were trimmed to 1 cm. length and were subjected to continuous clean water flow for 15 min followed by sterilization with dilute detergent solution (0.1%) for 15 more min. The explants were agitated in detergent solution to wash away debris. Successive washings excluded soapiness. Explants were further sterilized with 70% alcohol for 90 sec and then with 0.05% mercuric chloride for 10 min before finally washing in sterile double distilled water 3-4 times.

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Short Communication

### Initiation, multiplication and rooting

Disinfected explants were further trimmed at the laminar airflow cabinet pre-set to sterile conditions to about 8-10 mm. length prior to inoculation, on half-strength Murashige and Skoog (1962) medium supplemented with 8.87 µM 6-benzyladenine (BA) and 30 g/l (0.087 M) sucrose. pH of the medium was adjusted to 5.8 using 1N NaOH and 1N HCl. Direct regeneration was observed within 15 days of inoculation. Shoot multiplication was also observed in the same medium. Since rooting was not concomitant with shoot growth and multiplication, MS medium with different growth regulators was used. Rooting was initiated when 1.5 cm long shoots were transferred to half-strength MS medium supplemented with 14.70 µM indole-3-butyric acid (IBA).

### *In vitro* conservation

20 replicates of *in vitro* obtained plantlets both from mature and seedling derived Jack were transferred from standard culture conditions (SCC; 16-h photoperiod, temperature: 25±2° C, light intensity: 31.55 $\mu m^{-2}s^{-1}$) after allowing an initial growth and establishment period of 3 weeks to reduced temperature and light conditions (10°C, low light intensity (2.97 $\mu m^{-2}s^{-1}$) as conservation treatment. Media composition was kept same as that used for multiplication (MS+8.87 µM BA)

Conservation gain is defined as the period of conservation of a given species under *ex situ* conditions without any drastic alteration in survival, re-growth and capability to perform normal functions similar to that grown under natural conditions (Rajasekharan *et al*. 2005).

Five parameters such as number of shoots, shoot length, internode length, number of nodes and number of leaves were identified to guide as conservation indices for recording observation; to estimate growth reduction and conservation gain among the treatment induced. These parameters were chosen as per IPGRI guidelines (CIAT/IPGRI 1994), modified to suit the species.

The seedling derived *in vitro* plantlets relocated to low temperature conservation treatment were monitored for growth and survival for a period of 3 years and *in vitro* plantlets derived from mature tree clones for 6 months respectively, recording observations on the above five conservation indices. Data were subjected to statistical analysis to indicate significance in the treatment induced.

### Statistical analysis

One-way ANOVA (Gomez and Gomez 1984) was applied to ascertain whether the different storage durations have a uniform effect on *in vitro* plantlet behavior as expressed by different biometrical characters. The technique was also used to elicit for uniform performance on observations made under different treatments. Further, in order to ascertain the influence of storage durations on *in vitro* plantlet behavior, one-way ANOVA under factorial set up was carried out for different treatments for *in vitro* plantlet material derived from seedling and mature tree clones. One sample paired *t*-test (Gomez and Gomez 1984) was then applied to: 1) Test the significance of Jack *in vitro* plantlets conserved under SCC with that conserved at 10°C for up to one year; 2) Test the significance of seedling derived *in vitro* plantlets conserved under SCC with that derived from mature plants separately for each of the biometrical characters up to a period of 6 months.

The use of the paired *t*-test was necessitated as the paired samples under comparison in both the cases are interrelated. Comparisons were made first, with *in vitro* plantlet culture sets derived from Jack seedlings conserved under SCC with that conserved at 10°C for 1, 3, 6 and 12 months respectively. In the second set, *in vitro* plantlet culture sets derived from Jack seedlings conserved under SCC were compared with *in vitro* plantlets derived from mature tree clones conserved for 1,3 and 6 months, to ascertain the differences in conservation parameters used in this study. Since there was depletion of medium among culture sets stored under SCC beyond one year, it was not possible to maintain them conserved without subculture under SCC. Hence, no comparison could be made with material continuously conserved at 10°C for 2 and 3 years respectively.

## RESULTS AND DISCUSSION

### *In vitro* establishment, multiplication, rooting and hardening

Asepsis of explants could be achieved up to 70% by sterilization with mercuric chloride, which proved to be a better sterilizing agent compared to sodium hypo chloride. In the latter, bleaching of explants were noticed during the process of sterilization and such bleached explants failed to regenerate. Optimal multiplication, rather than mass multiplication, was desired for *in vitro* conservation studies and this could be achieved when half strength MS medium supplemented with 8.87 µM BA was used as multiplication media. A multiplication rate of 4-5 shoots/explant could be obtained. Apical explants responded faster than nodal explants, but multiplication rate reduced to an average of 2 shoots/ explant. On the contrary, nodal explants took at least one week more to respond, but multiplication rate was faster, averaging to 5-6 shoots/explant. Rooting being fastidious, response was observed only when IBA was used for its stimulation. Rooting was observed to occur in half-strength MS medium supplemented with IBA concentrations ranging from 4.90 to 29.40 µM. Optimal rooting was observed in 14.70 µM IBA medium; the percentage of shoots rooting reduced when the concentration of IBA was either decreased or increased.

### *In vitro* conservation

One-way ANOVA elicited differential behavior for storage duration with *in vitro* plantlets for shoot length (seedling derived, under SCC) number of leaves (seedling derived, under 10°C); shoot length, and number of nodes (derived from mature tree clones) under SCC. For the remaining parameters, there was no significant effect on storage duration (**Table 1**).

Seedling derived non-rooted shoots were successfully conserved at 10°C with reduced light for a period of 3 years with and without intervening subculture, retaining healthy *in vitro* morphology. A number of these cultures are still being maintained under the same conditions and the maximum storage period is yet to be ascertained. Shoot material derived from mature jackfruit trees have been successfully conserved for 6 months at SCC. *In vitro* plantlet multiplication is in progress for relocation from SCC to 10°C.

Among seedling-derived *in vitro* plantlets, it could be established that storage at 10°C resulted in reduced depletion of medium leading to delay in first subculture beyond one year. Beyond this period under SCC, there was considerable depletion of medium and subculture was imminent. At one year, in comparison to *in vitro* plantlets conserved at SCC, there was a significant reduction in the number of shoots for *in vitro* plantlets conserved at 10°C ($t$ = 7.031) and shoot length ($t$ = 3.417), which favors conservation. There was no significant change in the internodal length, number of nodes and number of leaves (**Table 2**).

Among *in vitro* plantlets derived from mature tree clones, comparison after 6 months with seedling derived *in vitro* plantlets conserved under SCC, no significant difference could be perceived in shoot number, internodal length and leaf number. However there was a significant reduction in number of nodes ($t$ = 3.371) and shoot length ($t$ = 4.602) in *in vitro* plantlets derived from mature tree clones (**Table 3**).

Rooted plantlets were established *extra vitrum* in 'Soilrite' potting mixture with 95% survival. Non-rooted conserved shoots stored at SCC and 10°C could be established *extra vitrum* after one year by dipping the cut ends in 100 mg/l IBA, recording 78% survival rate. Few seedling derived *in vitro* plantlet cultures, which were stored for 2 years were relocated to SCC, sub cultured and successfully regenerated. These *in vitro* plantlets showed no anomalies and normal growth response was restored.

**Table 1** Results of one-way ANOVA for testing the differential behavior of storage durations.

| Conservation index | Mean values (cm) | | | |
|---|---|---|---|---|
| | 1 month | 3 months | 6months | 12 months |
| Shoot length (seedling derived) SCC CD at P=0.05=0.763 Significance-* | 1.74 | 2.48 | 3.42 | 3.46 |
| Number of leaves (seedling derived) 100C CD at P=0.05=0.899 Significance-* | 1.60 | 1.80 | 3.00 | 4.60 |
| Shoot length (mature tree clone derived) SCC CD at P=0.05=0.784 Significance-* | 0.84 | 1.42 | 2.22 | N I |
| Number of nodes (mature tree clone derived) SCC CD at P=0.05=0.871 Significance-* | 1.80 | 2.80 | 3.40 | N I |

(NI Not Indexed)

**Table 2** Paired *t*-test for comparison of seedling derived jack *in vitro* plantlets conserved at SCC and 10°C for one year.

| Conservation indices | Mean values (cm) | | *t* value |
|---|---|---|---|
| | SCC | 10°C | |
| Number of shoots | 4.5 | 2.3 | 7.031* |
| Shoot length | 2.8 | 1.8 | 3.417* |
| Internode length | 0.5 | 0.4 | 1.715 NS |
| Number of nodes | 3.7 | 3.1 | 1.717 NS |
| Number of leaves | 2.8 | 2.8 | 0 NS |

**Table 3** Paired *t*-test for comparison of seedling derived and mature tree clone derived jack *in vitro* plantlets conserved at SCC for six months.

| Conservation indices | Mean values (cm) | | *t* value |
|---|---|---|---|
| | SCC-SD | SCC-MT | |
| Number of shoots | 4.6 | 5.1 | -0.875NS |
| Shoot length | 2.5 | 1.5 | 4.603* |
| Internode length | 0.4 | 0.5 | -0.425NS |
| Number of nodes | 3.8 | 2.7 | 1.371* |
| Number of leaves | 2.8 | 2.6 | 0.526 NS |

SD = seedling derived; MT = mature tree; NS = not significant;* Significant

## DISCUSSION

The diversity in jackfruit trees are lost due to logging and clearing land for agriculture, and the market demand for jackfruit may lead to the replacement of local diversity (Khan *et al*. 2010). The application of tissue culture methods for improvement, large-scale propagation and conservation of fruit trees have been well demonstrated (Litz *et al.* 1985; Engelmann 2011). Successful *in vitro* propagation of jackfruit seedlings has been achieved (Rahman and Blake 1988). Regeneration of plantlets from bud and nodal explants of mature jackfruit trees has been reported (Jaiswal and Amin 1990; Roy *et al*. 1990). For *in vitro* conservation most widely applied technique is temperature reduction, which can be combined with a decrease in light intensity or culture in the dark. Tropical species are often cold-sensitive and have to be stored at higher temperatures, which depend on the cold sensitivity of the species. *In vitro* slow growth storage techniques are being routinely used for medium-term conservation of numerous species, both from temperate and tropical origin, including crop plants, *e.g.* potato, *Musa*, yam, cassava (Ashmore 1997; Razdan and Cocking 1997; Engelmann 1999) and rare and endangered species (Sarasan *et al*. 2006). Moreover, it is not always possible to apply one single protocol for conserving genetically diverse material. In the present study, protocols have been developed for jackfruit *in vitro* conservation using low temperature and low light and *in vitro* plantlet could be maintained at 10°C with low light intensity beyond one year up to four years so far. After one year of conserving seedling derived *in vitro* plantlets, there was significant conservation gain at 10°C in terms of reduction in shoot length and number of shoots, indicating that lowering of temperature and reducing light intensity favors slow growth. In context to plant regeneration, it has been reported that genetic variability occurs due to somaclonal variation increase with the number of multiplication cycles or with duration in culture (Ashmore 1997). Hence, the multiplication rate was restricted to minimum when *in vitro* conservation treatment is employed for this species.

While the objective of an in *vitro* conservation program would primarily be to conserve elite clonal material, it may be advantageous to study the variability among seedling populations in jack conserved *in vitro*, which was hitherto not attempted or conserved. Hence, *in vitro* conservation protocols are established seedling derived *in vitro* plantlets and *in vitro* plantlets derived from mature jackfruit trees. Because jackfruit is almost entirely cross-pollinated, this long-lived, woody tree species is expected to exhibit large variation within-population (Hamrick and Godt 1990). Though tropical, *A. heterophyllus* can be conserved for 4 years under reduced light and temperature conditions, retaining their lush and healthy visible *in vitro* features. During storage at 10°C and SCC, polyphenols were exuded into the media rendering it brown-black. Despite this, no morphological or physiological alterations like bleaching, drying, etc., was observed. Since these exudates were not impedimental, use of PVP and activated charcoal was avoided to satisfy the need for minimal additives in medium during *in vitro* conservation. Thus, an effective protocol for *in vitro* conservation of *A. heterophyllus* is reported for 4 years at 10°C with annual subculture schedules for establishment of IVAG for Jackfruit. The traditional *ex situ* conservation method for these categories of plant species is in the form of field collections. Conservation in the field presents major drawbacks, which limit its efficacy and threaten the safety of plant genetic resources conserved in this way. So IVAG will act as a back up.

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