In Vitro Conservation of Artocarpus heterophyllus Lam.

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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. 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 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. 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 diarrhoea, 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 (Sankaradar 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 pre-vailing 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 of 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.
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 μm/s²) after allowing an initial growth and establishment period of 3 weeks to reduced temperature and light conditions (10°C, low light intensity (2.97 μm/s²) as conservation treatment. Media composition was kept same as that used for multiplication (MS+8.87 μM IBA).

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 depict 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 duration. 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

Aspesis 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 chlorite. 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 at SCC, no significant difference could be perceived in shoot number, internodal length and leaf number. However there was a significant reduction in the 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.
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. 1995; Engelmann 2011). Successful in vitro propagation of jackfruit seedlings has been achieved (Rahman and Blake 1988). Replication 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 (Saras 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 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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