Micropropagation of breadfruit (*A. altilis*) enhanced using a bioreactor system

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Abstract

Breadfruit (*Artocarpus altilis*) is an important starchy food crop with much potential to support livelihoods in the Pacific region. The region has the greatest diversity of breadfruit, with three known species and hybrids. Seeded varieties are largely found in Melanesia and Micronesia, while seedless varieties are found in Polynesia. The seedless varieties are propagated using marcotts, root suckers and root cuttings, and the seeded varieties use seeds. To support increased demands for food for the growing population and to supply market demands, improved methods of propagation are needed. The use of conventional in vitro technology, combined with a temporary immersion bioreactor system, reduced the field-planting readiness time from 44 to 30 weeks. Plantlets produced using the bioreactor were more vigorous, sturdier and taller than those cultured in the conventional semi-solid static tissue culture system. Breadfruit seedlings generated via the bioreactor system are undergoing field evaluation in three breadfruit orchards in Nadi, Fiji, alongside marcotts and root suckers. The analysis is based on the best source of planting material in terms of stem girth, plant height, early fruiting, fruiting patterns, and nutrition and production aspects of varieties. Bioreactor-generated plantlets have also been distributed to Pacific Island countries for evaluation. The research by the Centre for Pacific Crops and Trees (CePaCT) of the Secretariat of the Pacific Community (SPC) based in Suva, Fiji is part of the Pacific breadfruit project funded by the Australian Centre for International Agricultural Research (ACIAR).

Keywords: breadfruit, micropropagation, bioreactor, Centre for Pacific Crops and Trees

INTRODUCTION

Breadfruit (*Artocarpus altilis)* Parkinson (Fosberg) is a species of the mulberry family, Moraceae (Ragone, 1997). A staple food crop in many tropical regions, especially in the Pacific, breadfruit is believed to have been taken by the early Lapita settlers from South-east Asia via Melanesia to the Polynesian islands (Zerega et al., 2004). The Pacific region, where they grow as a backyard tree crop, is home to the greatest diversity, with three known species and many hybrids.

Seeded varieties of breadfruit are largely found in Melanesia and Micronesia, while seedless varieties are mainly found in Polynesia (McLean, 2014). The seedless varieties are propagated using marcotts, root suckers and root cuttings, whilst the seeded varieties use seeds.

Breadfruit is a nutrient-rich crop, with some varieties such as mejwaan in Marshall Islands identified as containing high levels of carotenoids (Englberger et al., 2014). Breadfruit has been identified as a potential export crop in Fiji and Samoa. To meet market demands for export markets, however, a sustainable market supply system must be supported by improved mass micropropagation systems.

A protocol developed by Tuia et al. (2007) using the conventional static in vitro system with glass vessels was successful in establishing a diversity of Fijian breadfruit in 2007. The Samoan diversity was established in 2008 and eventually the protocol was used to culture other breadfruit varieties growing in the SPC regional field genebank at Centre for Pacific Crops and Trees (CePaCT) in Suva, Fiji. Research by Murch et al. (2007) and Tuia et al. (2007) noted the impact of the addition of the growth regulator 6-benzylaminopurine (BAP) on

enhancing breadfruit multiplication and shoot growth. However, more research was needed to explore advanced technologies for improving the production of planting material. Research by Murch et al. (2007) on the temporary immersion bioreactor tilting system with cultured breadfruits exposed to 0.46 mg L^{-1} of BAP for six months produced 100% establishment in the screen house. The use of the bioreactor system was initially developed for biotechnological production of microbes for antiserum use in vaccination (Oliveira et al., 2011).

This research aims at addressing one of the constraints in the shortage and non-availability of planting material of market-preferred varieties for year-round production, by supplying them with preferred varieties of plantlets raised on the bioreactor. The overall objective of the project is to enhance livelihoods through the development of an effective and sustainable breadfruit supply chain system.

MATERIALS AND METHODS

The research was carried out at the SPC CePaCT based in Suva, Fiji. Explants were sourced from two varieties (Samoan *ma'afala* (AA/SM/08) and Fijian *koqo* (AA/FJ/10)) from the SPC in vitro collection established by previous research (Tuia et al., 2007). The explants had been cultured on a hormone-free nutrient medium, Woody Plant Medium (WPM) (Lloyd and McCown, 1980), with 7.75 g L^{-1} of agar prior to the start of the experiment. The cultures were exposed to a temperature of 25±2°C and a light intensity of 2668 lux irradiance with 18 hours day length.

There were four stages to the research: the first three stages were conducted in the laboratory and Stage 4 in the screen house.

Stage 1

Fifteen explants a month old were used in this experiment, by removing the root and shoot tip, stems from each breadfruit variety were multiplied on Woody Plant Medium (WPM) (Lloyd and McCown, 1980) supplemented with BAP 2.5 mg L^{-1} + 7.75 g L^{-1} agar with pH of 5.6-7.2 in glass vessels. During this stage, an average of five shoots per explant was produced within two weeks.

Stage 2

Fifteen explants with shoots from Stage 1 for each treatment were transferred on solid WPM medium supplemented with 2 g L^{-1} activated charcoal in glass vessels for four weeks.

Stage 3

Fifteen shoots of 30 mm in height from Stage 2 were sorted into three treatments: Treatment 1 (control, WPM), Treatment 2 (WPM+BAP 0.5 mg L^{-1}) and Treatment 3 (WPM+BAP 2.5 mg L^{-1}). The media for all 3 treatments also included 2 g L^{-1} of activated charcoal. The impact of using two tissue culture systems; glass vessel (cospak jar) and bioreactor tilting immersion liquid system (manufactured at Caisson Laboratories based in United States, the vessel is 27×10×10 cm and includes a lid and the vessel includes a microporous patch for gas exchange) was investigated and assessed the root length, plant height and number of plants survived at 12 weeks.

Stage 4

Five plantlets were randomly selected from each treatment for transplanting in the screen house. The plantlets were potted in 50% potting mix and 50% foam perlite. The plant height and number of plants survived were assessed for 12 weeks.

The overall experiment proceeded for 30 weeks. The glass-treated plantlets nurtured in the screen house were kept back for another 14 weeks until they were ready for field planting (26 weeks altogether in the screen house). The bioreactor-treated plantlets, however, were ready for field planting after being nurtured in the screen house for only 12 weeks.

RESULTS AND DISCUSSION

After 30 weeks of the experiment, only the bioreactor-treated plantlets were ready for field planting. The glass-treated plantlets needed another 14 weeks for further acclimatization in the screen house as they were too small, both in leaf size and height, were not turgid enough and their root system was not developed enough for field planting.

In the laboratory, in stage 1, the multiplication of plantlets was enhanced with the addition of BAP 2.5 mg L^{-1} to the medium, producing an average of five shoots per explant within two weeks (Figure 1). Based on preliminary experiments, this concentration produced normal plantlets whereas higher concentrations (BAP 3.5, 4.5, 5.5 mg L^{-1}) produced abnormal shoots and calluses.

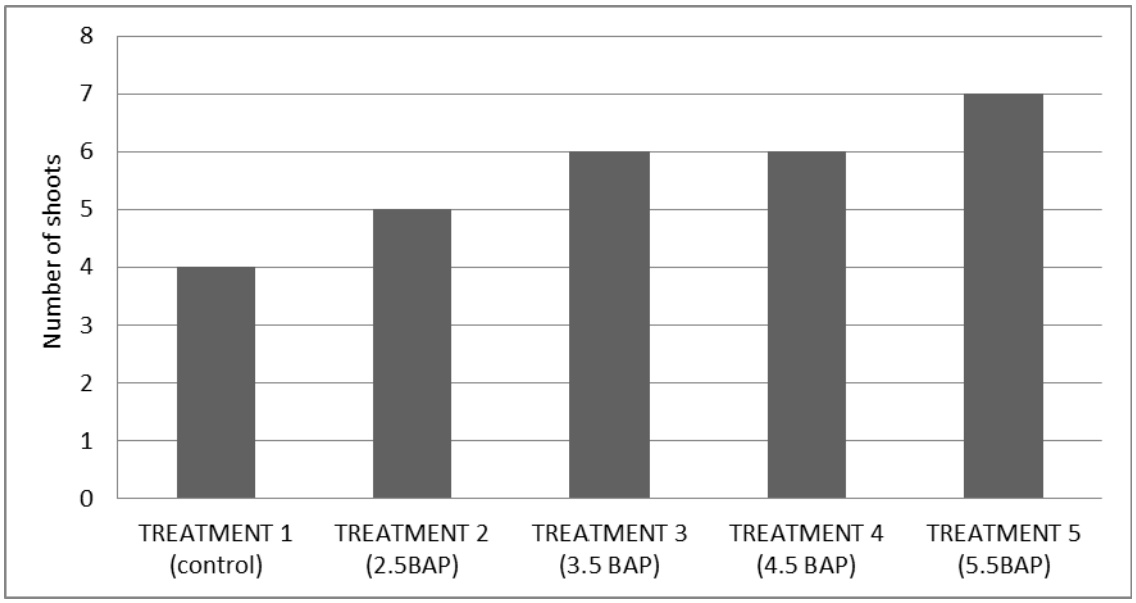

Figure 1. Average number of shoots for *ma'afala* (SM) and *koqo* (FJ) breadfruit varieties after 2 weeks in the laboratory. *n*=15.

The two varieties (*ma'afala* and *koqo*) responded differently to the bioreactor treatment when compared with the glass treatment in terms of height and rooting. The bioreactor had a significantly positive effect on root length of *ma'afala* plantlets (F prob for medium × chamber < 0.05), and it had no effect on either the height (F prob for medium × chamber > 0.05) or the root length of the *koqo* plantlets (F prob for medium × chamber > 0.05) when analyzed by GenStat.

In the screen house, the bioreactor-treated *koqo* plantlets grew taller than the glass-treated plantlets, but there was no obvious difference between these treatments for the *ma'afala* plantlets (F prob for variety × chamber < 0.05).

The bioreactor-treated plantlets had 100% average survival in the screen house for both breadfruit varieties, whilst 83% survival was observed for the glass-treated plantlets (Figure 2). Furthermore, the glass-treated plantlets needed to be grown for an extra 14 weeks before being ready for field planting.

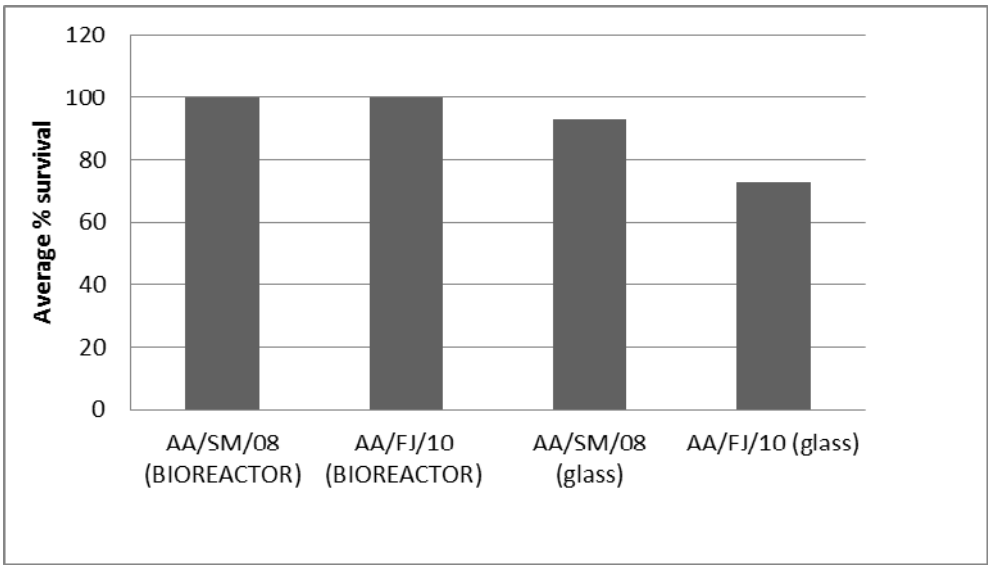

Figure 2. Average survival of *ma'afala* (SM) and *koqo* (FJ) breadfruit varieties after 12 weeks growth in the screen house. *n*=5.

The readiness of the plantlets for field planting was 30 weeks for the bioreactor-treated plants as against 44 weeks for the glass-treated plantlets. The results of this research on glass-treated plantlets support the findings of the previous research by Tuia et al. (2007), in which 44 weeks was the total production system from the tissue culture stage to the field stage for glass-treated plantlets.

CONCLUSIONS

This research has developed a quicker system for field-readiness of planting material at 30 weeks by using a bioreactor system. The advantages observed with plantlets generated with this system include production of good quality plantlets with increased height and good root systems, thus better establishment and acclimatisation in the screen house. The results of this research have already facilitated the mass-propagation and distribution of breadfruit varieties to the Pacific breadfruit project, including seven Pacific Island countries (Fiji, Federated States of Micronesia, Nauru, Palau, American Samoa, Tokelau and Marshall Islands) under the auspices of the FAO *International Treaty on Plant Genetic Resources for Food and Agriculture*. Future distributions of breadfruit will be made to Solomon Islands, Niue, Papua New Guinea, French Polynesia, Pitcairn Island, Samoa, Guam, Tonga, Kiribati, Vanuatu, Cook Islands, New Caledonia, Tuvalu, and Wallis and Futuna. The protocol is being further refined to support increased demands for planting material by farmers and the commercial sector.

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