Studies on In Vitro Culture of Breadfruit Cultivars in the Pacific

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Abstract

This paper outlines an in vitro protocol for breadfruit, *Artocarpus altilis*. Juvenile explants obtained from trimmed root suckers potted in the screenhouse proved to be the optimum source of material for in vitro establishment with a mean survival rate of 78%. In contrast, a 64% mean survival rate was obtained with explants derived directly from field-grown, trimmed root suckers. Explants less than 10 mm rooted in 1-2 months, compared to explants greater than 10 mm, where roots developed after 3-4 months in culture. The immersion of explants in 100 mg/L citric acid during treatment minimized browning. Woody plant medium (WPM) was the optimal medium in terms of survival, root production and shoot growth, with multiplication enhanced with the addition of 2.5 mg/L of 6-benzylaminopurine (BAP) to the medium.

INTRODUCTION

Breadfruit, *Artocarpus altilis* (Parkinson) Fosberg (Moraceae), is an important tree crop in the Pacific especially amongst the Polynesians and Micronesians. It is a food security crop that requires less management compared with other tree crops. It has a wide range of uses, including traditional, social and medicinal. Breadfruit has become a potential export crop for Samoa and Fiji since 2001 (NWCL, 2005). Ragone (1995, 1997) and Zerega et al. (2004, 2005) reported only two species, *A. altilis* and *A. mariannensis* Trécul and possible hybrids, found in the Pacific Islands. *Artocarpus altilis* is the more commonly found species. Conventional propagation methods are either by seeds, or root suckers, root cuttings and marcotts (airlayers). These methods are often slow, thus limiting production and supply of plant material. Likewise, movement of plant material across borders, either between islands or countries, also restricts using vegetative material to prevent the spread of unwanted pests and diseases. Consequently, tissue culture technology can play a critical role in the long-term conservation and rapid mass propagation of difficult plants and provide a relatively safe method for the distribution of plant material.

In vitro propagation has been exploited commercially for the propagation of many plant species, in particular ornamental plants such as orchids (Taji et al., 1992). For in vitro propagation of *Artocarpus* species, research has largely been carried out on jackfruit (*A. heterophyllus* Lam.) (Au Rahman and Blake, 1988a, b; Au Roy et al., 1990; Au Roy and Hadiuzzaman, 1991; Auamin and Jaiswal, 1993; Auroy et al., 1993). Successful in vitro culture of *A. altilis* was reported by Rouse-Miller and Duncan (1999) and Nazario (2003). Some initial diagnosis work was carried out by the Queensland University of Technology (QUT) on the detection of viruses using leaf samples of 20 core cultivars from the National Tropical Botanical Garden (NTBG) in Hawaii. This preliminary work has indicated an absence of viruses in the NTBG breadfruit collection (Harding and Tuia, 2005). The absence of viruses combined with the use of in vitro culture can greatly facilitate the distribution of breadfruit around the Pacific and abroad.

This paper outlines studies to develop an in vitro protocol for selected Pacific Island cultivars carried out in the Centre for Pacific Crops and Trees (CePaCT) of the

Secretariat of the Pacific Community (SPC) based in Suva, Fiji. Such a protocol is aimed at establishing the NTBG core collection in culture for conservation and distribution after virus indexing.

MATERIALS AND METHODS

The study was conducted in CePaCT from December 2004 to December 2006. The first step required developing an effective sterilization procedure to culture breadfruit and investigating: different explant sizes (3, 5, 10, 15 and 20 mm), source of explant material (screenhouse and field), culture initiation medium, and the effect of 6-benzylaminopurine (BAP) on budding of 30-mm-long nodal cutting explants.

Explants were obtained from juvenile auxiliary shoot buds or tips from either trimmed breadfruit suckers grown in the screen house or in the field. For the screenhouse material, source plants of named cultivars of varying sizes were collected, depending on availability in the field, from Tailevu province in Fiji. Three source plants of the Samoan variety 'Ma'afala' planted in the screenhouse originated from tissue culture material from Hawaii. These plants, up to 5 m tall, were decapitated to 3-4 m, all leaves removed, roots trimmed to 10 mm and planted in pots (190 mm × 210 mm) in the screenhouse. All screenhouse plants were watered three times/week and fertilized with N-P-K (10-10-20) every 2 m. Some explants were collected directly from field material in Fiji. In this case, the tips and leaves were removed from the source plants to allow rejuvenation. Due to a limited availability of stock materials, explants required for all experiments were obtained at different times on two different occasions within 3 w, whenever the required explant size was available.

The sterilization procedure was selected after preliminary trials investigating a range of different sodium hypochlorite solutions (obtained from domestic bleach "White King" 4.2% sodium hypochlorite) used for varying periods of time. After disinfection, all explants were thoroughly rinsed well with sterile distilled water and then planted on culture medium.

The media investigated included basal Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), MS supplemented with myo-inositol 100 mg/L, glycine 2 mg/L, Thiamine HCl 0.1 mg/L and pyridoxine 0.5 mg/L, half strength MS, MS + 2.5 mg/L BAP, woody plant medium (WPM) (Lloyd and McCown, 1980) and cassava (CAS) medium (CIAT, pers commun.). Sucrose at 2% was added to WPM and CAS media, whereas all other media were supplemented with 3% sucrose. The medium was solidified with 7.8 g/L agar, after the pH was adjusted to 5.7-5.8. 10 ml of medium was dispersed into each McCartney (81 mm in height and 28 ml volume) for experiments 1, 2 and 3, whereas in experiment 4, 22 ml of medium was dispersed in Cospak glass (84 mm in height and 120 ml volume) bottles prior to autoclaving at 121°C at 15 psi for 20 m.

All cultures were placed in growth room at a temperature of 25°C ± 2°C and provided with a light intensity of 2668 lux irradiance and daylength of 18 h. Explant survival and establishment in tissue culture, plant height, root development, contamination by fungi or bacteria and production of phenolics were recorded monthly for 4 m followed by subculturing and transfer to fresh medium.

Prior to transplanting in the screenhouse, all plantlets were cultured on WPM for 4 m. Two sturdy plantlets were selected for each cultivar ('Koqo', 'Puou' and 'Ma'afala') when the plantlet had reached the top of the 120 ml Cospak glass jar (100% height) and had also produced healthy roots measuring an average 4-5 cm. Only two plantlets were selected as the majority of plantlets were retained in culture for further mass propagation. A porous substrate mix of 3 parts per litre to 1 part potting mix was used for transplanting to the screenhouse. Growth was monitored every month over a period of 5 m when the plants were ready for field planting.

EXPERIMENTS

The effectiveness of the selected sterilization procedure on explants, obtained either from the screenhouse or from the field, was investigated. For the screenhouse

material, six cultivars were used, including five Fijian cultivars: FJ01 ‘Samoa balavu’, FJ02 ‘Samoa’, FJ03 ‘Dreu Lo’, FJ05 ‘Votovoto’ and FJ10 ‘Koqo’, and one Samoan cultivar, SM02 ‘Ma'afala’, with an average of 38 plants per treatment per cultivar. For the field material, three unknown cultivars were used, with an average of 21 plants per treatment. For both field and screenhouse-derived material, ½ MS was the standard culture medium, and the experiments were monitored for 4 m.

The effect of five different sizes (3, 5, 10, 15 and 20 mm) of explant on the establishment of breadfruit in tissue culture was investigated. Four Fijian cultivars were used (FJ01, FJ02, FJ03 and FJ10), with an average of 20 explants per treatment per cultivar. Explants were cultured on standard medium of ½ MS.

Four different media (½ MS, MS, WM and CAS) were evaluated for initiating and establishing breadfruit in tissue culture. Two Fijian cultivars (FJ03 and FJ10) were used with five explants per treatment per cultivar.

The effect of BAP on budding and multiplication of breadfruit nodal cuttings was investigated. Four media were used (½ MS, WPM, MS supplemented with vitamins, and MS + 2.5 mg/L BAP). Only one cultivar, ‘Ma'afala’, was used with 12 explants per treatment media. For each medium treatment, six explants were placed vertically and six horizontally on the culture medium. Explants were taken from plants already established on MS in tissue culture. For each nodal cutting explant, stems were trimmed to a size of 30 mm, leaves removed and the explant inoculated vertically and horizontally on the four media and observed for budding.

RESULTS AND DISCUSSION

A 78% survival rate was achieved using the established sterilization procedure with explants derived from screenhouse plants, compared to a 64% survival rate with field-derived explants (Fig. 1). Field explants contained higher levels of both fungi (11%) and bacteria (29%) compared to 6% and 15%, respectively, found with screenhouse explants. Screenhouse-derived explants provided cleaner source material with which to establish tissue cultures. Additional advantages are contamination can be minimized, trimming and harvesting of explants is easier, and conditions can be controlled compared to field conditions. Preliminary experiments on explants obtained from mature trees showed low survival rates with explants remaining dormant throughout the culture period despite transfer onto several media containing different concentrations of BAP.

The larger explants (10 mm and 20 mm) achieved relatively higher survival rates (79% and 85%) than the smaller explants of 3 mm and 5 mm, where the survival rate was 75% and 72% (Table 1 and Fig. 1). Small explants produced roots after 3-4 m of culture compared to larger explants which rooted after 5 m. Higher contamination levels were observed with 10 mm and 15 mm explants and phenolics were apparent with 20 mm explants (Table 1). These experiments showed that smaller explants (< 10 mm), taken from screenhouse-grown plants, provide the optimum source of explants for in vitro culture.

Optimum plantlet growth was obtained on WPM. Plants cultured on this medium achieved good growth in terms of height (30%), root production (76%) and root length (2.7 cm) (Table 2). WPM has been reported as being optimal for in vitro establishment of woody species because of the need for a low ionic macro salt medium (Lloyd and McCown, 1980). CAS medium, the only medium containing growth regulators, enhanced the presence of phenolics. Nazario (2003) also found a similar correlation between production of phenolics and medium containing growth regulators.

The addition of BAP to the medium (MS + 2.5 mg/L BAP) increased nodal budding to an average of six buds per explant compared to less than four buds per explant with the other three media (½ MS, WPM and MS+ 4 vitamins) containing no growth regulators (Table 3 and Fig. 2). Auroy et al. (1993) reported that the addition of benzyladenine (BA) and napthaleneacetic acid (NAA) to MS nutrient medium induced the maximum number of shoot buds with jackfruit. Work on *A. chaplasha* Roxb. and *A. heterophyllus* (Au Roy and Hadiuzzaman, 1991) showed the formation of multiple shoots

when explants were cultured on medium containing different concentrations of BAP. Similarly, multiple bud break occurred with four cultivars of *A. altilis* when the culture medium contained 12 mg/L BAP (Nazario, 2003). It was observed that explants cultured vertically, rather than horizontally, produced more buds (Table 4).

Transfer to the screenhouse was successfully achieved with plantlets of two cultivars ('Koqo' and 'Puou') using a porous substrate mix of 3 parts perlite to 1 part potting mix (Fig. 3). Plantlets of 'Ma'afala' had previously been transferred to the screenhouse successfully using the same potting mix. The porosity of the substrate supported good aeration and uptake of nutrients by the roots, important as breadfruit is sensitive to waterlogging.

CONCLUSION

This protocol requires further optimization and is the development of a method allowing explants to be successfully cultured directly from mature trees. The lack of such a protocol has hindered the establishment in tissue culture of the "utility" core collection maintained in the field at the NTBG in Hawaii. Such a method must take into account the likelihood of increased contamination and phenolic production associated with mature trees. Contamination, mainly from bacteria, and phenolics production can be a problem when establishing woody species in tissue cultures. This was the case with breadfruit when large explants were excised and cultured. These studies demonstrated that breadfruit can be established in tissue culture and successfully transferred to the screenhouse using a porous substrate. Multiplication can be improved with the addition of BAP to the culture medium. Explants derived from screenhouse-grown source plants achieved good establishment and growth compared to explants obtained directly from field-grown material. Similarly, smaller explants (less than 10 mm) proved to be optimum with respect to lower contamination levels and faster rooting.

This research has implications for on-going studies to optimize tissue culture methods for breadfruit and has applications for in vitro culture of difficult-to-culture tree species. The goal is to facilitate rapid mass propagation of tree species for adequate supply and to distribute virus-indexed plant material to promote germplasm exchange and conservation.

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Tables

Table 1. Results showing response of varying sized explants of breadfruit in tissue culture after four months in culture.

	Size of explant				
	3 mm	5 mm	10 mm	15 mm	20 mm
Plants established in tissue culture (%)	75	72	79	71	85
Height as % of tube height (81 mm)	10	12	7	10	10
Explants with roots (%)	26	7	0	0	0
Average root length (cm)	0.47	0.14	0	0	0
Plants with contamination (%)					
Fungi	8	6	13	0	3
Bacteria	9	21	15	29	12
Plants with phenolics (%)	0	4	1	0	16

Table 2. Response of breadfruit explants on four different establishment culture media in tissue culture after 4 months in culture.

Culture Medium	½ MS	CAS	MS	WPM
% plants established in tissue culture	66	80	83	96
Height as % of tube height (81 mm)	24	28	13	30
% explants with roots	57	22	17	76
Average root length (cm)	2.3	0.7	0.5	2.7
% plants with contamination				
Fungi	7	3	0	4
Bacteria	17	0	0	0
% plants with phenolics	0	3	0	0

Table 3. Response of breadfruit explants to different culture medium supplemented with and without BAP growth regulator after 4 months in culture.

Culture Media>	MS+ 4 vitamins	WPM	½ MS	MS +2.5 mg/L BAP
Plants survived (%)	100	100	100	100
Average number of buds per treatment	3	2	4	6
Height as % of tube height (81 mm)	4	1	7	10
Plants with roots (%)	17	33	50	0
Average root length (cm)	0.15	0.33	0.62	0

Table 4. Response of breadfruit explants to different culture medium and different inoculation (horizontal and vertical) methods of explants after 4 months.

Culture media	MS+ 4 vitamins		WPM		½ MS		MS +2.5 mg/L BAP	
Placement of explants on culture medium[1]	H	V	H	V	H	V	H	V
Plants survived (%)	100	100	100	100	100	100	100	100
Average number of buds per treatment	3	3	2	3	3	5	5	7
Height as % of tube height (84 mm)	50	30	20	10	50	90	100	100
Plants with roots (%)	33	0	33	33	67	33	0	0
Average root length (cm)	0.3	0	0.3	0.3	0.6	0.7	0	0
Plants with phenolics (%)	0	0	0	0	0	0	0	0

[1] H=Horizontal; V=Vertical.

Figures

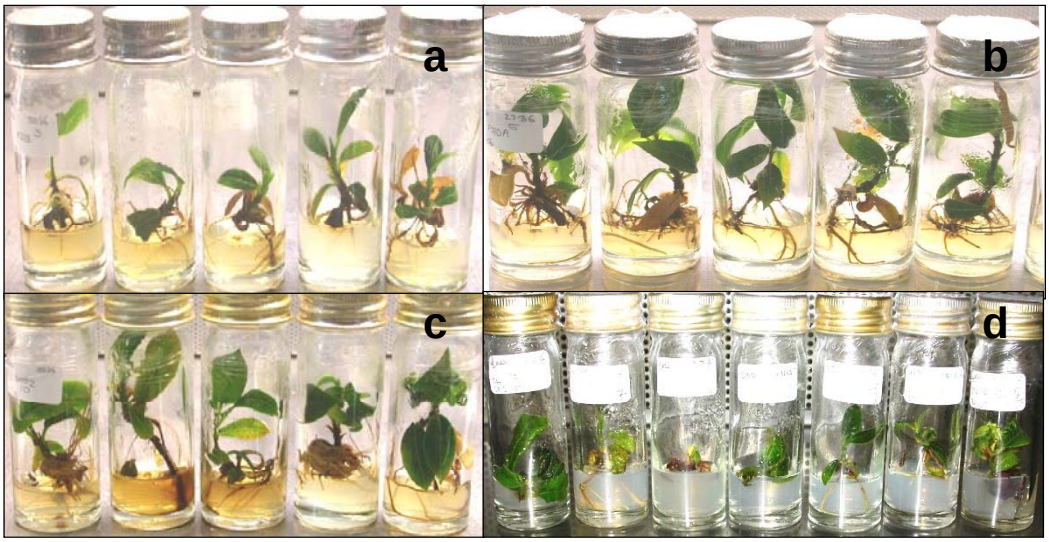

Fig. 1. Response of breadfruit explants in tissue culture after four months in culture. a. FJ 03 ‘Dreu Lo’ (3 mm); b. FJ10 ‘Koqo’ (5 mm); c. SM02 ‘Ma'afala’ (10 mm); d. seven cultivars cultured on ½ MS medium; from left to right: ‘Samoa balavu’, ‘Dreu Lo’, ‘Buco’, ‘Votovoto’, ‘Koqo’, ‘Balekana’ and ‘Ma'afala’.

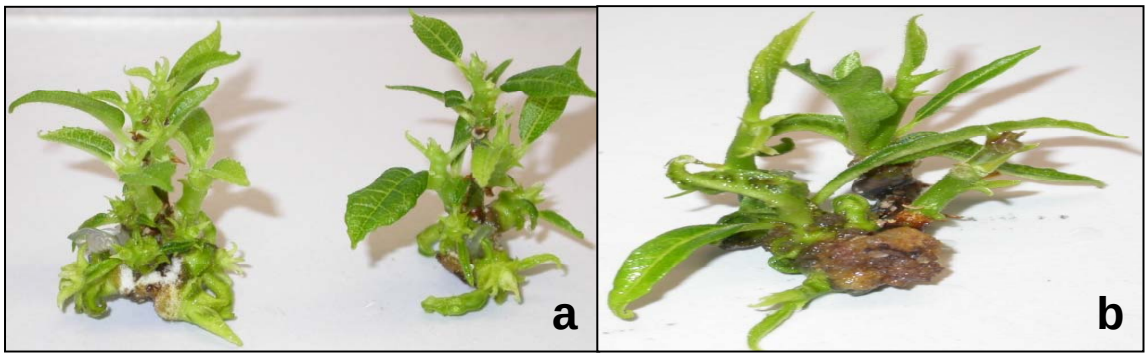

Fig. 2. Growth of ‘Ma'afala’ explants after 1.5 months; a. placed vertically; b. placed horizontally.

Fig. 3. Plants successfully acclimatized in screenhouse at 5 months. a. ‘Koqo’ (left) and ‘Puou’ (right); b. ‘Ma'afala’.