GUAVA CALLUS PRODUCTION UNDER DIFFERENT CULTURE MEDIUM AND PLANT GROWTH REGULATOR CONDITIONS

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ABSTRACT

This research evaluated culture media and plant growth regulators for their influences on callus initiation. Guava (*Psidium guajava* L.) is an important tropical fruit species that is rich in vitamins, minerals, organic acids, and pectins. Different concentrations of 6-benzyladenine (BA), kinetin, or 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthaleneacetic acid (NAA) were added to Murashige and Skoog (MS) and woody plant medium (WPM) and tested for their influences. There are differences in callus initiation and morphology between MS and WPM, and among PGR concentration treatments.

Additional index words: Psidium guajava, plant growth regulators, cultivar.

INTRODUCTION

Guava (*Psidium guajava* L.) is a rich source of vitamins, minerals, organic acids, and pectins (Chan et al 1971, Rathore 1976, Wilson 1980, Campbell 1984, Menzel 1985, Loh and Rao 1989, Yadava 1994). Seventy-nine different phytochemicals provide guava with many unique properties and actions including anti-microbial, astringent, bactericidal, cicatrizant, emmenagogue, hypoglycemic, laxative, nutritive, and spasmolytic. Singh et al (1992) reported that regular consumption of guava fruit significantly increases the good cholesterol level (high density lipoprotein) and significantly decreases serum total cholesterole and blood pressures. Through pharmacological studies of guava with mice, Olajide et al (1999) demonstrated that guava leaf extract considerably inhibited paw oedema, reduced pain, exhibited an antipyretic effect, and prevented diarrhea. Medicinal uses of guava have been reported involving gastroenteritis, dysentery, wounds, ulcers, rheumatics, and toothache (Rathore 1976). Our own preliminary research has demonstrated antimicrobial activity of guava juice, fruit, and leaf extract against *E. coli* O157:H7 (Yang et al., 2004). Because of its considerable store of vitamins and vitamin precursors, minerals, organic acids, and pectins, there is a need to fully explore the possibilities of using this tropical fruit for phytochemical production and antimicrobial applications. It is our belief that with appropriate biotechnological approaches it is possible to generate production of specific phytochemicals in guava that can be used to improve overall health and well-being of the general public. The research objectives were to study suitable PGR concentration and combination for guava callus production; to investigate cultivar responses on callus production; and to determine optimum culture medium strength for guava callus production. By achieving these three objectives we believed we would have advance our knowledge base that could facilitate phytochemical production and extraction.

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MATERIALS AND METHODS

MS (Murashige and Skoog, 1962) and WPM (Lloyd and McCown, 1981) were used as the base media. These media were supplemented with 3% sucrose and 0.7% agar. Medium pH was adjusted to 5.8 using 1N KOH or 1 N HCl before agar was added. The culture media were sterilized by autoclaving at 121^{o}C for 20 minutes.

Softwood shoots from greenhouse-grown stock guava plants were used as explant materials, which were disinfested for 15 minutes in 15% bleach solution (0.78% NaOCl) containing 20 drops Tween-20 per liter, and rinsed immediately with sterile distilled water 4 times. The softwood shoots was cut into 0.5 cm segments as explants. Each explant was transferred onto 10 ml of a particular treatment medium in 25 x 95 mm shell vials with transparent polypropylene closures. Each shell vial containing one explant represented an experiment unit.

A completely randomized design (CRD) was used. Each individual explant in a shell vial was a replication, as there was only one explant per shell vial. Callus fresh weight (mg) per explant was recorded after 10-12 weeks in culture. Average weights were obtained for replication groups and compared using the Least Significant Difference procedure in SAS version 8 (SAS Institute, Cary, NC, USA). Treatment differences were considered significant at the α=0.05 level.

The cultures were placed under 16 hours of light per day provided by cool white fluorescent tubes at 37.6±10.1 μmol $m^{-2}s^{-1}$ and at 23±1^{o}C. Cultures were transferred onto the same fresh media every four weeks.

Different concentrations of 2,4-D (0.8, 1.0, 1.2, 1.4, 1.6, or 1.8 mg/l) were added as treatments to the base MS or WPM. Explants were cultured on each treatment medium. Two sets of experiments were conducted one using MS base medium, the other using the WPM. Each concentration treatment was tested with 14 explant replications. One explant was transferred onto each shell vial as one experiment unit or replication.

The test cultivars include Ruby Supreme, RDF, Pear, Allahabad, RDE, L-49, and Beaumont. Explants (0.5 cm in length) from each cultivar were cultured on MS or WPM with supplements of 2,4-D at 0.5 mg/l and BA at 1.0 mg/l, plus the base supplements of 3% of sucrose and 0.7% agar. The same procedure used for PGR concentration comparison experiments was followed here. There were 14 replications for each cultivar treatment.

MS base medium strengths (½ x, 1x, or 2x) were used as treatments. Each strength MS base medium was supplemented with 3% sucrose, 0.7% agar, 2,4-D at 0.5 mg/l and BA at 1.0 mg/l. ‘RDF’ explants (0.5 cm in length) were cultured on each strength MS treatment medium. The same procedure used for PGR concentration comparison experiments was followed here, except the strength treatment trials used 18 shell vials resulting in 18 replications.

RESULTS AND DISCUSSION

Concentration levels of 2,4-D significantly affected callus production by Guava ‘Beaumont’ (Fig. 1). Explants cultured on 2,4-D at 1.6 mg/l treatment produced the most amount of callus. The callus fresh weight difference among 2,4-D concentration treatments was significant at α=0.05 level. There were different cultivars responses in callus production to the MS and WPM base culture media. General observation of callus fresh weight data indicated that there was the largest amount of 'Beaumont' callus growth occurred when cultured on WPM than explants cultured on MS (Fig. 1). By contrast, visual assessment of the 'RDF' explants produced more callus when cultured on MS in comparison with explants cultured on WPM (Fig. 2). There were not enough data on this to conduct statistical analysis on these observations.

Our preliminary research has indicated that the combination of 2,4-D at 0.5 mg/l and BA at 1.0 mg/l was the best for callus initiation. This combination was selected for the cultivar comparison tests. Significant differences were observed among the cultivars (Fig. 3). 'Ruby Supreme', 'RDF', 'L-49', or 'Beaumont' produced much more callus than 'Pear', 'Allahabad', or 'RDE' on MS. In contrast, 'Ruby Supreme', 'RDF', 'RDE', or 'Beaumont' produced more callus than 'Pear', 'Allahabad', or 'L-49' on WPM. 'Ruby Supreme', 'RDF', or 'Beaumont' produced favorable results in callus production, regardless of the base culture medium.

'RDF' explants were cultured on different strength MS plus 2,4-D at 0.5 mg/l and BA at 1.0 mg/l. Significant difference in callus fresh weight were observed among MS strength treatments. Both visual assessment (Fig. 4) and callus fresh weight data (Fig. 5) indicated that 2x MS treatment enhanced callus production. Callus also displayed good morphological quality. However, about 40% of calli produced from this treatment tended to become brown, and eventually die (data not shown). No similar observation was noticed from ½ or 1x MS treatment.

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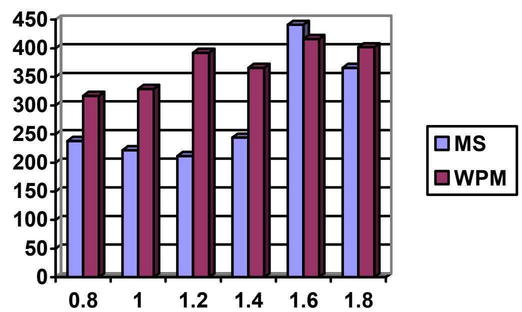

Figure 1. Guava (var. Beaumont) callus fresh weight (mg) from MS (left) or WPM (right) plus 2,4-D at 0.8, 1.0, 1.2, 1.4, 1.6 or 1.8 mg/l.

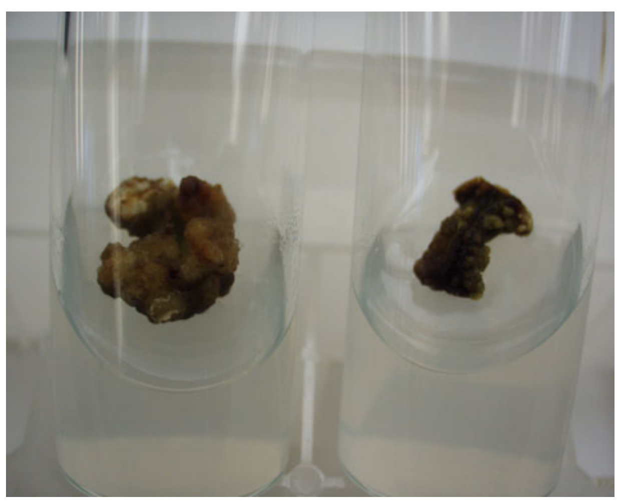

Figure 2. Guava (var. RDF) callus initiation on MS (left) and WPM (right) media.

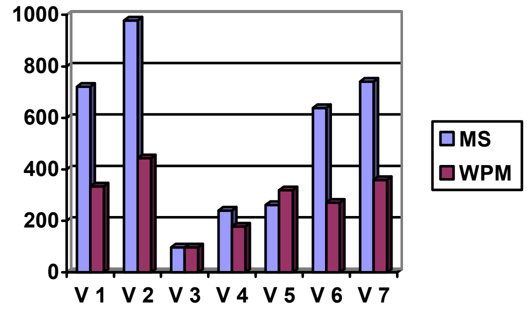

Figure 3. Guava callus fresh weight (mg) from seven cultivars (V1-Ruby Supreme, V2-RDF, V3-Pear, V4-Allahabad, V5-RDE, V6-L49, V7-Beaumont) cultured on MS (left) or WPM (right) basic medium plus 2,4-D at 0.5 mg/l and BA at 1.0 mg/l.

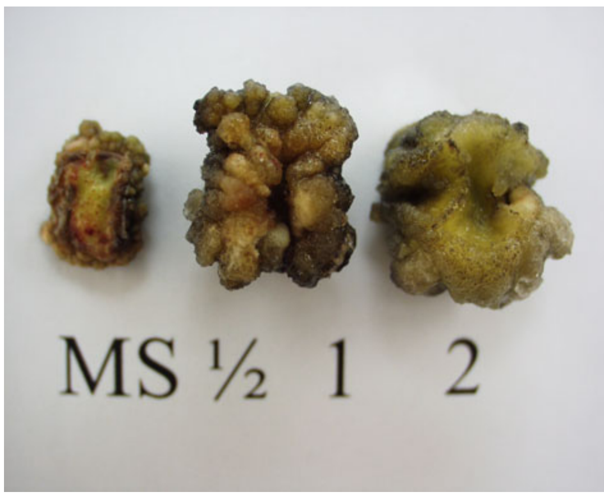

Figure 4. Guava (var. RDF) callus initiation on different strengths of basic MS medium.

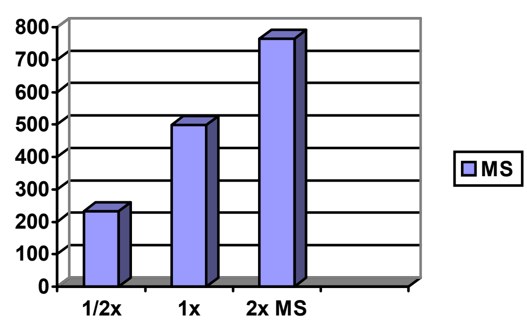

Figure 5. Guava (var. RDF) callus fresh weight (mg) from ½, 1, or 2x MS plus 2,4-D at 0.5 mg/l and BA at 1.0 mg/l.