TISSUE CULTURE STUDIES ON MAHOGANY TREE, Sweitenia

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1. ABSTRACT

The mahogany tree, Sweitenia, which grows as natural populations in tropical America and parts of Asia and Africa, is a highly valued timber tree known for its redwood color, strength, durability, water resistance and aesthetic appeal. Callus tissues were established from aseptic segments of cotyledons and leaves of mahogany (Sweitenia mahogani Jacquin and S. macrophylla King). They were maintained on a modified Murashige & Skoog (MS) medium supplemented with 2 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 mg/l of -naphthalene acetic acid (NAA). The explants proliferated extensively on agar medium in the growth chamber at 25 + 1°C in 2-3 weeks. The callus from cotyledonary tissues were very soft and white in color and further subcultures resulted in a loose "snow-flake" appearance. Callus obtained from leaves were almost transparent and colorless. Experiments on in vitro culture and micropropagation of mahogany suggest that mass propagation of this tree is possible using this method, particularly because of decline in natural populations.

2. INTRODUCTION

The Mahogany tree, Sweitenia is a large tropical American tree growing over 100 ft high and reaching around 4-5 ft in diameter. The heartwood of this tree is well-known and is considered as a highly-valued timber because of its strength, durability and water resistance. The wood is reddish, pinkish, salmon-colored or yellowish when fresh and deepens with age to a reddish brown golden luster. Because of these qualities, the wood is used in the manufacture of furniture, interior decoration, boat construction and various other miscellaneous uses.

There are three recognized species of Sweitenia having separate geographic distributions, viz., S. mahogani jacquin, S. macrophylla King and S. humilis Succ (10). Although these natural populations exist mainly in Central America, and parts of Asia and Africa, unfortunately, population pressures on the land, colonization programs, commercial logging and other deforestation programs have been depleting the natural forests of mahogany there and in other parts of the world. Besides, political uncertainties in these areas and demand for the wood in other parts of the world warrant examining other avenues for establishing and growing large numbers of these populations. Somatic cell genetics using tissue, cell and protoplast culture technology and methods of micropropagation and cloning offer an alternate route for mass propagation and maintenance of large populations. Investigations carried out on in vitro establishment of callus tissue and micropropagation on two species of mahogany, viz., S. mahogani Jacquin and S. macrophylla King are described.
3. MATERIALS AND METHODS

3.1. Seed material

Seeds of *S. mahogani* were obtained from Sri Lanka and of *S. macrophylla* from Western Samoa. Seeds were germinated and planted in the greenhouse and they develop into vigorously-growing plants. The young plants were maintained in the greenhouse (Fig. 1).

3.2. Methods

3.2.1. Callus Initiation. Seeds were surface-sterilized in 10% Chlorox for 10 min and after several rinses, they were soaked in sterile distilled water overnight, since the seed coat is hard. The seed coats were removed the next day and they were surface-sterilized again in 10% Chlorox for 5 min. and washed several times before they were transferred to the agar culture medium. Aseptic 1 cm stem segments and squares of leaves (1 cm.) were also used to initiate callus.

3.2.2. Culture Media. A modified Murashige & Skoog (7) medium referred as MS medium was used. In addition to the normal salts, 30 mg/l of glutamine was added. 30 g/l of sucrose and Difco-Bacto agar (1% w/v) was used. for some experiments the woody plant medium (WPM) (6) was used. The culture medium was supplemented with 1.0, 2.0 or 5.0 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 mg/l of -naphthalene acetic acid (NAA). In certain experiments, a combination of 1.0 mg/l of kinetin and 1.0 or 5.0 mg/l of 2,4-D were used. The pH of the medium was adjusted to 5.6 or 5.8 before autoclaving.

Cultures were maintained in a growth chamber at 25 ± 1°C with 12 hr/day illumination.

3.2.3. Micropropagation. The method based on growth of axillary buds from branch segments of plants (2) was employed. 2-3 cm segments of shoots containing an axillary bud were surface-sterilized in 10% Chlorox for 10 min and rinsed several times in sterile distilled water. They were planted on MS or WPM agar medium containing 1.0 mg/l of kinetin (K) or 4.0, 10.0 or 20.0 mg/l of 6-benzylaminopurine (BAP). In some experiments activated charcoal (0.2 or 0.5%, w/v) was added to the medium.

4. RESULTS

4.1 General growth response. The two species of mahogany, viz., *S. mahogani* and *S. macrophylla* essentially showed similar response to the culture conditions, viz., media, growth substances, etc. Also, the plant materials did not show any specific difference in response to the two culture medium, viz., MS or WPM. Therefore, most of the experiments were carried out with MS medium only.

4.2 Response of cotyledons. Explants of seeds without seed coat, viz., cotyledons with embryo when transferred to the culture medium produced callus within 2-3 weeks. The cotyledons exposed directly to the agar surface produced large proliferations of callus within 6 weeks (Fig. 2). These proliferations appeared soft and very white in color and further subcultures resulted in a loose "snow-flake" appearance. Microscopic observations of these cells revealed cells with very active protoplasm and contents. The cells appeared elongated ranging from 15-100 μ in length. The tissues were very loose; but attempts to initiate liquid cultures from these were not successful.

Callus initiation and further growth occurred in media containing combinations of 2,4-D (1.0, 2.0 or 5.0 mg/l) + kinetin (1.0 mg/l) or combinations of 2,4-D (2.0 mg/l) + NAA (1.0 mg/l). Subsequent routine subcultures of the callus has been carried out only on a culture medium containing 2,4-D (2.0 mg/l) + NAA (1.0 mg/l).