CALLUS FORMATION FROM LEAVES OF CASSAVA, *MANIHOT ESCULENTA* CRANTZ*

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SUMÁRIO

Estudos para a determinação das concentrações ideais de 2,4-D (ácido 2,4-diclo-fenoxiacético), visando a formação de calo e uma futura regeneração da planta de mandioca (*Manihot esculenta* Crantz), foram desenvolvidos a partir de segmentos de suas folhas, na Universidade do Arkansas, Fayetteville, Arkansas, USA. O meio de cultura foi uma modificação daquele idealizado por Murashige e Skoog (1962), o qual continha 2% sacarose, 0.6% agar, 0.1 mg/l ácido nicotínico, 0.001 mg/l ácido giberélico, 0.005 mg/l benziladenina e uma das quatro concentrações de 2,4-D (1; 0,1; 0,01, e 0,001 mg/l). Após cinco semanas de cultura, foi demonstrado que as concentrações de 1 e 0. 1 mg/l foram melhores para a produção de calo do que as concentrações de 0,01 e 0,001 mg/l.

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1. INTRODUCTION

Cassava, *Manihot esculenta* Crantz, a major starch-calorie-production crop in the tropics, has potential as a major industrial crop in terms of commercial energy production in the future. It is vegetatively propagated in commercial use and in milpa agriculture, but it can be hybridized by standard breeding procedures. Interspecific hybrids, however, have met with little success. The monoecious plants of *manihot* sp. often fail to synchronize receptivity with anthesis, and pollen-storage attempts to date have not been successful.

There is merit in hybridizing *M. esculenta* and *M. glaziovii* to exploit the resistance of the latter to cassava bacterial blitht, *Xanthomonas manihotis* (Arthaud-berthet) Starr. Earlier attempts to make interspecific crosses between these two species have not yielded expected recombinants because of barriers that appear to protect the integrity of the separate species. Recent advances in plant cell and protoplast culture have drawn considerable attention because they form the basis of a novel technology to induce and recover agronomically desirable mutations, to

rapidly screen naturally occurring variability and to extend the range of plant hybridization beyond the bounds of sexual compatibility (Bajaj, 1974). The regeneration of potato plants from leaf cell protoplast was reported by Shepard (1982) as a new approach to cloning or asexual propagation. The method yields variants that promise future crop improvements. Hybrids have been developed from fused protoplasts isolated from cell cultures of a male fertile cultivar of *Nicotiana tabacum* L. and from three different male sterile cultivars (Bonnett and Glimelius, 1982).

The purpose of this note is to report the development of callus in *Manihot esculenta* as a prerequisite to morphogenesis and, ultimately, to somatic hybridization.

2. MATERIALS AND METHODS

The Cassava stems were obtained from the Department of Agronomy, University of Florida. The plants were developed in our greenhouse at 22°C and 60% humidity. Young leaves were excised and were treated in 70% ethanol for 20 seconds and then held in a 0.25% sodium hypochlorite solution for 20 minutes. These then were washed thoroughly several times with sterile distilled water. These leaves were further excised into 7 to 10 mm sections and were placed on a solid modified Murashige and Skoog (1962) medium with 2% sucrose, 0.6% agar, 0.1 mg/l nicotinic acid, 0.001 mg/l GA_3 (gibberellic acid), 0.005 mg/l benzyladenine and one of four concentrations of 2,4-dichloro-phenoxyacetic acid (1, 0.1, 0.01, and 0.001 mg/l). They were maintained under a photoperiod of 12 hours with 300 ft-c, 40% relative humidity, and 27°C day and 25°C night temperatures, Ten replicates were made for each 2,4-D concentration. The objective was to determine which level of 2,4-D would give acceptable callus production under these conditions.

Figure 1 — An actively growing callus of Cassava, *Manihot esculenta* Crantz, showing early development of an adventitious shoot.

Figure 2 — An actively growing callus of *Manihot esculenta* Crantz showing early development of an adventitious root that appeared some three weeks after the shoot appeared as shown in Fig. 1.

3. RESULTS AND DISCUSSION

After 10 days, callus formation was observed in the 1 mg/l and 0.01 mg/l 2,4-D concentration media and, at the end of five weeks, considerable amounts (initial weight: 0.026 g, five-week weight: 0.578 g) of callus were produced from the concentrations of 1 and 0.1 mg/l 2,4-D media (Figure 1). The concentrations of 0.01 and 0.001 mg/l 2,4-D media resulted in very little tissue that could be identified as callus. It was therefore concluded that the concentration of 2,4-D at the level of 1 to 0.1 mg/l was better than the concentration of 0.01 to 0.001 mg/l 2,4-D media. The callus produced in this way is now being maintained for morphogenesis. An adventitious root can be seen in Figure 2.

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