EFFECT OF GIBBERELLIC ACID ON *IPOMOEA BATATAS* REGENERATION FROM MERISTEM CULTURE[1]

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ABSTRACT - The elongation of the explants is one of the crucial aspects in developing protocols for regeneration of sweet potato from meristem culture. In order to promote elongation and accelerate explant development, gibberellic acid (GA3) was added to the culture medium. Experimental results were strongly influenced by the cultivars employed. The effect of GA3 was to promote multiple shoot growth and elongation of cv. Mãe de Família; on the other hand, the addition of GA3 had no effect on explant growth in cv. Coração Alado.

Index terms: sweet potato, micropropagation, gibberellic acid.

EFEITO DO ÁCIDO GIBERÉLICO SOBRE A REGENERAÇÃO DE *IPOMOEA BATATAS* A PARTIR DE CULTURA DE MERISTEMAS

RESUMO - O alongamento dos explantes é uma das etapas críticas no desenvolvimento dos protocolos para regeneração de batata-doce a partir da cultura de meristema. Para estimular o alongamento e acelerar o desenvolvimento dos explantes, foi adicionado ao meio de cultura ácido giberélico (AG3). A utilização de duas cultivares distintas mostrou clara influência dos genótipos no resultado experimental. A adição de AG3 ao meio de cultura possibilitou não somente o alongamento do meristema apical mas também aumento do número de gemas desenvolvidos por meristema na cultivar Mãe de Família. A cultivar Coração Alado não teve seu desenvolvimento alterado pela adição de AG3.

Termos para indexação: batata-doce, micropropagação, ácido giberélico.

INTRODUCTION

Sweet potato is traditionally propagated vegetatively by vine cuttings. This technique favours the propagation of viral infected material which is less productive than healthy ones. The consequent low productivity discourage the growers to continue the cultivation of such crop.

The species has a great economic importance and is grown in most of the tropical and temperate regions. In Brazil it is the fourth most consumed crop. Sweet potato is a good source of starch, mineral salts and vitamins.

The technique of *in vitro* culture of meristems has been commonly used to obtain disease free plants of a number of species (Hu & Wang 1983). The application of this technique for *Ipomoea batatas* culture has already been described (Alconero et al. 1975, Elliott 1969, Mori 1971, Gama 1988). Plant regeneration from meristems is slow, time consuming and it generally results on the development of only one plant from each meristem cultured (Frison & Ng 1981).

The aim of this work was to acellerate the development and increase the multiplication rate per cultured meristem. The improvement in the technique efficiency will give, as a first consequence, an increase in the number of healthy plants; with this purpose, gibberellic acid was added to the culture medium. The

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gibberellin effects *in vivo* are very well known (Fig & Street 1980), but it has been rarely used in plant tissue culture (Metraux 1987).

MATERIALS AND METHODS

Shoot tips from sweet potato cultivars Coração Alado and Mãe de Famflia used for meristem isolation were obtained from branches with about 50 cm grown in tubers mantained in the dark. The tubers were kept in a plate with water, in a growth chamber at a temperature of 25 ± 2ºC, for a period of one to three months; tips could be excised after the first month. The explants were surface sterilized as follows: the shoots with about 5 cm were dipped for 20 min in 0,2% tween 80, washed with sterile water; dipped in sodium hipochlorite 2% for 20 min and rinsed three times with sterile water. In the laminar flow cabinet the shoots were dipped in ethanol 70% for one minute, and again rinsed three times with sterile water. Under stereoscopic microscope the meristem plus 1 or 2 leaf primordia were isolated from the shoot tips.

The explants were cultured on solid medium with salt nutrients as prescribed by Murashige & Skoog (1962) supplemented with 100 mg/l myo-inositol, 0.5 mg/l thiamine and 3% sucrose (MS). Growth regulators were added at the following concentration: 0.5 mg/l Kin (Kinetin) and 0,2 mg/l IAA (Indole-3-acetic acid) as described by Alconero et al. (1975). GA3 (40 mg/l) was added or not to this medium. All media were sterilized by autoclaving for 15 min at 120ºC and solidified with 0.8% agar. The pH was ajusted to 5.7 prior to autoclaving.

Explants were cultured in Petri dishes until the development of more than three buds, when they were transfered to glass recipients with 15 ml of solid medium.

The cultures were kept in the dark for the first week; in the second one, they were submitted to photoperiod of 16 h, till the end of the experiment. The light intensity was of 2,500 lux, supplied by fluorescent bulbs Gro lux, and the temperature was 25 ± 2ºC.

After six weeks in culture, the regenerated shoots bearing more than three axilary buds were micropropagated by cutting them in 2 cm long segments with at least one bud. These segments, as the callus with the remaining buds, were transfered to MS medium without growth regulators; they were kept in growth chamber in the same conditions of light and temperature as above described.

RESULTS AND DISCUSSION

The described desinfection methodology made possible the utilization of all excised meristems, as none of them was lost by contamination.

In the medium (MS) supplemented with 0.5 mg/l Kin and 0.2 mg/l IAA, the cultivars Mãe de Famflia and Coração Alado had the same development pattern characterized by the formation of green calli; it was not observed any differentiation (Fig. 1). Consequently, this medium was considered inadequate for the multiplication of cultivars under experimental tests.

Responses of both cultivars to the media supplemented with 40 mg/l GA3 were distinct. The addition of GA3 to the medium had no influence on the growth of cultured meristems of cv. Coração Alado; the plant was not regenerated and only calluses were observed. On the other hand, cultured meristems of cv. Mãe de Famflia formed multiple shoots (2-7 shoots per meristem) and elongated quickly. The length of the induced shoots varied

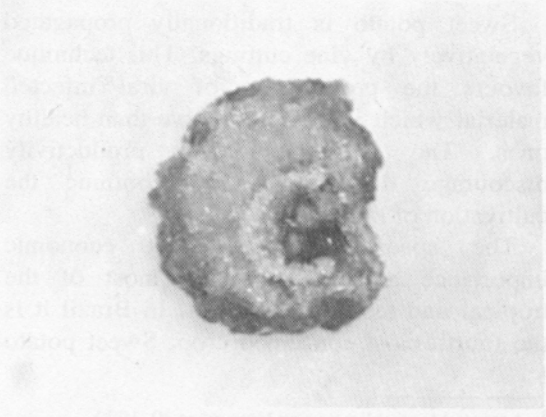

FIG. 1. Callus developed from meristem of cv. Mãe de Famflia cultured in Murashige & Skoog (MS) medium supplemented with 0.2 mg/l IAA plus 0.5 mg/l Kin; 45 days after inoculation.

greatly, from 3 mm to 2 cm. Poorly developed leaves were also observed (Fig. 2). The results for cultivar Mãe de Famílía are contradictory to those obtained for hyacinth, chicory and tobacco, where gibberellin is described as inhibiting adventitious shoot formation (Pierik 1987).

Rey & Mroginski (1985) described a 50% increase in the number of regenerated meristems from *Ipomoea batatas* when GA3 was added to the medium. However, they only observed the regeneration of one plant per meristem and not a multiple shoot regeneration as reported here.

Nodal segments with one axilary bud on MS medium without growth regulators produced roots after the first week of culture. The buds developed shoots with expanded leaves morphologically similar to those plants grown *in vivo*. After three weeks of culture in that medium it was observed the complete development of new plants (Fig. 3).

After the excision of axilary buds, the basal region grown arround the meristem explant (Fig. 4) kept budding as the above micropropagation described, when transfered to MS medium without growth regulators.

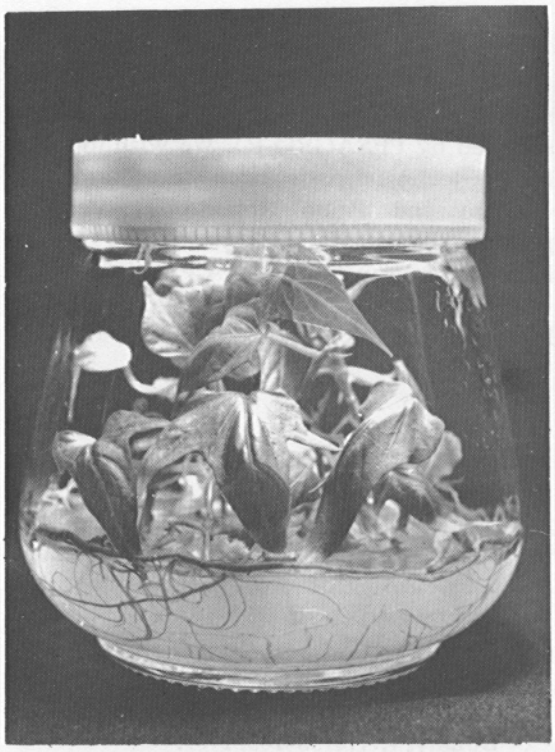

FIG. 3. Cultivar Mãe de Famílía regenerated from meristem, 30 days after transference of axilary buds to MS without growth regulators.

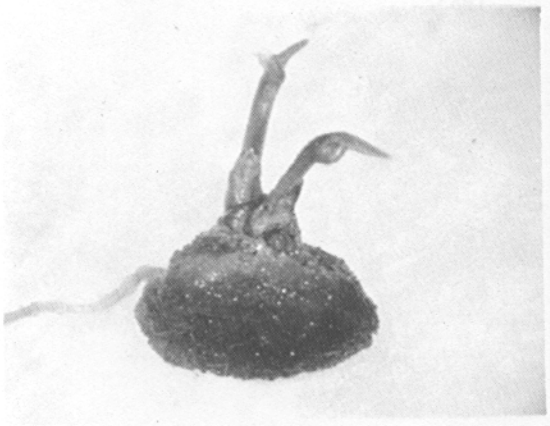

FIG. 2. Shoots formed by meristem of cv. Mãe de Famílía cultured in MS plus 0.2 mg/l IAA plus 0.5 mg/l Kin plus 40 mg/l GA3, 25 days after inoculation.

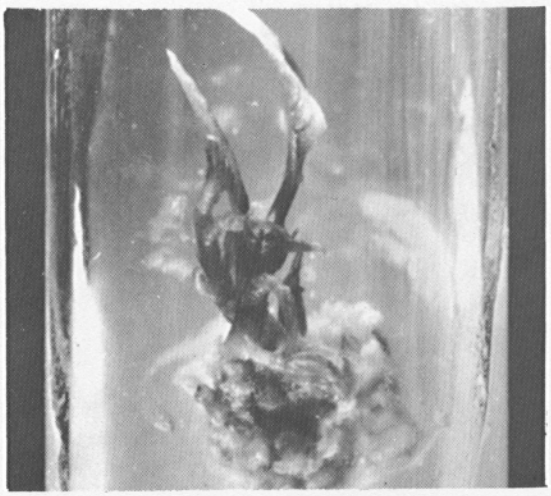

FIG. 4. Further development of buds in remaining callus from the first micropropagation series.

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