Bud Source, Asepsis and Benzylaminopurine (BAP) Effect on Yacon (*Polymnia sonchifolia*) Micropropagation

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Abstract

The yacon (*Polymnia sonchifolia*) is used largely for the high fructan content of its tubers; consequently, it is a good alternative for diabetics. One of the more important restricting factors of the commercial production of yacon is its susceptibility to nematode attack. This, as well as germplasm bank maintenance, justifies the importance of in vitro propagation of this species. In this way, our work aimed to verify the best asepsis method for yacon for the in vitro establishment from the rhizophore and the axillary buds of the aerial parts, and the effect of benzylaminopurine (BAP) addition to the culture medium. The number of contaminated cultures, the occurrence of phenolic oxidation and the occurrence of a vitreous aspect, showed differences with bud source, immersion time for asepsis, and BAP use. The results contribute to establishing a yacon micro propagation procedure.

INTRODUCTION

Polymnia sonchifolia belongs to the Asteraceae (Compositae) family, and grows commonly in the Andes region (National Research Council, 1989). It was introduced to Brazil in 1991, in Capão Bonito Country, São Paulo State (Kakihara et al., 1997) and gave rise to economic potential because of the high fructan content of its tubers and its application in phytotherapy. The tubers contain inulin and are, therefore, a good alternative for diabetics (Lizarraga et al., 1997). The tubers are consumed naturally or processed, and the leaves, following drying, are consumed as tea (Vilhena et al., 1998).

In the field, growers encounter difficulties in adapting their usual knowledge to yacon cultivation, although, satisfactory economic results are obtained.

The extension services are supporting crop development and have identified the major problem in crop loss as the root-knot nematode (*Meloidogyne incognita*). It is estimated that a 30% increase in production is possible with the utilization of effective disease free „seed pieces", as the first step towards a good yield.

Tissue of numerous plant species have been cultured in vitro and became, consequently, free of pathogens (Hollings, 1965). The purpose of this study was to develop a technique for rapid clonal propagation of yacon by tissue culture, by evaluating a bud source, immersion time for asepsis and the effect of BAP addition to the culture medium.

MATERIALS AND METHODS

The experiment was carried out in the micro propagation laboratory of the Plant Production Department of the Agronomic Science College of UNESP in 1999. Buds, which emerged on rhizophores, were excised and washed with running water for five minutes; the same procedure was applied for shoot axillary buds. Disinfection was performed with a 20% aqueous solution of sodium hypochlorite, with two different immersion times, either 20 (treatment A) or 40 min (treatment) B. Twenty rhizophores and twenty-shoot axillary buds were submitted to treatment A, and the same number of buds were submitted to treatment B. All buds were placed on Murashige and Skoog medium (1962) with complete strength macronutrients and supplemented with 2 mg L^{-1}

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Eds. J. Bernáth et al.
Acta Hort. 597, ISHS 2003

benzylaminopurine (BAP), 100 mg L^{-1} inositol, 1g L^{-1} thiamine HCl, 6 g L^{-1} agar and 20 g L^{-1} sucrose. The pH of the medium was adjusted to 5.8 before autoclaving (Krajewska et al., 1987, 1989; Balvanyos et al., 2000). The culture was established in culture tube glasses (2,5 x 15,0 cm). A completely randomized block was applied. Cultures were evaluated 20 and 40 days after the beginning of the experiment, for numbers of developing buds (callus or shoots), numbers of contaminated cultures, the occurrence of phenolic oxidation and the occurrence of a vitreous aspect.

RESULTS

In the cultures established from rhizophore buds, we have observed in A (20 min. immersion in 20% aqueous solution of sodium hypochlorite) and B (40 min. immersion in 20% aqueous solution of sodium hypochlorite) treatments a high fungal and bacterial contamination in the first evaluation. The remaining cultures were eliminated in the second evaluation because they exhibited phenolic oxidation. In the cultures established from shoot axillary buds with treatment B, we have observed high phenolic oxidation, although, in treatment A the buds showed callus formation and, after replication in medium without BAP, which led to a vitreous appearance, satisfactory development was obtained.

DISCUSSION

Buds excised from the rhizophore show a high bacterial and fungal contamination ratio, in all disinfection treatments, suggesting the presence of endogenous microorganisms associated with yacon subterraneous parts. The endogenous microorganisms, in many cases, defy concentration and immersion in disinfectant solutions (Grattapaglia et al., 1998), although the drive to use high sodium hypochlorite concentrations induces phenolic oxidation.

Cultures established from shoot axillary buds show a low contamination ratio in disinfection treatment A. However, callus development with a vitreous aspect occurs (Fig 1.A.). Gaspar (1986) defines a vitreous aspect in some *in vitro* developing plants as a morphological response to stress conditions. This abnormality is frequently related to plant physiological alterations due to high cytokinin concentration. Fig.1.B shows a good developing yacon plant after replication in medium without BAP.

Shoot axillary buds submitted to disinfection treatment B exhibited phenolic oxidation. This indicates a sensibility of yacon buds to 40 min. immersion time in a 20% sodium hypochlorite aqueous solution and, if a long immersion is necessary, the results suggest research is needed on the addition of an anti-oxidant to the medium.

This work presents a viable procedure for in vitro propagation of yacon, suggesting shoot axillary buds as ideal explants, and 20 min. immersion time in 20% sodium hypo- chlorite aqueous solution a safe disinfection procedure. According to our results, BAP addition to the culture medium will be unnecessary, and can induce a vitreous aspect.

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Tables

Table 1. Bud source and asepsis during in vitro development of yacon explants

	20 days after beginning					40 days after beginning		
Treatments	**DE**	**DEAP**	**CA**	**OE**	**BCE**	**FCE**	**OE**	**DE**
1A	3 a	2 a	1 a	0	12 a	5 a	3 a	0
1B	7 b	5 b	2 b	0	7 b	6a	7 b	0
2A	15 c	15 c	0	0	5 bc	0	0	15 a
2B	9 b	9 d	0	7	4 bc	0	0	9 b
F	9.33*	12.70*	7.21*	0	0.25*	0	4.82*	17.85*

***** Means within a column followed by different letters are significantly different according to the protected least significant difference test at Tukey ($p<0,05\%$).

1A:rhizophore buds submitted to A asepsis treatment; 1B:rhizophore buds submitted to B asepsis treatment; 2A:shoot axillary buds submitted to A asepsis treatment; 2B:shoot axillary buds submitted to B asepsis treatment; DE:developing explants; DEAP:developing explants with aerial part; CA:callus; OE:oxidized explants; BCE:bacterial contaminated explants; FCE:fungal contaminated explants.

Figures

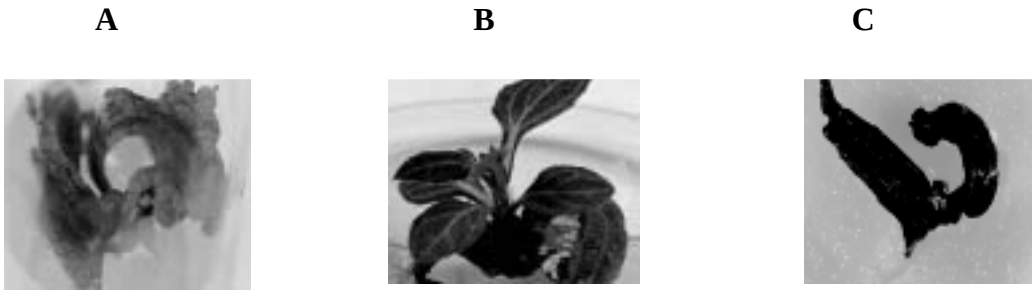

Fig.1. Tissue culture of *Polymnia sonchifolia.*
A: callus development with vitreous aspect. **B:** yacon plant with normal development after replication in medium without BAP. **C:** phenolic oxidation observed in shoot developed from rhizophore bud and submitted to 40 min. immersion in 20% aqueous solution of sodium hypochlorite.