



In Vitro Tuberization in Genotypes of Cassava

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Abstract

The objective of this research was to induce in vitro tuberization in two cassava genotypes grown in liquid media with addition of 6-benzylaminopurine (BAP) and α -naphthaleneacetic acid (NAA) in different concentration of sucrose. Nodal segments (3 cm height) from the cultivars Mantiqueira and Parazinha were used as explants and kept in vitro MS media containing also mio-inositol (100 mg L⁻¹) and sucrose (30 g L⁻¹). The pH of the media was adjusted to 5.7 ± 0.1 before autoclaving. The root inducing media was composed by MS media plus 30 g L⁻¹ sucrose (T1), MS plus 0.4 μ M BAP + 1.6 μ M NAA, in different concentrations of sucrose including 30 (T2), 60 (T3) and 80 g L⁻¹ (T4), and MS media supplemented with 22 μ M BAP, grown in 30 (T5), 60 (T6), 80 g L⁻¹ sucrose (T7). The experiments were undertaken at 50 to 60 μ mol light irradiance for eight hours at 27 ± 1 °C. Number, length and pattern of the induced tuber roots were recorded. The length and number of roots were higher for T1, especially in Mantiqueira, but no formation of tuberous roots was observed. Tuber roots was observed after using T4 in both cultivars.

Resumo

O presente trabalho teve como objetivo a indução in vitro de raízes tuberiformes de dois genótipos de mandioca cultivadas em meio líquido, sob a ação de 6-benzilaminopurina (BAP) e ácido α -naftalenoacético (ANA), e diferentes concentrações de sacarose. Como explantes foram utilizados segmentos nodais, com cerca de 3 cm de comprimento, excisados de plantas de mandioca das cultivares Mantiqueira e Parazinha, mantidas in vitro mediante subcultivos em meio constituído dos sais de MS, mio-inositol (100 mg L⁻¹) e sacarose (30 g L⁻¹). O pH dos meios foi corrigido para 5,7

$\pm 0,1$, antes da autoclavagem. Os meios de indução constaram do meio MS com 30 g L⁻¹ de sacarose (T1), MS suplementado com 0,4 μ M BAP + 1,6 μ M NAA, sob diferentes concentrações de sacarose 30 (T2), 60 (T3), 80 g L⁻¹ (T4), e MS com 22 μ M de BAP também sob diferentes concentrações de sacarose 30 (T5), 60 (T6) e 80 g L⁻¹ (T7). Os experimentos conduzidos sob regime luminoso de 16 horas escuro e 8 horas diárias de luz à temperatura de 27 \pm 1oC e irradiância de 50-60 μ mol m⁻² s⁻¹. Foram avaliados para a indução das raízes tuberiformes o número, comprimento e do padrão de desenvolvimento das raízes. O T1 foi o melhor tratamento para as características comprimento da raiz (CR) e número de raízes (NR), principalmente para a cultivar Mantiqueira, no entanto, não foi constatada microtuberização. Já no T4, o desenvolvimento das raízes tuberiformes foi mais eficiente, apresentando uniformidade tanto no aspecto, como na textura em ambas as cultivares.

Introduction

Cassava is a perennial plant originated from South America, which stores starch in a storage taproot. This dicotyledonous plant, belong to the family Euphorbiaceae, and the genus *Manihot* has about 200 species grown mainly in regions with tropical and subtropical climate (Figueiredo 1996). Although the cassava plant cannot be considered a crop with all needed human nutritional requirements, its storage roots are among the most important sources of cheap carbohydrate for developing countries (Junqueira 2001). Cassava is the third most common source of carbohydrate in the tropics, after rice and maize. Brazil is the world-leading producer, with an annual production of 25 million t (FAO, 2000). Cassava cropping is widespread in most of the Brazilian territory due to its high rusticity and well adapted to different soils and climates. The crop is usually grown by small farmers. In most of the growing regions the productivity is still low, mainly due to the low technological level of cultural practices adopted by farmers (Reis 1987).

The cassava is crop restricted to tropical and subtropical regions, which may account for the scanty available literature regarding environmental and endogenous factors involved in the control of the tuberization process. Most of the information available about tuberization was from potato (*Solanum tuberosum* L.). It is well known that the tuberization is a complex process, which is coordinated, by the environment conditions, hormonal control, nutrition and genetics (Villafranca et al. 1998). Potato tuberization is characterized by anatomical modifications, hormone and physiological changes. The use of in vitro growth of plants for production of microtuber has the advantage of higher control of the different factors that might affect the tuber formation, compared to plants grown in soil (Veramendi et al. 1999). Furthermore, by using microtubers it is possible to maintain genebank accessions in a much smaller space, and to remove virus- infection in asexually propagated species (Ranalli et al. 1994, Zobayed et al. 2001). Another advantage of in vitro propagation viz. a viz. conventional methods is the possibility to produce plantlets of asexual propagated plants in a much large scale in a shorter period, without being affected by field seasonality for producing tubers (Hussey and Stacey 1984, Debon et al. 1998). Previous potato research focused on the influence of growth regulators and photoperiod on the induction of tuberization (Hussey and Stacey 1984, Nowak and Colborne 1989, Villafranca et al. 1998, Silva et al. 2001, Lopez-Delgado and Scott 1997). Short day and sugars influence potato tuberization (Xu et al. 1998, Omokolo et al. 2003). The addition of carbohydrate on the growing media shifts the source/sink ratio affecting the development of shoots and tuber (Peres et al. 2005). Additionally, other factors such as temperature and nitrogen influence tuberization induction of potato (Hussey and Stacey 1984, Ulloa et al. 1997). The objective of this research was therefore to investigate the role of benzylaminopurine, naphthaleneacetic acid and sucrose on the development of tuberous roots in two commercial cassava cultivars grown in vitro using liquid media.

Material and Methods

Nodal segments (3 cm height) were used as explants. They were taken from plants of cv. Mantiqueira, micropropagated in the Tissue Culture Laboratory of UFV/MG. The cv. Parazinha used in the experiment was donated by the EMBRAPA/CENARGEN. All the cultivars were micro-propagated in three consecutive sub-growths for 42 days each. We used MS media (Murashige and Skoog 1962) containing 100 mg L⁻¹ mio-inositol, vitamin complex for the MS media (0.2 g L⁻¹ glycine, 0.05 g L⁻¹ nicotinic acid, 0.05 g L⁻¹ pyridoxin-HCl and 0.01 g L⁻¹ thiamine-HCl), and 30 g L⁻¹ sucrose in solidified 2.3 g L⁻¹ Fitagel (Sigma, USA). The pH of the media was adjusted to 5.7 \pm 1 before placing in autoclave at 115 oC for 30 min. For the tuberization liquid media it was used the MS composition supplemented with 30 g L⁻¹ sucrose (T1), MS media plus 0.4 μ M de BAP and 1,6 μ M NAA (Figueiredo 1996), with different concentrations of sucrose as follow: 30 (T2), 60 (T3) e 80 g L⁻¹ (T4). A MS media with 22 μ M de BAP (Silva et al. 2001) plus 30 (T5), 60 (T6) e 80 g L⁻¹ sucrose (T7) was also tested. The experiments were conducted at light regime conditions of 16 h under light and 8 h at dark in a room illuminated with white fluorescent light kept at 27 \pm 1oC with 50 to 60 μ mol m⁻² s⁻¹ of irradiance.

After 45 days of growth in the tuberization media, the length of aerial portion (LAP), number of roots (NR), length of the largest roots (LR), and pattern of the roots development were recorded. The experimental design was completely random with eight replications per treatment, and each experimental unit was one flask containing two plantlets each.

The data were subjected to the analysis of variance using the software SAEG/UFV (Euclides 1983), and the mean separation was performed using the Tukey test at $P \leq 0.05$.

Results and Discussion

The different treatments, under the light regime for tuberization induction resulted in distinct morphological changes in the plantlets grown after 45 days in liquid media. The treatment with MS0 + 30 g L⁻¹ sucrose (T1) resulted in longer aerial part of the plantlets (LAP), length of the longer roots (LR) as well as number of roots (NR) compared to the remaining treatments, containing BAP, NAA or higher levels of sucrose in the media (Tables 1 and 2).

Table 1. Influence of the media composition on the length of aerial portion (LAP), length of largest roots (LR), and number of roots (NR) 45 days after growth in the tuberization liquid media of cv. Mantiqueira

Treatments	LAP	LR	NR
T1= MS0 + 30 g L ⁻¹ sucrose	25.37 a	14.62 a	21.50 a
T2= 0.4 µM BAP + 1.6 µM ANA + 30 g L ⁻¹ sucrose	11.00 b	5.12 b	7.25 bc
T3= 0.4 µM BAP + 1.6 µM ANA + 60 g L ⁻¹ sucrose	6.12 c	4.75 b	12.50 b
T4= 0.4 µM BAP + 1.6 µM ANA + 80 g L ⁻¹ sucrose	6.25 c	3.50 b	4.50 bc
T5= 22 µM BAP + 30 g L ⁻¹ sucrose	3.37 c	4.00 b	4.62 bc
T6= 22 µM BAP + 60 g L ⁻¹ sucrose	4.50 c	4.37 b	3.75 c
T7= 22 µM BAP + 80 g L ⁻¹ sucrose	3.87 c	5.12 b	5.25 bc

Means separation within the columns was done by the Tukey's studentized range test at $P \leq 0.05$.

Table 2. Influence of the media composition on the length of aerial portion (LAP), length of largest roots (LR), and number of roots (NR) 45 days after growth in the tuberization liquid media of cv. Parazinha

Treatments	CPA	LR	NR
T1 = MS0 + 30 g L ⁻¹ sucrose	12.82 a	5.29 a	7.37 a
T2 = 0.4 µM BAP + 1.6 µM NAA + 30 g L ⁻¹ sucrose	8.57 bc	0.76 b	3.12 ab
T3 = 0.4 µM BAP + 1.6 µM NAA + 60 g L ⁻¹ sucrose	8.21 bc	0.47 b	1.25 b
T4 = 0.4 µM BAP + 1.6 µM NAA + 80 g L ⁻¹ sucrose	9.21 ab	1.52 b	2.37 b
T5 = 22 µM BAP + 30 g L ⁻¹ sucrose	5.04 c	1.82 b	3.12 ab
T6 = 22 µM BAP + 60 g L ⁻¹ sucrose	6.35 bc	1.86 b	4.62 ab
T7 = 22 µM BAP + 80 g L ⁻¹ sucrose	5.09 c	2.05 b	5.62 ab

Means separation within the columns was done by the Tukey's studentized range test at $P \leq 0.05$.

The MS0 + 30 g L⁻¹ sucrose (T1), which favored mostly leaf and root growth, did not induce any tuberization in either cultivar, and influenced mainly the vegetative growth without affecting the secondary growth in the roots. Furthermore, these effects were higher in Mantiqueira than in Parazinha (Table 3). The former cultivar was affected by the MS0 + 30 g L⁻¹ sucrose (T1) in extending the length of the leaves and roots, and the number of roots (Table 3).

Table 3. Development of cassava cvs. Mantiqueira and Parazinha grown in MS media supplemented with 30 g L⁻¹ sucrose

Cultivars	LAP	LR	NR
Mantiqueira	25.37 a	14.62 a	21.50 a
Parazinha	12.82 b	5.29 b	7.37 b

Means separation within the columns was done by the Tukey's studentized range test at $P \leq 0.05$. LAP: length of the aerial portion; LG: length of the largest roots; NR: number of roots.

The treatment containing 0.4 μM BAP + 1.6 μM ANA + 80 g L⁻¹ sucrose was the most effective to induce tuberous roots in cassava, which was well differentiated after 45 days of growth in the inducing media, and observed only in Parazinha (Fig. 1). This cultivar changed its pattern of growth compared to Mantiqueira. The former had significant smaller growth of aerial portion and roots (Table 3), which led to the initiation of tuberous roots 20 to 30 days after being under tuber inducing conditions. These favorable conditions for tuberous root initiation included exposure to cytokinin, auxin, high content of sucrose, and in a short dark environment. The most effective treatment for cassava had a small amount of BAP, and requires NAA, but with a similar high concentration of sucrose (Fig. 1). Similar results showing the interaction between cytokinin, auxin and sucrose were observed by Estrada et al. (1986) working with the induction of microtubers of potato. Most of the literature indicates that high sucrose levels are needed for potato tuberization (Hussey and Stacey 1984, Ranalli et al. 1994). Cytokinins play an important role in creating the sink during plant development, and through regulating the expression of a gene involved in the partition of assimilates towards the stolons as observed in potato (Prat 2004).

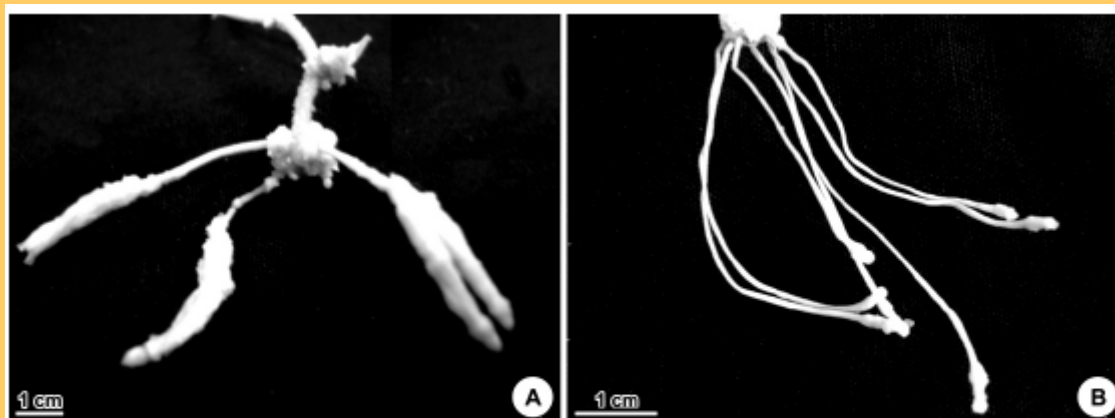


Fig. 1 – Root development in cvs. Parazinha (A) and Mantiqueira (B) cultivated in tuberization inducing media MS supplemented with 0.4 μM BAP + 1.6 μM NAA + 80 g L⁻¹ sucrose 45 days after growth

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