Effect of Various Factors on Minimal Growth in Tissue Culture Storage of Cassava Germplasm

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ABSTRACT

Several experiments evaluated influence of various factors in tissue culture storage on growth rate and viability of cassava germplasm stored in vitro. Cultivars responded differently to low temperature; thus use of this factor alone was not completely suited for adequate storage. Osmotic concentration, due to presence of mannitol, was only effective if sucrose was also present in the medium and only at 22°C storage. Inclusion of activated charcoal reduced shoot growth rate and increased culture viability, however. In addition, intermediate levels of nitrogen supported viability at 22°C, and larger storage vessels promoted propagation potential of the cultures. It was determined that sufficient growth limitation and high viability could be achieved through storage at 22°C to 24°C and by using media with low osmotic concentration, activated charcoal, and large vessels. Furthermore, minimal-growth storage could be linked to cryogenic techiques as a system for long-term conservation and international exchange of germplasm.

Introduction

Conventional maintenance of cassava germplasm in the field often exposes the materials to various pests and diseases and to soil and climatic problems. Requirements of land, labor, and other inputs are also high. Stakes can be kept only for a short period due to premature sprouting and insect or microbial attack; in addition, stakes might harbor systemic contaminants. Preservation of varieties by seed propagation may be limited by low or no-seed setting in numerous accessions, strong inbreeding depression, and high genetic segregation. Thus a large number of seed must be collected to preserve the nonfixed alleles of an accession (IBPGR, 1982). Early recognition of these problems stimulated research on tissue culture methods for conservation and international exchange of cassava germplasm.

The ability to consistently regenerate plants from somatic cells in culture is still lacking in cassava. However, methodologies are available to grow plants to maturity from apical meristems of a wide range of germplasm (Roca et al., 1982a). Shoot apical meristem cultures are particularly suited for germplasm conservation, because of their freedom from microorganisms, small space requirements, and high genotype stability.

Limiting culture growth, primarily by means of reduced temperature, has increased the transfer period of cassava clones in vitro (Roca et al., 1982a) by 18

to 24 months, depending upon cultivar (CIAT, 1981). However, temperature stress resulted in culture deterioration in some genotypes (Roca et al., 1982b).

This report summarizes results of experiments to evaluate effects of various physical and chemical factors on rate of growth and the viability of cassava cultures stored in vitro.

Materials and Methods

Single node cuttings of two varieties obtained from meristem-cultured plantlets were used as explants for the storage experiments. Unless otherwise stated, cultures were stored in Murashige and Skoog (1962) medium with each liter supplemented with 3% sucrose, 1.0 mg thiamine, 100 mg inositol, 0.02 mg 6-benzyladenine, 0.1 mg gibberellic acid, and 0.01 mg naphathalene acetic acid. Test tubes were 25x150 mm with an 0.8% Difco agar (Roca et al., 1982a) and were placed under 1,500 lux illumination, with a photoperiod of 12 hours. Rate of shoot elongation, proportion of leaf fall, number of shoots per culture, and number of nodes formed on each shoot were determined, with six to eight replications per treatment, after 8 to 9 months of storage.

The results by variety were either averaged or presented separately, depending on whether or not the cultivars' responses were similar or different, respectively.

Results

Effect of temperature

Storage temperatures lower than 20°C were detrimental to culture survival of many cassava varieties (Roca et al., 1982b). In this experiment, the cultures were stored at 18°, 22° and 30°C, under illuminations of 500, 1,000, and 2,000 lux.

Shoot elongation was directly related to storage temperature in both cultivars, and M Col 1467 grew faster than M Col 22 in all the treatments. Illumination had less effect on growth and viability than temperature. Although the number of shoots of both cultivars increased to 4-6 per culture, with storage temperature, irrespective of illumination, the number of nodes formed per shoot remained either unchanged in M Col 22 or slightly decreased in M Col 1467 when the cultures were maintained at the higher temperature. Thus, viability increased with temperature in M Col 22, but decreased at 22° and 30°C in M Col 1467.

Effect of osmotic concentration

Although earlier studies showed that high sucrose levels reduced shoot growth, enhanced rooting, and caused phenolic deterioration of some cassava genotypes in storage (Roca et al., 1982a), it was suggested that metabolically inactive osmotica could be more effective than sucrose in limiting culture growth. Thus, nodal cuttings were cultured in three levels of sucrose, combined with four of mannitol, and kept at 22° and 30°C.

At both storage temperatures, mannitol was completely detrimental to culture survival in the absence of sucrose. However, in the presence of 3% sucrose, the addition of minimum levels of mannitol greatly increased the number of shoots per culture and nodes formed per shoot, although it did not change shoot elongation

rates. Shoot elongation increased with 0.25% mannitol, whereas viability did not, however, both decreased at the highest mannitol level. With 6% sucrose, only the lowest mannitol concentration resulted in slow growth at 22°C and much higher viability. Higher mannitol levels however, greatly reduced shoot growth and viability at 22°C, but not at 30°C.

Effect of nitrogen concentration

The nodal cuttings were explanted in media containing 0 to 60 mM of total nitrogen, based in the Murashige and Skoog medium (1962), and stored at 22° and 30°C (Table 1).

Table 1. Effect of nitrogen concentration on shoot elongation and viability of cassava cultures stored in vitro at two temperatures.

Temperature (°C)	Total N (mM)	Shoot elongation (cm/month)	Leaf fall (%)	Viability	
				Shoots per culture (no.)	Nodes per shoot (no.)
22	0	0.2	20	1.0	6.0
	10	0.3	65	2.0	12.0
	20	0.4	85	2.0	13.5
	40	0.6	75	1.0	20.0
	60	0.4	50	3.0	23.0
30	0	0.2	50	1.0	6.0
	10	0.3	60	2.0	15.0
	20	0.5	70	2.0	17.5
	40	0.6	80	5.0	18.0
	60	0.8	60	6.0	17.0

At either temperature, rate of shoot elongation was directly related to concentration of nitrogen in the medium, becoming detrimental at 0 and 10mM, as shown by predominance of single shoot cultures with very few nodes per shoot. Slow growth rates and high viability were obtained at 22°C with 40 to 60 mM of total nitrogen, and at 30°C with 10 to 20 mM. With 60 mM total nitrogen, the growth rate was too high in the cultures maintained at 30°C, but was low enough at 22°C (Table 1).

Effect of activated charcoal

Deterioration of cultures stored at 20° to 22°C was previously characterized by root browning, defoliation, and dehydration (CIAT, 1981). The inclusion of 4% to 6% sucrose accentuated browning, whereas the increase in cytokinins and gibberellins tended to reduce culture deterioration during storage (Roca et al., 1982b). To further test the reduction of deterioration, nodal cuttings were stored at 22° and 30°C in media containing 0.25% activated charcoal (Table 2).

The addition of charcoal to the medium not only reduced defoliation in both varieties, but also nearly halved shoot growth in M Col 22, without changes in the viability of the cultures stored at both temperatures (Table 2). The cultures retained more leaf chlorophyll and had less browned roots than without charcoal.

Table 2. Effect of activated charcoal on shoot elongation and viability of cassava cultures stored in vitro at two temperatures.

Temperature (°C)	Activated charcoal (%)	Leaf fall (%)	Shoot elongation (cm/month)	Viability	
				Shoots per culture (no.)	Nodes per shoot (no.)
M Col 22					
22	0.00	50	1.5	1.7	17.0
	0.25	30	0.8	1.5	23.0
30	0.00	45	1.2	2.0	13.5
	0.25	40	0.8	2.3	11.5
M Col 1467					
22	0.00	65	2.4	2.0	12.0
	0.25	50	2.2	3.0	13.0
30	0.00	60	2.3	4.0	9.5
	0.25	40	2.1	4.0	8.7

Effect of culture vessel size

Leaf contact with the vessel inner surface had earlier been shown to result in bleaching and leaf drop (CIAT, 1981). To try to prevent such contact, the nodal cuttings were cultured in test tubes of 25x150 mm and bottles of 50x140 mm in size, containing 10 and 70 ml of medium, respectively, and maintained at 22° and 30°C (Table 3).

Table 3. Effect of vessel size on shoot elongation and viability of cassava cultures stored in vitro at two temperatures.

Temperature (°C)	Size of vessel (mm)	Shoot elongation (cm/month)	Leaf fall (%)	Viability	
				Shoots per culture (no.)	Nodes per shoot (no.)
22	25 x 150 (10 ml)	1.0	75	9.2	8.0
	50 x 140 (70 ml)	1.8	50	17.0	10.5
30	25 x 150 (10 ml)	1.2	60	8.0	9.6
	50 x 140 (70 ml)	2.1	35	10.0	9.8

At either storage temperature, rate of shoot elongation in large vessels almost doubled; however, leaf fall diminished and culture viability greatly increased, especially at 22°C (Table 3). In addition, leaves and roots remained healthier in the large than in the small vessels.

Effect of growth inhibitors

Among the growth inhibitors, abscisic acid has effectively slowed down growth in other species (Roca et al., 1982b). To test its effect on cassava, 2.5 through 10.0 mg/l of abscisic acid were added to the storage medium. Culture growth was completely suppressed even at the lowest concentration, at both 22° and 30°C. However, the shoots did not deteriorate completely as expected, but rather remained green.

Discussion

An in vitro conservation method largely depends on achieving sufficient growth limitation and maximal viability. This study has demonstrated that temperature, osmotic and nutritional stresses, and the cassava genotype can influence the rate of growth and the viability of the cultures during storage. Cultivars such as M Col 1467 grow faster at reduced temperature than do others such as M Col 22. Because M Col 1467 prevails in cooler growing regions, it would probably require more frequent subculturing. Lower storage temperatures than 20°C would drastically reduce viability in varieties such as M Col 22. It seems, therefore, that reduced temperature alone will not completely provide adequate storage conditions for cassava collections.

Growth limitation due to osmotic stress may be exerted through the reduction of water and nutrient uptake and thus, it may be detrimental to culture survival as observed in this study when mannitol alone was present, even at the lowest concentration. High culture viability occured only if sucrose was added to the medium, at a level directly related to the storage temperature. The increase in viability observed when 0.12% mannitol was added to the 3% and the 6% sucrose media, remains without explanation.

Low nitrogen concentration delayed vegetative growth more at 22° than at 30°C; however, intermediate levels of nitrogen, directly related to storage temperature, were needed to support viability, and the lack of nitrogen in the medium was highly detrimental to culture survival.

Low temperature, high osmotic concentration, low mineral nutrition, growth inhibitors, on one hand, and rapid growth and longevity on the other, tend to cause tissue browning, followed by defoliation, and eventual deterioration of the cassava cultures stored in vitro. Browning could be attributed to phenolic oxidation, and gradual shoot deterioration to ethylene-induced senescence, especially when the culture vessels were small or have been tightly capped.

Reduction of defoliation rates and increase in viability of cultures stored in large vessels may be attributed to the dilution of toxic exudates and emanations from the tissues. The increase in culture viability and the reduction in the rate of shoot elongation due to the inclusion of activated charcoal can be explained by the removal of toxic compounds and certain growth hormones through absorbance in the medium. Therefore, sufficient growth limitation and high viability can be obtained through storage at 22° to 24°C with active osmotica at low

concentration, sucrose and activated charcoal included in the media and using large culture vessels.

Nearly 6,000 cassava clonal accessions can be stored in a 5x6x3 m room, using five vessels of 50x140 mm per accession. At present, more than 1,300 cassava cultivars from CIAT's germplasm collection have been transformed into in vitro cultures for storage. Most materials have been maintained for 6 to 24 months, with a total of 0.6 to 1.2 subcultures, respectively. Given the recent advances in the cryopreservation of cassava meristems (Kartha, Leung and Mroginski, 1982), a dual conservation system can be envisaged: the base collection under cryogenic storage, using liquid nitrogen, and the active or working collection, under minimal-growth storage. The latter constitutes a source of materials for quick micropropagation and use in international exchange.

The exchange of cassava clones in vitro reduces the risks of disease dissemination across country boundaries. This is a suitable method for the transfer of genetic resources both from the collection site to the principal germplasm center and from the center to the national programs. In the last 4 years, selected cassava materials have been distributed from CIAT to 15 countries, and over 700 introductions to CIAT have been carried out using in vitro methodologies.

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