SCIENTIFIC NOTE

IMPORTANCE OF ABSCISIC ACID (ABA) IN THE *IN VITRO* CONSERVATION OF CASSAVA (*Manihot esculenta* CRANTZ)

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A B S T R A C T

The conventional technology for *in vitro* plant conservation for cassava (*Manihot esculenta* Crantz) germplasm collections is laborious due to the need for several sub-culturing procedures per year. This practice implies high costs for medium preparation, tissue culture tubes, time-consuming labor, risks of contamination, mislabeling of accession, and the need for large growth chambers. We have developed a new procedure using *in vitro* cultivated nodal axillary buds treated with different abscisic acid (ABA) concentrations to reduce the time for recycling transplants cultivated in a SP basic nutritive medium. Nodal explants were stored for three months with ABA. Plants were obtained after nodal axillary buds were placed in SP medium without ABA. Results indicated that 20 and 30 µM ABA induced bud dormancy and delayed sprouting without affecting subsequent growth of plants after treatment.

Key words: tissue culture, dormancy, *Manihot esculenta*.

INTRODUCTION

In vitro conservation of cassava germplasm collections involves laborious work due to the high frequency of sub-culturing, which is carried out every 60 to 70 d, depending on the access. Alternative procedures have been proposed based on manipulation of environmental factors, such as room temperature, medium composition and alterations in concentration focused on sucrose, mannitol (Roca, 1984) and silver nitrate (Mafla *et al*., 2004). However, results have been variable and accession-dependent. New germplasm accession was recently introduced at the Genetic Resources and Biotechnology of Empresa Brasileira de Pesquisa Agropecuária, Brasilia (EMBRAPA) for conservation that misses information of *in vitro* culture behavior and response. *In vitro* storage is very important because with this technique the troublesome process of maintenance and conservation of genetic resources under field condition can be eliminated. For *in vitro* conservation, the preservation protocols should reflect the objective of achieving a maximum survival rate, as well as genetic stability with minimum subculture frequency (Naidu and Sreenath, 1999).

Given the commercial interest in this genetic material, as well as its rare occurrence as a spontaneous mutant (Carvalho *et al.*, 2000; 2004), there is a need to develop new *in vitro* conservation technology. An alternative approach could be to add hormones as growth retardant agents in SP medium, which is a modified Murashige and Skoog (1962) medium (MS medium) developed by Barrueto Cid (2005) based on the dilution of a macronutrient composition that allows overall nutrient balance, and was applied for cassava (Barrueto Cid *et al*., 2007), and several other species (Barrueto Cid and Durzan, 2003; Barrueto Cid *et al*., 2004). Abscisic acid (ABA) has been reported to show strong pleiotropic inhibitory effects on plant growth and seed embryo germination (Fong *et al*., 1983; Leung and Giraudat, 1998; Lemos, 2005).

Given the properties of ABA, it has been used in tissue culture for different purposes in *Vitis vinifera* L. (Perl *et al*., 1995), *Poa pratensis* L. (Ark *et al*., 1991), several forest trees (Gupta and Kreitinger, 1993), coffee (Naidu and Sreenath, 1999) and *Medicago sativa* L. (Rudús *et al*., 2006). No attempt was found to explore the properties of ABA in tissue culture conservation of cassava germplasm (Unnikrishnan and Sheela, 2000; Fauguet, 2001; Alves, 2004).

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The objective of this experiment was to test the possibility of using ABA at different concentration in SP medium to retard bud sprouting and *in vitro* plant growth, as well as to recover explants.

MATERIALS AND METHODS

The experiments were carried out at the tissue culture facilities of EMBRAPA Genetic Resources and Biotechnology, Brasilia, DF., Brazil, in 2007. *In vitro* plants of sugary cassava were used as donors of experimental nodal axillary buds. All experiments were carried out under standard *in vitro* plant culture conditions: 25 ± 2 °C with 16:8 h photoperiod at a photon flux density of 50 µm m^{-2} s^{-1} from a cool fluorescent tube. *In vitro* plants were standardized for size (50-70 mm long) and used to collect node axillary buds that were cultured in Petri dishes with SP medium supplemented with 0, 5, 10, 20, and 30 µM of ABA. SP basal medium consisted of half-strength MS macrosalt, MS microsalt without IK (mg L^{-1}): $MnSO_4$.$4H_2O$ 22.0, $ZnSO_4$.$7H_2O$ 8.0, H_3BO_3 6.0, Na_2MoO_4 .$2H_2O$ 0.25, $CuSO_4$.$5H_2O$ 0.25, CoCl .$6H_2O$ 0.25 and half –strength MS Fe-EDTA; 1 mg L^{-1} each of: calcium panthotenate, pyridoxine HCl, thiamine HCl and nicotinic acid; inositol 50 mg L^{-1}, sucrose 20 g L^{-1} and Phytagel 2.5 g L^{-1}. The pH was adjusted to 5.8 before autoclaving at 121 °C (0.2MPa) for 20 min.

Five explants from accession CAS36.18 and CAS36.3 were cultured for 90 d and replicated three times. After this period, the explants were allowed to germinate and grow in SP medium without ABA for a period of 45 d. Bud sprouting, *in vitro* plant recover after bud dormancy period, and *in vitro* plant growth were recorded by photo documentation, counting germinated sprouted buds and aerial plant part lengths.

Several preliminary tests were performed before full application of a completely randomized experimental design that was performed once. Explants distribution in the growth chamber bench was randomized twice a week. Data analyses were performed by variance estimation (ANOVA) and the Tukey test for the mean values with significance probability of $P \leq 0.05$. Values of sprouting percentage were transformed to arc sine before estimating the variance.

RESULTS AND DISCUSSION

ABA affects both bud sprout and shoots growth with the same pattern, depending on the concentration. Observations made at 90 d after treatment indicate full inhibition of bud sprouts and shoots growth for ABA concentration greater than 10 µM (Table 1). ABA concentration of 0 and 5 µM ABA showed no significant inhibitory effect on either parameter, but concentrations of 10 µM inhibited bud sprouting and shoot lengths by approximately 4-fold. With 10 µM of ABA, roots were only partially affected (Figure 1B) while shoots showed a 4-fold inhibition (Table 1). However, ABA showed differential effects on shoot sprouts and roots at higher concentrations. While both roots and shoots showed full development in the control (without ABA), the same was not observed at 20 µM of ABA (Figure 1C) and 30 µM (picture not shown). Cumulatively, these results confirmed our initial hypothesis that ABA represses cassava axillary bud sprouting and shoot growth. In addition, it is possible that the response to ABA is not dependent on the cassava genotype.

After 45 d in SP medium without ABA, all explants showed growth and development. Fig. 1D shows plant aspect especially at level of 20 µM, at 30 µM was seen

Table 1. Percentage of axillary bud sprouting and shoot lengths at 90 days of treatment with different ABA concentrations in SP medium on cassava genotypes Cas36.18 and Cas36.3.

ABA (µM)	Cas36.18		Cas36.3	
	Bud sprouting	Shoot length	Bud sprouting	Shoot length
	(%)	(mm)	(%)	(mm)
0	100.0a	31.0a	100.0a	28.0a
5	80.0a	29.0a	83.3a	27.3a
10	26.7b	8.7b	33.3b	9.0b
20	0.0c	0.0c	0.0c	0.0c
30	0.0c	0.0c	0.0c	0.0c

Same letter in a column indicates no significant difference according to the Tukey test ($P \leq 0.05$).

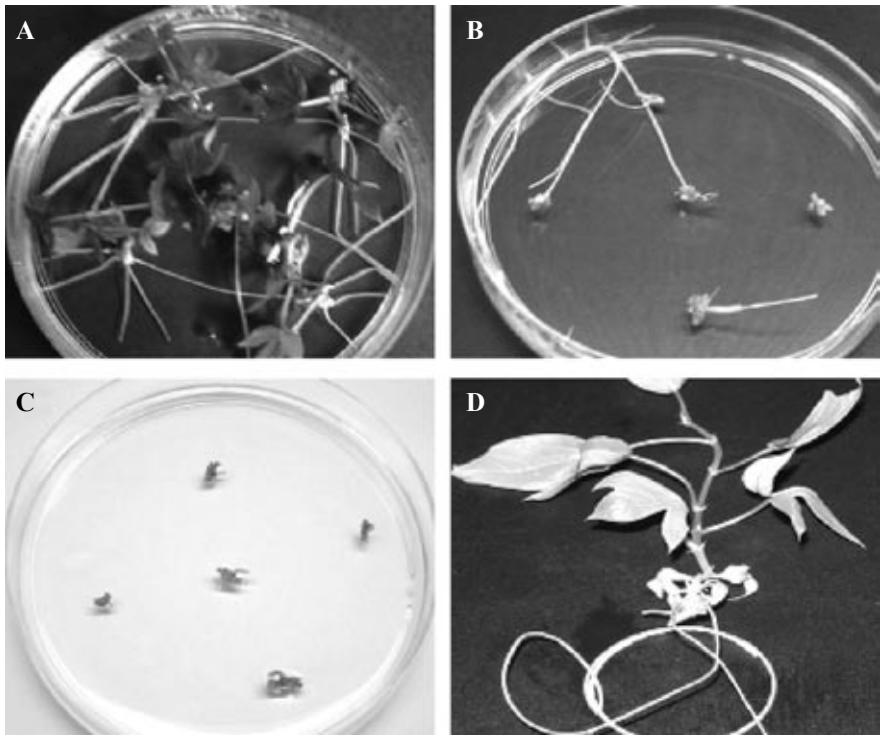

Figure 1. Effect of different abscisic acid (ABA) concentrations on nodes derived from *in vitro* plants and recover of landrace CAS36.18. A) Control (no ABA) bud sprouting; B) 10 µM ABA effect on nodal explants for 90 d; C) 20 µM ABA effect on nodal explants during 90 d; D) Regenerated *in vitro* plant after treatment of node with 20 µM ABA and growth on basic SP medium for 45 d.

similar result (picture not shown). On the other hand, Figure 2 illustrates the vigorous stem length of both accessions in all ABA concentrations. The plantlet sizes reached, in terms of tissue culture, is very adequate and denotes favorable *in vitro* conditions and shows as well that 20 and 30 µM ABA did not affect subsequent plant growth. In addition, from in field conditions it is possible to assume that such results do not exclude the possibility of ABA having a protective role during the dry season, when cassava axillary buds remain in dormancy. However, during the raining season, when there are not stressful conditions, axillary bud can reassume sprouting and growth, and consequently ABA catabolism may be stimulated.

With the *in vitro* technique, ABA seems to be incorporated into axillary buds in the prior period. This could have resulted in decreased ABA levels due to its catabolism and in the absence of abiotic stress under the new *in vitro* conditions. More research is needed to understand ABA catabolism, i.e., degradation and conjugation process, in cassava and in other plants. More information is also needed for signaling pathways, gene expression, messenger RNA, enzymes, binding sites and regulatory mechanisms (Milborrow, 2001; Hirayama and Shinozaki, 2007; Ren *et al*., 2007).

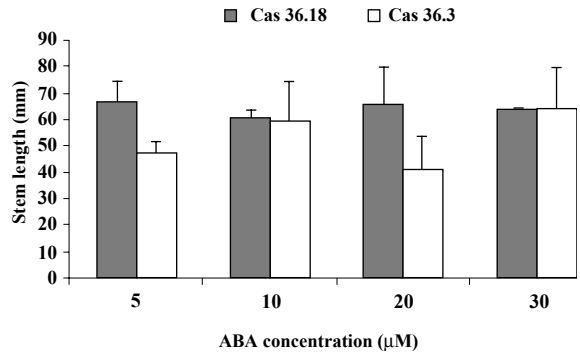

Figure 2. Plant growth after abscisic acid (ABA) treatment of buds of two genotypes and regrowth in basic SP medium for 45 d.

This is the first report of the effect of ABA on *in vitro* plant bud sprouting and growth retardation with cassava. Results demonstrated that ABA treatment of *in vitro* plant buds at the appropriate concentration can be used to improve *in vitro* germplasm collection management, allowing for storing buds for longer periods than *in vitro* plants. This could reduce the transplanting cycle of accessions in our large germplasm collection. The use of other ABA concentrations with other plant species, such as potato (*Solanum tuberosum* L.), garlic (*Allium sativum* L.), and onion (*Allium cepa* L.) could replace the use of toxic maleic hydrazide.

CONCLUSIONS

The present work confirmed the inhibitory effect of ABA on cassava axillary bud sprouting and on *in vitro* plant growth. ABA added to a nutritive medium, strongly inhibited bud sprouting at 20 and 30 µM for 90 days without affecting subsequent recovery of plants in a basic medium without ABA. ABA could now be used in SP medium for *in vitro* cassava culture and to improve management of large germplasm collections by reducing labor requirements.

R E S U M E N

Importancia del ácido abscísico (ABA) en la conservación *in vitro* de la yuca (*Manihot esculenta* Crantz). L. Pedro Barrueto Cid[1*], y Luiz L.C.B. Carvalho[1]. La tecnología usual para conservación *in vitro* de colecciones de germoplasma de yuca (*Manihot esculenta* Crantz) es corrientemente laboriosa y emplea varias transferencias por año. Este procedimiento envuelve altos costos en preparación de medios, consumo de tiempo, riesgos de manipulación y necesidad de mucho espacio para la mantención de colecciones en cámaras de cultivos. Se desarrolló un nuevo procedimiento usando yemas axilares nodales cultivadas *in vitro* con diferentes concentraciones de ácido abscísico (ABA), con el objetivo de reducir los ciclos de transferencia de los cultivos mantenidos en un medio nutritivo básico tal como el SP. Los segmentos nodales fueron almacenados por tres meses en presencia de ABA. Las plantas fueron obtenidas después que los segmentos nodales fueron transferidos al medio SP sin ABA. Los resultados indican que 20 y 30 µM de ABA indujeron una completa dormancia de yemas, sin afectar el desarrollo posterior de las yemas nodales y su consecuente conversión en planta.

Palabras clave: cultivo de tejidos, dormancia, *Manihot esculenta*.

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