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Chapter 3

BIOTECHNOLOGY APPLIED TO CASSAVA PROPAGATION IN ARGENTINA

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ABSTRACT

Cassava is a staple food to millions of people in tropical and subtropical countries. Although it is traditionally cultivated from stem cuttings, which is a simple and inexpensive technique, this method presents serious problems such as low multiplication rates, difficulties to conserve stems, and dissemination of pests and diseases. Many of these problems would be solved through *in vitro* tissue culture. In this work, we evaluated the *in vitro* establishment and multiplication of 28 cassava

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clones of agronomic interest for the Northeastern Argentina, a boundary area for this crop. Since the transfer of *in vitro* plants to *ex vitro* conditions is a critical phase of micropropagation, we evaluated the effect of different acclimatization treatments on survival and growth parameters of plants (cv EC118) grown in a culture chamber. We also scored their field survival and performance by comparing them with plants obtained by the conventional planting technique. After disinfection, uninodal segment culture in Murashige and Skoog medium supplemented with 0.01 mg/L BAP + 0.01 mg/L NAA + 0.1 mg/L GA3 allowed the *in vitro* establishment of 100% of the clones and their subsequent multiplication. Cultures were maintained at 27°±2°C with a 14 h photoperiod. During establishment, sprouting occurred in 100% of the clones and rooting in 93% of them; the remaining clones formed roots during the multiplication phase. Thirty days after multiplication, the plants presented significant differences in plant height, average number of nodes per plant and number of roots per plant. During acclimatization, five treatments were evaluated: three substrates (perlite, T1; sand + vermicompost, T2; commercial substrate composed of peat and perlite, T3), and two hydroponic treatments (tapwater, T4; Arnon and Hoagland nutrient solution, T5). Although in chamber growth conditions the acclimatized plants showed statistical differences in several growth parameters depending on the treatments, no differences were observed in the survival percentage. Shoot and root fresh and dry weight and leaf area were highest in T5 and lowest in T2 and T4. Field survival differed significantly between treatments, discriminating a group with high survival rates (T5: 73.3%, T3: 86.7%, and control treatment: 100%) and another with low survival rates (T2: 33.3%; T1: 35% and T4: 36.7%). At harvest, there were no significant differences in the total fresh weight. However, the percentage of biomass partitioned to roots was significantly higher in T3 and T5, which resulted in a higher tuberous roots yield than that of the control treatment.

Keywords: *acclimatization, cassava, in vitro plant regeneration, Manihot esculenta, tuberous roots, yields*

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a staple crop with great economic importance, constituting a basic component in the dietary of over 1,000 million people in tropical and subtropical countries (FAO/FIDA, 2000; Ceballos, 2002).

The broad diversity of uses that can be given to the whole plant, its flexibility regarding the timing of planting and harvesting, and its ability to be produced under a wide range of edaphoclimatic conditions (Puonti-Kaerlas, 1998; Ceballos, 2002) make this species fulfill a prominent role in the context of food security (FAO/FIDA, 2000).

World production of the cassava tuberous root is estimated to be 214 million tons (FAO, 2009). In Argentina, cassava is cultivated mainly by small scale and subsistence farmers in the Northeast region, reaching a tuberous root production of 200,000 tons (De Bernardi, 2001).

Traditionally, cassava is propagated asexually by stem cuttings. Although this is a simple and inexpensive method that simultaneously preserves the varietal features, it provides very low multiplication rates and it is the main mode of transmission and spread of pests and diseases, thus affecting the quality and quantity of the planting material and crop yield (Roca and Jayasinghe, 1982; Puonti-Kaerlas, 1998; Pedroso de Oliveira et al., 2000; Bellotti et al., 2002; Albarrán et al., 2003). Therefore, to ensure regional development of this crop, it is necessary to multiply plants from breeding lines, elite cultivars or systemic pathogen-free materials (Smith et al., 1986; Puonti- Kaerlas, 1998; Thro et al., 1999).

In this regard, the techniques of *in vitro* tissue culture can provide solutions to these problems. The cassava meristem culture has allowed the propagation of virus and other systemic pathogens-free plants (Roca et al., 1991), while the uninodal segment culture has facilitated the rapid plant multiplication and *in vitro* conservation of different varieties (Smith et al., 1986; Roca et al., 1991; Pedroso de Oliveira et al., 2000; Albarrán et al., 2003). According to Roca et al. (1991), the use of this technique allows obtaining three to five plants per month from each nodal segment. It should be noted that the efficiency of the technique varies with the genotype, as some clones are more easily adapted to the process of *in vitro* propagation than others (Pedroso de Oliveira et al., 2000; Albarrán et al., 2003).

The most critical phase of micropropagation is acclimatization, which is the gradual transfer of plants from the *in vitro* environment to *ex vitro* conditions, where they undergo a lower relative humidity and a comparatively much higher light intensity (Pospíšilová et al., 1999; Jorge et al., 2000). Moreover, these plants have a lower nutrient availability, mechanical damage to the roots (Segovia et al., 2002) and exposure to saprophytic and eventually phytopathogenic microorganisms (Grattapaglia and Machado, 1990).

Several studies have reported a high mortality of cassava plants in the acclimatization stage (Zok et al., 1993; Da Silva et al., 1995; Azcón Aguilar et

al., 1999; Jorge et al, 2000; Zimmerman et al ., 2007, Marín et al. 2008). Mortality can reach 95% if the right technology is not used (Segovia et al., 2002). According to Jorge et al. (2000), the low survival in the acclimatization phase and field establishment may be one of the reasons why *in vitro* tissue culture has not been adopted as a tool for the propagation of cassava in a large scale.

It is important to note that while production costs of plants increase with the implementation of these techniques, *in vitro* tissue culture has the potential to produce large numbers of quality plants at any time of the year, hundreds of times faster than traditional techniques (Roca, 1984; Thro et al., 1999; Ceballos, 2002).

The aim of this chapter was to evaluate the *in vitro* establishment and multiplication of 28 cassava clones of agronomic interest for the Northeast region of Argentina and to assess the effect of different acclimatization treatments on plant survival and growth of the EC118 clone in growth chamber and field conditions. In addition, we determined the tuberous root yield of acclimatized plants in comparison with the traditional planting technique.

MATERIALS AND METHODS

The plant material consisted of 28 clones of cassava (*Manihot esculenta* Crantz) provided by the germplasm bank of the "Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria (INTA EEA)" El Colorado (Formosa, Argentina) (Table 1).

In Vitro Establishment and Multiplication

Cassava (*Manihot esculenta* Crantz) stem cuttings with approximately six buds were planted in pots with a mixture of 1:1 of black soil and fine sand to improve drainage, and then maintained under greenhouse conditions. Uninodal segments (approximately 10 mm long) dissected from cassava plants grown under greenhouse conditions were used as source of explants for all experiments. Explants were disinfected in 70% ethanol for 1 min and then immersed in 1.1% sodium hypochlorite plus 0.05% (v/v) Triton X-100® for 20 min, and finally rinsed three times with sterile distilled water.

Table 1. Origin and main features of cassava clones provided by the germplasm bank of the INTA EEA El Colorado (Formosa, Argentina)

Clone	Origin	kg/plant	Plant height (m)	Root color
EC 22	Col. Drifin Porá, Corrientes	2.20	2.85	white
EC 107	EEA EC Mariño	3.03	2,80	brown
EC 44	Picada Sur Javier, Misiones	3.30	2.45	white
EC 42	Paraje López, Misiones	5.33	2.25	brown
EC 6	Monte Caseros, Corrientes	2.93	3.00	white
EC 20	Ingeniero Juárez, Formosa	1.76	2.30	brown
EC 88	Cahuare, Misiones	3.70	2,00	brown
EC 19	Paso Itá, Corrientes	1.73	1.60	white
EC 26	Col. San Justo, Corrientes	1.66	1.50	brown
EC24-10	Col. Drifin Porá, Corrientes	1.92	1.50	brown
EC 29-9	Col. San Justo, Corrientes	2.03	1.70	brown
EC 3	Col. Santa Ana, Corrientes	3.16	2.10	brown
EC 90	Cahuare, Misiones	4.20	1.90	brown
EC 157	Manantiales, Corrientes	2.40	1.90	brown
EC 165	Manantiales, Corrientes	2.90	1.80	white
EC 161	Yrigoyen, Formosa	2.66	1.90	brown
EC 110	Pomberí	2,73	2.00	white
EC 111	Campeona C.B.	2.86	1.80	brown
EC 74	Km 1124 San Javier, Ctes	1.80	2.05	white
EC 113	EEA, E.C., Negra, Formosa	1.86	1.80	white
EC 23	Drifin Porá	0.90	1.30	white
EC 118	C.A.6 3, Misiones	2.16	1.80	white
EC 124	Yerutí	3.86	2.20	white
EC 1-1	Col. Santa Ana, Entre Ríos	1.86	3.00	white
EC 121	C.A. 25.1, Misiones	3.00	2.90	brown
EC 163	Manantiales, Corrientes	1.16	2.20	white
EC 27-4	San Justo, Corrientes	3.73	2.00	brown
EC 162	No characterization	-	-	-

Resource: EEA INTA El Colorado. Unpublished data.

Uninodal segments were cultured aseptically on Murashige and Skoog (1962) basal medium (MS) additioned with 0.01 mg/L naphthaleneacetic acid (NAA), 0.01 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L gibberellic acid (GA_3). The culture media pH were adjusted to 5.8 with KOH and/or HCl and solidified with 0.75% agar (Sigma® A1296). Test tubes (43 mL capacity) were covered with aluminium foil and autoclaved at 1.46 kg cm^{-2} for 20 min. The cultures were covered with Resinite AF-50® film (Casco S. A. C. Company, Buenos Aires) and incubated in a growth chamber at 27±2ºC under a 14 h

photoperiod regime with an irradiance of 116 μmol.m^{-2}.s^{-1} provided by cool white fluorescent lamps.

After 30 days of *in vitro* establishment, we evaluated the percentage of contamination, sprouting and establishment, as well as plant height, number of nodes per plant, rooting percentage and average root number per plant. An explant was considered as established when it was alive and disinfected after 30 days of culture.

Subsequently, regenerated plants were subjected to a first multiplication cycle. *In vitro* uninodal segments were cultured in MS supplemented with 0.01 mg/L NAA, 0.01 mg/L BAP and 0.1 mg/L GA_3, and after 30 days the parameters evaluated in the establishment phase were assessed again.

Acclimatization of Plants in Growth Chamber

Fifty days after the second multiplication cycle, *in vitro* plants of the EC118 cultivar were removed from test tubes, soaked in tapwater to remove the remaining culture medium and rinsed carefully. Then, they were submerged in fungicide solution (2% w/v Captan®) for 10 min, and subjected to five treatments of acclimatization (Table 2): three consisting in the use of different solid substrates placed in 180 cm^3 plastic pots (Fig. 1A), and two conducted under hydroponic conditions, with a device designed to allow autonomous and constant aeration (Figs. 1 B and C).

Table 2. Description of the acclimatization treatments of cassava *in vitro* plants (EC118 cultivar)

Treatments		Conditions
Solid substrates	T_1	Perlite
	T_2	sand + 3% (w/w) vermicompost
	T_3	D1 Dynamics® a commercial substrate composed of peat and perlite, 9:1 respectively
Hydroponic system	T_4	Tapwater
	T_5	Arnon and Hoagland (1940) nutrient solution

In all treatments, plants were protected by transparent plastic covers (like a moist chamber) to avoid dehydration, and kept in a growth chamber at 27±2ºC with 14 h photoperiod and an irradiance of 215 $\mu mol.m^{-2}.s^{-1}$ for 20 days. The relative humidity was gradually decreased until the complete elimination of the plastic cover. Plants of treatments 1, 2 and 3 were drenched with fungicide (2% w/v Captan®) and irrigated with 20 cm^3 of Arnon and Hoagland (1940) nutrient solution per plant, three times a week, while plants of the hydroponic treatments maintained the initial level nutrient solution or tapwater as appropriate.

The variables evaluated were: survival rate, plant height, number of nodes per plant, leaf area (measured with a portable meter L-3000C LI-COR®), and fresh and dry aerial and radical weight, total fresh and dry weight, and dry matter percentage for both *in vitro* regenerated plants (initial state) and acclimatized plants incubated for 20 days in growth chamber (final state).

Figure 1. Acclimatization phase in growth chamber: (A) Treatment of acclimatization in multicell trays filled with three different types of substrates and covered with a transparent plastic. (B) Treatment of acclimatization under hydroponic conditions. (C) Detail of the device used to promote aeration in the hydroponic system.

Survival and Growth Performance under Field Conditions

Field trials with acclimatized plants of the EC118 clone were carried out at the experimental farm of the Universidad Nacional del Nordeste (UNNE), Corrientes (27º28`S, 58º16`W), Argentina. The soil of the experimental site is a mixed hyperthermic alfic Udipsamment (Escobar et al., 1994). The climate of this zone is subtropical. This zone has an average annual precipitation of 1500 mm, which represents a positive hydric balance, an average annual temperature of 21.5ºC with an average minimum temperature of the coldest month (July) between 13 to 16ºC and a frost-free period of 320 to 360 days. The annual frost frequency is 0.4 (between June and July) and no frost probability expected between October and April (Bruniard, 1999).

Donor plants of the control stem cuttings were taken from a clonal orchard of the Facultad de Ciencias Agrarias (UNNE), where they were grown under the same environmental and agrotechnical conditions.

At this phase, the performance of the field acclimatized plants was compared to plants grown from stem cuttings with three-four nodes (control treatment; Tc). The plant spacing for all treatments was 1 m x 1 m. Plants were irrigated after planting and then three times a week during the first three weeks. All plants were harvested at 5 ½ months after planting.

Field survival was recorded at 20, 30, 60, 90, 120 and 165 days after planting. The following parameters were evaluated at harvest time: plant height, main branch length, number of branches per plant, number of nodes per plant, percentage of branched plants and shoot and tuberous root fresh weight per plant. The yield of each treatment was expressed as a percentage of the yield obtained in the control treatment (Tc).

Experimental Design and Statistical Analysis

The experimental design of the *in vitro* establishment and multiplication phase of the 28 clones of cassava, and the acclimatization of the EC118 clone in growth chamber was completely randomized, with three repetitions for each treatment. For the evaluation of plant performance and behavior under field conditions, the experimental design was a randomized complete block with three replications for each treatment. Data were subjected to one-way analysis of variance (ANOVA) after verifying the normality of the variables, and the means were compared by Duncan's multiple comparison test ($p \leq 0.05$;

p≤0.01), using InfoStat software professional version 1.1 (InfoStat, 2002). Means are presented with standard error (±SE).

RESULTS AND DISCUSSION

In Vitro Establishment

All cassava clones evaluated were established *in vitro*. The *in vitro* establishment percentage varied between 38.7% and 100%. Fungal and bacterial contamination was observed in 89% of the clones. Although fungal infection was more frequent, affecting 71% of the clones, it ranged between 4.6% and 18%, while bacterial infection reached 56.4%, affecting fewer clones (51%). The percentage of unresponsive explants ranged between 0% and 6%, with the exception of the EC 19 clone, which presented 20% of unresponsive explants.

In the establishment phase, the percentage of sprouting showed significant differences between clones (p≤0.05), ranging between 38.7% and 95.5%. Almost 90% of the clones tested exceeded 50% of sprouted explants, while more than half of the clones (61%) had values above 75% (Fig. 2).

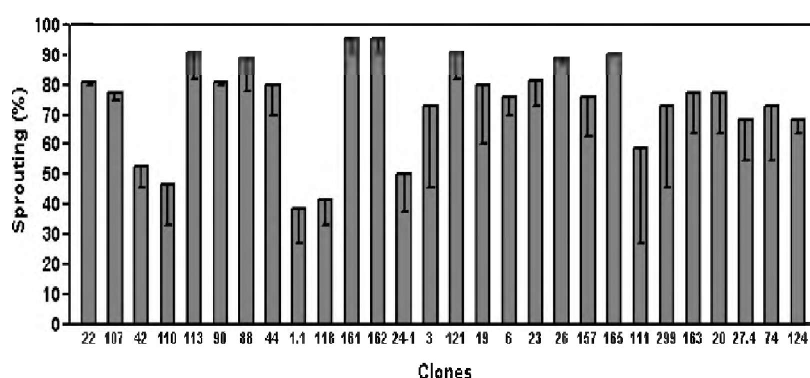

Figure 2. Sprouting percentage of the 28 cassava clones after 30 days of culture under *in vitro* conditions.

We found highly significant differences between clones with respect to plant height (p≤0.01), ranging between 3.3 and 69.4 mm; clones EC29 and EC26-9 presented the shortest plants, whereas EC121, EC107 and EC20 the tallest ones. The number of nodes per plant also differed significantly between

clones ($p \leq 0.01$), with average values between 2.4 and 9.7 nodes. In general, clones that produced the shortest plants were those that formed the lowest number of nodes per plant, and clones that produced the tallest plants were those that formed the largest number of nodes per plant.

Rooting was observed in 93% of the clones during the establishment phase. In addition, significant differences in the rooting percentage, which ranged between 6.3% and 91%, were found between clones ($p \leq 0.01$).

It should be noted that 57% of the clones showed a rooting percentage above 50%. Only two clones (EC26 and EC299) were not rooted in the establishment phase. The average root number per plant ($p \leq 0.01$) also differed between clones, ranging between 0 and 4 roots per plant.

In Vitro Multiplication

In the multiplication phase, 100% of the *in vitro* established clones regenerated plants from the uninodal segment culture. Maximal sprouting (100%) was found in 71% of the clones, while the remaining clones recorded values above 77% (Fig. 3). These values are higher than those reported by Albarrán et al. (2003), who obtained plants in the 34 clones evaluated, reporting percentages of regeneration between 60 and 90%. Acedo (2002) obtained 100% plant regeneration of the cassava cultivar "Golden Yellow" when using MS medium free of plant growth regulators (PGRs), and low levels of regeneration (13-21%) when using MS supplemented with NAA, BAP and GA_3.

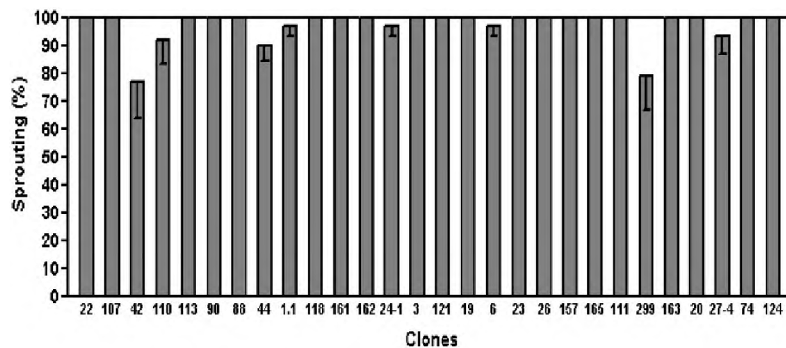

Figure 3. Sprouting percentage of the 28 cassava clones after 30 days of the first *in vitro* multiplication.

In our study, the plants were morphologically normal in phenotype (size, shape and color) independently of the clones tested. Therefore, we can state that the plants established *in vitro* are phenotypically stable compared to the original material.

Plant height varied significantly between clones ($p \leq 0.01$), with average values between 2.9 mm and 57 mm, slightly shorter than those observed in the establishment phase. In general, the clones that had the tallest stem in the establishment phase also developed the tallest stem in the multiplication phase. The number of nodes per plant also differed significantly between clones ($p \leq 0.01$), with average values between 2.3 and 6.6 nodes per stem. Marín et al. (2008) reported plant height values of 30 to 70 mm and a node number per plant of 3.5 and 7, for 19 elite cassava clones after 60 days of culture (twice the time used in our experiment) in a culture medium consising of ⅓ MS and 0.02 mg/L NAA. Smith et al. (1986) reported a significant increase in the average node number per plant in relation to the addition of PGRs in the basal medium MS, being the addition of NAA and BAP more effective than IBA alone. These authors explained this increase in the number of nodes per plant as a consequence of the promotion of multiple shoots. On the other hand, Pedroso de Oliveira et al. (2000) reported an increase in plant height in the first cycle of multiplication from 8.6 to 17.7 mm for six varieties of cassava from Brazil, using a culture medium composed of 35% macronutrients and micronutrients of MS supplemented with 1 mg/L thiamine, 100 mg/L inositol, 0.01 mg/L NAA, 0.01 mg/L GA_3 and 2 % sucrose. According to these authors, the presence of poorly developed plants was more frequent than the presence of etiolated plants.

In our experiment, all the clones tested were able to root in the multiplication phase, the rooting percentage ranging between 20% and 100%. It should be noted that 89% of the rooting clones had values above 50% (Fig. 4). The average root number per plant also differed significantly between clones ($p \leq 0.01$), ranging from 1 to 5 roots. Pedroso de Oliveira et al. (2000) reported rooting in the first cycle of multiplication in four of the six clones tested, with values between 30 and 70%, reaching 100% effectiveness just in the third cycle of multiplication.

In the multiplication phase, clones that showed higher plant height were those reporting the highest average root number per plant like in the establishment phase. Pedroso de Oliveira et al., (2000) argued that the presence of roots in cassava seedlings is beneficial to the multiplication process because it promotes the absorption nutrients and therefore a good production of buds that will serve as explants for the following culture cycles.

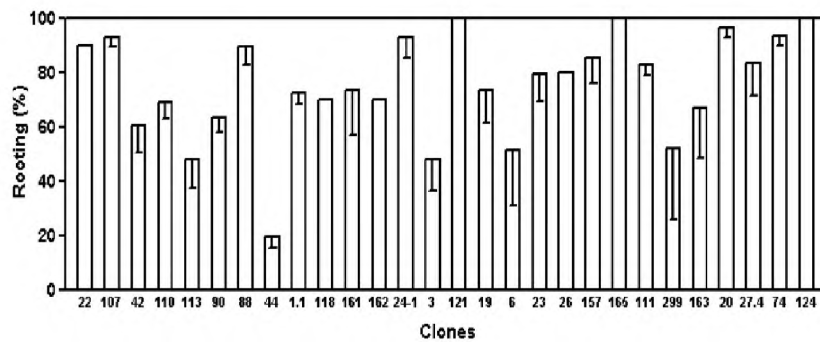

Figure 4. Percentage of rooted explants of 28 cassava clones grown *in vitro* 30 days after the first multiplication.

In general, the results indicated a great variability between clones (Fig. 5) with respect to all the parameters evaluated. This pronounced effect of the genotype on *in vitro* cassava plant development has been reported by other authors (Roca, 1984; Pedroso de Oliveira et al., 2000; Albarrán, et al., 2003; Marín et al., 2008). In this regard, Smith et al. (1986) proposed adjustments to the culture medium to stimulate the growth of cassava varieties that have low multiplication efficiency.

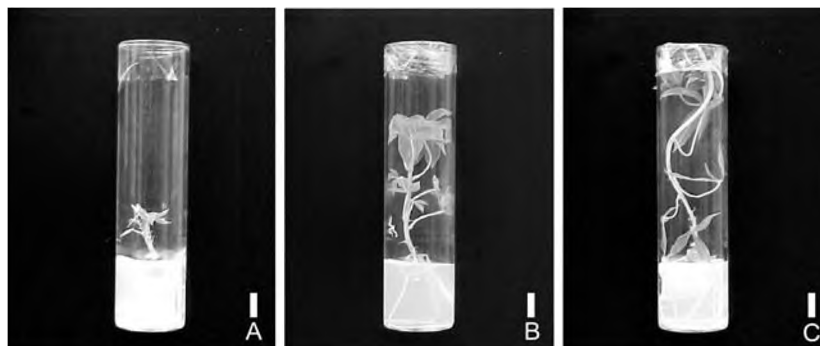

Figure 5. Variability of cassava clones with respect to plant height, node and root number per plant after 30 days of *in vitro* establishment. (A) clone EC24 (B) clone EC161, (C) clone EC121. Bar: 10 mm.

Acclimatization of Plants in Growth Chamber

The *ex vitro* survival, recovery and growth of cassava plants obtained *in vitro* were successful, regardless of the acclimatization method assayed. The

survival rate of plants at the end of the acclimatization phase in growth chamber varied between 96% (T_1, T_4 and T_5) and 100% (T_2 and T_3). These values were similar to those reported by Pedroso de Oliveira et al. (2000) for six Brazilian cassava varieties (92%) and higher than those obtained by Broomes and Lacon (1995) at the end of the first week of acclimatization of plants regenerated *in vitro* in liquid medium (82%). The values of our results are also higher than those found by Azcón Aguilar et al. (1997), who inoculated cassava plants with *Glomus desserticola* as a strategy to increase *ex vitro* survival during the acclimatization phase (75%). Zimmerman et al. (2007) acclimatized cassava plants rooted in vermiculite instead of gelling agent, and reported survival values greater than 95%; probably, this way to promote rhizogenesis provides the possibility to preserve the integrity of roots when removing them from the substrate.

Zok et al. (1993) analyzed the survival of acclimatized cassava plants using different combinations of soil with vermiculite, sawdust and coffee husks, using soil alone as control treatment. These authors reported survival values of 5% to 55% and obtained better performance with the mixture of soil with vermiculite, and remarked the importance of providing the substrate with good aeration and water retention so as to achieve successful plant acclimatization.

Le et al. (2007) achieved 93% survival after maintaining the plants for 7 days in water and then 20 to 25 days in a nutrient solution. Using a similar procedure but then transplanting plants to pots, Marín et al. (2008) obtained only between 0% and 57% survival for 19 elite cassava clones. However, Albarrán et al. (2003) reported survival values above 50% in 32 of the 34 clones of cassava plants evaluated following the method used by Marín et al. (2008). In our study, we observed significant differences between acclimatization treatments ($p \leq 0.01$) with respect to all the parameters evaluated. Higher values were obtained in plants growing under hydroponic conditions with Arnon and Hoagland nutrient solution (T_5) (Table 3).

All parameters, except for radical fresh weight, showed significant differences among the initial (T0) and final condition after the different treatments of acclimatization (Table 3). Radical fresh weight differed significantly ($p \leq 0.01$) from the initial condition (T0) only when plants were grown under hydroponic conditions with Arnon and Hoagland nutrient solution (T_5). The number of nodes per plant and leaf area was high in T_5 and T_3, differing significantly from T0, T_2 and T_4 (Table 3). These data are in contrast to those reported by Da Silva et al. (1995), who found no significant differences in survival, leaf area and node number, using different substrates

for the acclimatization of cassava plants. These authors obtained survival values ranging between 49% and 56% and inferred a relationship between survival and age of the rooted shoots at the beginning of the acclimatization process.

Table 3. Effects of different acclimatization treatments of cassava plants (clone EC118) on several growth parameters, evaluated at 20 days of *ex vitro* culture, with respect to initial *in vitro* conditions (T0): plant height (PH), number of nodes per plant (NNP), leaf area (LA), aerial fresh weight (AFW), radical fresh weight (RFW), total fresh weight (TFW), aerial dry weight (ADW), radical dry weight (RDW), total dry weight (TDW) and dry matter (DM)

Treatment	PH (cm)	NNP	LA (cm^2)	AFW (g)	RFW (g)	TFW (g)	ADW (g)	RDW (g)	TDW (g)	DM (%)
T_0	8.41^a	7.67^a	7.08^a	0.18^a	0.12^a	0.30^a	0.02^a	0.01^a	0.04^a	11.75^a
T_1	11.31^b	11.56^{cd}	33.57^{cd}	0.63^{bc}	0.19^a	0.82^b	0.09^b	0.03^b	0.13^b	14.12^{ab}
T_2	10.38^b	10.78^{bc}	17.86^b	0.42^b	0.15^a	0.58^b	0.07^b	0.03^b	0.10^b	18.02^c
T_3	11.89^b	12.11^{de}	44.26^d	0.77^c	0.17^a	0.94^b	0.11^b	0.02^b	0.13^b	13.62^{ab}
T_4	11.02^b	10.00^b	21.12^{bc}	0.50^{bc}	0.16^a	0.66^b	0.10^b	0.02^b	0.12^b	19.20^c
T_5	14.44^c	13.22^e	90.66^{de}	1.93^d	0.44^b	2.37^c	0.30^c	0.07^c	0.36^c	15.12^b

Different letters within columns indicate significant differences ($p \leq 0.01$).

Figure 6 shows the appearance of *in vitro* plants (T0) which were subjected to different acclimatization procedures (T_1 to T_5). In addition, it is possible to observe that the commercial substrate Dynamics® (T_3) and the hydroponic treatment with nutrient solution of Arnon and Hoagland (1940) (T_5) were beneficial on the vegetative growth, and that the hydroponic treatment improved radical development remarkably.

The use of the commercial substrate Dynamics® (T_3) and nutrient solution (T_5) led to an increase of 3.3 and 9 times in the *in vitro* dry weight values, respectively (Fig. 7). This significant increase in the biomass of acclimatized cassava plants is consistent with the findings of Pospíšilová et al. (1999), who worked with *Nicotiana tabacum*. These authors argued that if the *ex vitro* transplantation is successful, it ensures higher plant growth.

The percentage of dry matter showed significantly higher values ($p \leq 0.01$) in plants derived from sand + vermicompost (T_2) and hydroponic conditions using tapwater (T_4). However, both treatments had lower values of total dry weight (Fig. 7), implying a lower water content in plants subjected to these treatments. This decrease in dry weight accompanied by an increase in the percentage of dry matter was informed by Clostre and Suni (2007) in *Lemna*

gibba L. and by Gerardeaux et al., (2009) in *Gossypium hirsutum* L., in both cases associated with a lower potassium content in the growth medium.

Figure 6. Cassava plants (clone EC118) at the beginning and at the end of the acclimatization phase in growth chamber. *In vitro* plant (initial state or T0), acclimatized plant using solid substrates as perlite (T_1), sandy ground + vermicompost (T_2), commercial mixture of peat and perlite (Dynamics ®) (T_3), and acclimatized plant using hydroponic treatment with tapwater (T_4), and Arnon and Hoagland nutrient solution (T_5).

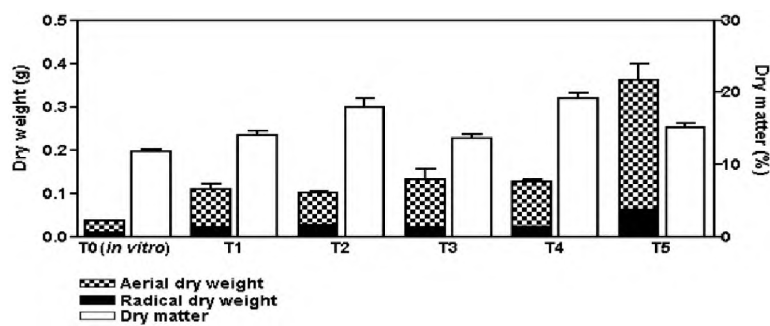

Figure 7. Aerial and radical dry weight and percentage of dry matter of the cassava plants derived from different treatments at the end of the acclimatization phase (clone EC118) in growth chamber, with respect to the initial condition (T0).

Survival and Growth Performance under Field Conditions

Field survival of plants showed significant differences between treatments at all the dates evaluated ($p \leq 0.05$), being higher in plants derived from stem cutting (Tc) and acclimatized plants in commercial substrate (T_3) and hydroponically with nutrient solution (T_5).

The largest reduction in the percentage of plant survival was remarked between 20 and 30 days of the transplantation field, and then remained constant up to 165 days of culture (Fig. 8). For this reason, we consider that the values recorded at 30 days of planting would be most appropriate to compare the survival of cassava plants acclimatized with different treatments. Two groups were distinguished: one with high survival rates (73.3%, 86.7% and 100% for T_5, T_3 and Tc, respectively) and another group with low survival rates (T_2: 33.3%; T_1: 35% and T_4: 36.7%).

Albarrán et al. (2003) reported values of field survival of acclimatized cassava plants between 22% and 100% depending on the clones, and emphasized that plants that survive the first two months in field conditions have a high probability to achieve productive age. This reduction in the survival rate was also observed in seed-derived cassava plants that were transplanted under field conditions, because for two to three months of culture they are more fragile than stem cutting-derived plants (Ceballos et al., 2002).

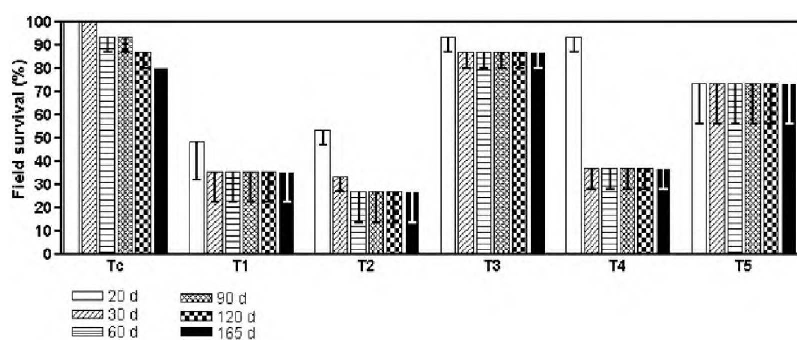

Figure 8. Field survival of cassava plants (clone EC118) acclimatized with different treatments compared to the control treatment (plants derived from stem cutting) during the crop cycle.

There were no significant differences between treatments ($p \leq 0.05$) with respect to the number of nodes per plant, main branch length, number of branches per plant and percentage of branched plants.

Fresh weight of the tuberous root, stem and leaf fresh weight and total fresh weight as well as the partition between tuberous roots and aerial organs showed significant differences between treatments ($p \leq 0.05$). Although plants grown from stem cuttings yielded the highest values of total fresh weight, they showed the lowest tuberous root fresh weight.

Table 5. Aerial fresh weight (AFW), radical fresh weight (RFW) and total fresh weight (TFW) of cassava plants (clone EC118) acclimatized with different treatments, transplanted under field conditions and evaluated at 165 days after planting. Tc (plants derived from stem cutting used as control treatment)

Treatment	AFW (g)	RFW (g)	TFW (g)	Partitioning to stem + leaves (%)	Partitioning to tuberous roots (%)
T_c	702.92^{c}	188.33^{a}	891.25^{b}	79.23^{c}	20.77^{a}
T_1	417.17^{ab}	135.38^{a}	552.54^{ab}	67.77^{b}	22.33^{a}
T_2	287.50^{ab}	130.00^{a}	417.50^{a}	77.67^{c}	32.23^{b}
T_3	399.17^{ab}	295.42^{b}	694.58^{ab}	57.09^{a}	42.91^{c}
T_4	268.75^{a}	112.50^{a}	381.25^{a}	68.32^{b}	31.68^{b}
T_5	531.25^{bc}	303.33^{b}	834.58^{b}	62.33^{ab}	37.67^{bc}

Different letters within columns indicate significant differences ($p \leq 0.05$).

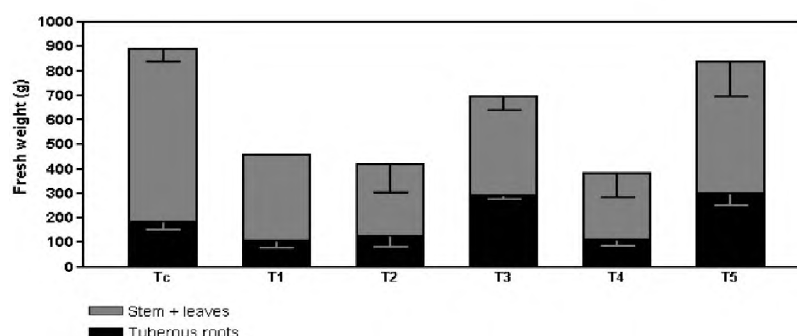

Figure 9. Aerial and radical fresh weight of cassava plants (clone EC118) acclimatized with different treatments and transplanted under field conditions, compared to control treatment (Tc: plants derived from stem cuttings).

On the other hand, total fresh weight of the acclimatized plants under treatments T_3 and T_5 did not differ significantly with respect to the control treatment (Tc), whereas tuberous root fresh weight showed statistical differences in both absolute value and percentage weight partitioned to

tuberous root (Table 5, Fig. 9). Probably as a consequence of late transplanting of plants (November) and a shortening of the crop cycle (5.5 months), it was not possible to achieve the tuberous root yield expected for the EC118 clone (Table 1).

Significant differences in tuberous root yield (expressed as a percentage of yield obtained in the control treatment) were observed between treatments ($p \leq 0.05$). The highest yield values were achieved in plants acclimatized in commercial substrate (T_3) and in hydroponic nutrient solution (T_5), which were 57% and 61% above the yield achieved by plants derived from stem cutting used as control treatment, respectively (Fig. 10).

Cassava plants acclimatized with T_3 and T_5 showed a behavior similar to those derived from stem cuttings. It is likely that the highest yield observed in these treatments was due to the higher initial development of these plants compared to other treatments and the slow growth typically shown by plants derived from stem cuttings under field conditions. According to Alves (2002), true leaves begin to expand just 30 days after stem cutting planting, at which photosynthesis begins to contribute positively to plant growth. For this reason, both tuberization and photoassimilate translocation would start later than in acclimatized plants, thus determining their production.

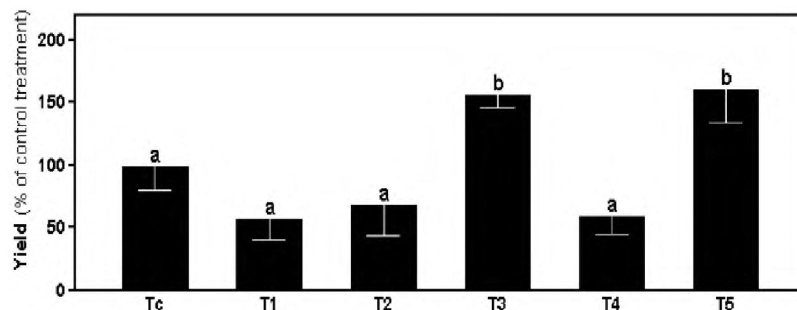

Figure 10. Tuberous root yield of cassava plants (clone EC118) derived from different acclimatization treatments compared to control treatment (Tc: plants derived from stem cuttings). The yield is expressed as a percentage of yield obtained in the control treatment).

While plant survival in the acclimatization phase in growth chamber did not differ between treatments, the differences observed in total weight, leaf area and root biomass at the end of this stage confirm that the plants obtained were significantly different, a condition that resulted in differential responses in the field plant survival.

It is likely that the greatest root development obtained with T_3 and T_5 favors further exploration of the soil, allowing better absorption of water and nutrients in field conditions compared to other treatments, including the control one.

CONCLUSIONS

- The basal medium MS supplemented with 0.01 mg/l NAA, 0.01 mg/l BAP and 0.1 mg/l GA_3 allowed the *in vitro* establishment, regeneration and multiplication of 28 clones from uninodal segment culture with optimal growth.
- In the establishment phase, *in vitro* rooting was achieved in 93% of the clones, of which 57% had a rooting percentage above 50%. In the first cycle of multiplication, all tested clones rooted and 89% of them showed a rooting percentage above 50%.
- It was possible to observe a pronounced effect of genotype on *in vitro* development of cassava plants. The average node number per plant at 30 days of culture ranged between 2.3 and 6.6 for the different clones during the first cycle of multiplication.
- Clones EC121, EC107 and EC20 were those with the best behavior *in vitro* both in the establishment and multiplication phases considering all the parameters evaluated.
- Acclimatization in growth chamber was successful, and a high survival of plants was found with all treatments (96% to 100%). Acclimatization treatments caused significant differences in leaf area and dry weight of aerial and radical parts, which resulted in a differential development of plants that subsequently affected the response of field grown plants, resulting in a higher yield of tuberous roots in plants acclimatized with the commercial substrate and with the nutrient solution in hydroponic conditions than that of plants derived from stem cuttings.
- With regard to field survival, it was possible to distinguish two groups: a group with high field survival (plants derived from stem cuttings and from acclimatization treatments with commercial substrate and hydroponic nutrient solution) and another group with lower field survival (plants derived from the treatments with sand + vermicompost, perlite and hydroponic tapwater).

- Although there were no differences with respect to the total fresh weight, the percentage of biomass partitioned to tuberous roots was significantly higher in the treatments with commercial substrate (T_3) and in hydroponic nutrient solution (T_5), which resulted in a higher yield tuberous root compared to plants derived from stem cuttings.
- The *in vitro* propagation process from the dissection of uninodal segments to field planting is carried out in approximately 10 weeks. Therefore, *in vitro* propagation is a promising alternative for the multiplication of cassava plants in Argentina, because it allows propagation rates and field yields higher than those obtained by traditional propagation methods.

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