In Vitro Germplasm Conservation of Habanero Pepper (*Capsicum chinense* Jacq.)

María del C. Montalvo-Peniche

Instituto Tecnológico de la Zona Maya, Carretera Chetumal-Escárcega, Km 21.5, Ej. Juan Sarabia, Quintana Roo, México

Lourdes G. Iglesias-Andreu

Laboratorio de Biotecnología y Ecología Aplicada, Universidad Veracruzana, Campus para la Cultura, las Artes y el Deporte. Av. de las Culturas Veracruzanos No. 101, Colonia Emiliano Zapata, C.P. 91090, Jalapa, Veracruz, México

Javier O. Mijangos-Cortés

Unidad de Recursos Naturales, Centro de Investigación Científica de Yucatán, Calle 43 #130, Chuburná de Hidalgo, C.P. 97200, Mérida, Yucatán, México

Sara L. Nahuat-Dzib

Instituto Tecnológico de Mérida, Km 6 carretera Mérida-Progreso, Mérida, Yucatán, México

Felipe Barahona-Pérez

Unidad de Materiales, Centro de Investigación Científica de Yucatán, Calle 43 #130, Chuburná de Hidalgo, C.P. 97200, Mérida, Yucatán, México

Adriana Canto-Flick and Nancy Santana-Buzzy¹

Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, Calle 43 #130, Chuburná de Hidalgo, C.P. 97200, Mérida, Yucatán, México

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Abstract. To determine the effect of different nitrogen sources and osmotic regulators on minimal growth of Habanero pepper (*Capsicum chinense* Jacq.) germplasm for in vitro conservation, different concentrations of nitrate, sucrose, mannitol, and sorbitol were evaluated. The micropropagation system based on Santana-Buzzy et al. (2006) culture medium was modified in its nitrate concentrations: reduced to 50% and increased to 150%, and osmoregulators were added to the basal culture media: sucrose (6% and 8%), mannitol (2%, 4%, and 8%), or sorbitol (2%, 4%, and 8%). The apical meristems of germinated plants were cultivated in the different treatments for 35 weeks without subculture. Results have demonstrated that mannitol at 2% had the better effect on minimal growth of the plantlets and did not affect the plant physiology and quality. The plantlets remained small in size, turgent, with green leaves and stems and looked like normal plants until to the end of the evaluation period. Changes in nitrogen media concentration did not prove to be adequate for conserving because they affected the plantlet quality (they became chlorotic). The presence of sorbitol and high osmolite concentrations induced minimal growth but reduced the plant quality. Sucrose at mid or low concentrations did not induce minimal growth.

Mexico has been recognized as the country with the greatest genetic diversity of peppers that belongs the *Capsicum* genus. Habanero pepper (*Capsicum chinense* Jacquin)

has several varieties and shows some of its variability in the fruit color; most fruits are green in color when growing and ripen into numerous colors like red, orange, salmon pink, yellowish, and brown. Habanero pepper is the hottest of the genus because it reaches 200,000 to 300,000 Scoville Units (Contreras-Padilla and Yahia, 1998).

Pepper plant regeneration is severely limited as a result of the formation of ill-defined buds or shoot-like structures either resisting elongation or producing rosettes of distorted leaves, which generally do not produce nor-

mal shoots (Franck-Duchenne et al., 1998; Ochoa-Alejo and Ramirez-Malagón, 2001; Steinitz et al., 1999). Additionally, radical differences in the regeneration response have been reported at the intervarietal level and explant source. Therefore, specific cultivar and tissue culture media have been devised to optimize regeneration from specific cultivars (Christopher and Rajam, 1994; Ramirez-Malagón and Ochoa-Alejo, 1996; Venkataiah and Subhash, 2001). Thus, the strong influence of the pepper variety (genotype) makes it necessary to optimize regeneration protocols for specific cultivars. *Capsicum chinense* Jacq. micropropagation was reported by Santana-Buzzy et al. (2005, 2006).

In vitro storage of plant genetic resources can be a very useful alternative to genetic variability conservation, crop improvement programs, and production of certified seeds, especially for recalcitrant species. The minimal growth plant method is a common midterm in vitro conservation system and involves the reduction of the plant metabolism and the increase of the subculture time without affecting the tissue or plant viability (Sarkar et al., 1999). To reduce the plant metabolism, the environment conditions (temperature, photoperiod, light intensity, and so on) or culture media composition (organic and inorganic nutrients, osmotic regulators, or growth inhibitors) can be modified along the incubation period (Roca et al., 1991). There are other alternatives to increase the in vitro conservation period such as the reduction of the oxygen flow and shoot defoliation (Bajaj, 1995). The addition of osmotic regulators such as mannitol, sorbitol, or sucrose has resulted in a substantial reduction of in vitro plant growth (Bajaj, 1995).

The aim of this study was to evaluate the modifications of plant tissue culture media: nitrates concentration and osmotic regulators such as sucrose, mannitol, and sorbitol on growth reduction of the apexes of *Capsicum chinense* Jacq. germplasm during in vitro conservation.

Materials and Methods

Plant material. Three cultivars (accessions) were selected: Nux, RPs, and MT, classified Habanero orange-colored type, red type, and purple type, respectively. The genetic material came from the *Capsicum chinense* Jacq. germplasm bank collected from farmers of the Yucatan Peninsula and being conserved and characterized in the Scientific Research Center of Yucatan. The apical meristems of 5-week-old Habanero pepper plants were cut off under sterile conditions after in vitro germination and cultured in different in vitro conditions.

In vitro culture conditions and measured variables. The Santana-Buzzy et al. (2006) in vitro culture system (coded as MS) was used as the control treatment and modified in the experimental strategy (Table 1). The control medium contained the mineral salts and vitamins but was modified in its nitrate

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¹To whom reprints requests should be addressed; e-mail buzzy@cicy.mx

concentrations: half strength (N50) and 50% over the normal concentration (N150), or supplemented with different carbon sources to function as osmotic regulators: sucrose 6% (SUC6), sucrose 8% (SUC8), mannitol 2% (MAN2), mannitol 4% (MAN4), mannitol 8% (MAN8), and sorbitol 2% (SOR2), sorbitol 4% (SOR4), sorbitol 8% (SOR8). The media were solidified with Gelrite (2.2 g·L⁻¹) and adjusted to a pH of 5.8 before sterilization. The meristems were incubated in a photoperiod condition of 16/8 h (light/dark) at 50 µmol·m⁻²·s⁻¹ of light intensity and 28 ± 2 °C.

The growth and development of the meristems were evaluated after 35 weeks of culture with no subculture. The variables measured were: plant height (cm) from the base to the top of the plantlet, number of leaves (over 1 cm long), and root length (cm) measured from the stem to the tip of the root. The number of leaves and root length data were transformed according to $\sqrt{x} + 0.75$ previous to statistical analysis.

Statistical analysis. The experiments were set up in a completely randomized factorial design with eight to 10 replicates (unbalanced). Analyses of variance were performed with the PROC ANOVA procedure of SAS (SAS Institute, 1985) for the three variables across genotypes and culture conditions (one of them was the control treatment). The multiple average comparisons among treatments were compared with Duncan's multiple comparison test ($P \leq 0.05$). With the aim of selecting the better in vitro storage condition for the Habanero pepper germplasm, the adjusted means of the three variables across the three genotypes by treatment were standardized to reduce the data to a common scale of mean 0 and variance 1, and two multivariate analyses were performed in a 3 × 11 matrix: cluster analysis with the PROC CLUSTER and the canonical discriminant analysis (CDA) with the PROC DISCRIM procedures of SAS, respectively (SAS Institute, 1985). For clustering the treatment behaviors, the distance matrix was performed with the standardized data with the dissimilarity Euclidian coefficient and an UPGMA cluster analysis was performed rendering a phenogram. The number of clusters was supported by the Hottelling's T² pseudostatistic test performed with the SAS procedure (SAS Institute, 1985). The clusters were confirmed by CDA analysis and the analyses of variance was then recalculated using the groups generated by CDA as a variation factor using PROC GLM of SAS; the multiple average comparisons among groups were performed using the Duncan test ($P \leq 0.05$) (SAS Institute, 1985).

Results

Genotype response to culture media conditions. The plantlets of the three accessions (different genotypes) of Habanero pepper showed statistical differences in plant height, number of leaves, and root length throughout the 11 culture media during in vitro storage. The analysis was performed

with the combined data of each accession throughout the 11 media conditions. For the three variables, the genotype red (RPs) showed the highest value, followed by the orange genotype and the purple genotype, which showed the lowest values (Table 2).

The factorial analysis of variance (accession × culture medium) showed statistical differences and some no significance among genotypes within culture media (Table 3). The genotypes did not show differences for the three variables measured when cultured in the control media, i.e., the plantlets independent of genotype had the same behavior or response (Fig. 1A).

Any culture modification showed differences among accessions in at least one variable, and response varied with accession (Table 3). The highest contrast was observed when meristems were cultured in the presence of sorbitol at 2% concentration (SOR2); in this case, all the accessions were different from each other.

Under modified culture conditions, it was observed that the accessions reduced their growth, but RPs (red-type pepper) showed the highest growth in almost all variables in comparison with MT and Nux accessions but with the same RPs tendency (Table 3). Opposite to RPs, the MT accession was the most sensitive with less plant growth in many of the culture conditions.

Culture media conditions on in vitro germplasm storage. The media nitrogen content changes had no positive effect because plantlets showed higher growth rates than most of the cultures supplemented with osmoregulators: sucrose, mannitol, or sorbitol (Table 3). When nitrogen culture media concentration was modified, 50% lower (N50) or 50% higher than control concentration (N150), there was differences between

genotypes, but in some cases, no response to the stimuli could be found like in the RPs genotype that grew at similar rates as control plantlets (Table 3). Also, no response was observed in root length of Nux at the N50 medium.

All accessions slowed down its growth rate because the culture media composition increased in osmolarity. RPs plantlets reduced the number of leaves when cultured in sorbitol, varying from 12.6 in MS media (control) to 10.3, 7.9, and zero as sorbitol concentrations increased to 2%, 4%, and 8%, respectively. In the presence of mannitol, Nux accession reduced its root length as concentration increased to 2%, 4%, and 8%, respectively. A reduction in the number of leaves in the MT accession took place when sucrose concentration was increased in the culture media. In normal conditions (MS media with sucrose 3%), 12.2 leaves were counted, but at concentrations of 6% and 8% of sucrose, the number of leaves decayed to 5 and 2.8, respectively (Table 3).

To clarify the culture media effect on growth of Habanero pepper germplasm during in vitro storage, the accession responses throughout culture condition were averaged and culture media were compared (Table 4). The highest growth was reached in control medium. On the other hand, MAN8 and SOR8 showed the lowest growth rate. The SUC6, N50, N150, and SUC8 culture conditions, although statistically different, reduced the growth rate in different grades in height and number of leaves, but root length varied from no response in N50 to low growth in N150. The MAN2, MAN4, and SOR4 conditions showed statistical differences and positive effect on the growth rate of plantlets, because plant height diminished to 2 cm like in the highest osmolarity treatments

Table 1. In vitro germplasm conservation experimental design for *Capsicum chinense* Jacq. germplasm modified from the micropropagation system of Santana-Buzzy et al. (2006).

Treatment label	Nitrates (%)	Sucrose (%)	Mannitol (%)	Sorbitol (%)
MS	100	3	—	—
N50	50	3	—	—
N150	150	3	—	—
SUC6	100	6	—	—
SUC8	100	8	—	—
MAN2	100	3	2	—
MAN4	100	3	4	—
MAN8	100	3	8	—
SOR2	100	3	—	2
SOR4	100	3	—	4
SOR8	100	3	—	8

Table 2. Minimal growth of accessions of *Capsicum chinense* Jacq. throughout in vitro conservation treatments.^z

Accession	Plant ht (cm)	Number of leaves	Root length (cm)
RPs	3.51 a	2.57 a	2.32 a
Nux	3.22 b	2.27 b	1.92 b
MT	2.45 c	1.94 c	1.58 c
CV ^y	21.7	22.1	29.0

^zMain effect means followed by the same letter (in columns) are not significantly different at $P \leq 0.05$ according to Duncan's multiple comparison test.

^yAnalysis of variance's coefficient variation.

Table 3. Effect of in vitro conservation culture condition by accession of *Capsicum chinense* Jacq. on minimal growth.^z

CC ^y	ACC ^{xa}	Plant ht (cm)	Number of leaves	Root length (cm)
MS ^y	RP _s	5.60 a	12.60 a	7.15 a
	Nux	5.53 a	12.20 a	6.23 ab
	MT	4.77 a	11.30 a	6.40 a
	CV ^w	19.9	8.6	6.4
N50	RP _s	4.01 a	9.67 a	7.28 a
	Nux	2.78 b	5.90 b	6.63 ab
	MT	2.39 b	4.30 b	4.69 b
	CV	17.8	16.7	22.0
N150	RP _s	4.52 a	10.20 a	7.00 a
	Nux	2.93 b	4.20 b	1.40 b
	MT	2.21 b	3.00 b	2.40 b
	CV	26.1	13.8	47.9
SUC6	RP _s	4.73 a	10.40 a	6.00 a
	Nux	5.15 a	11.00 a	7.20 a
	MT	2.75 b	5.00 b	3.10 b
	CV	29.3	17.9	31.0
SUC8	RP _s	3.60 a	8.00 a	5.88 a
	Nux	4.24 a	10.60 a	4.65 a
	MT	2.17 b	2.80 b	1.40 b
	CV	25.9	21.9	31.5
MAN2	RP _s	2.50 a	3.70 a	4.80 a
	Nux	2.28 ab	4.20 a	4.06 a
	MT	2.19 b	3.60 a	2.74 a
	CV	12.1	22.8	29.2
MAN4	RP _s	2.28 a	4.50 a	5.55 a
	Nux	2.15 b	1.30 b	1.50 b
	MT	2.08 b	1.90 ab	0.69 b
	CV	5.8	34.7	27.0
MAN8	RP _s	2.02 ab	0.10 b	0.81 a
	Nux	2.12 a	0.70 a	0.28 b
	MT	2.00 b	0.80 a	0.20 b
	CV	5.8	22.7	20.1
SOR2	RP _s	4.31 a	10.25 a	6.81 a
	Nux	3.19 b	6.75 b	3.50 b
	MT	2.15 c	1.70 c	0.87 c
	CV	30.6	21.6	25.4
SOR4	RP _s	3.00 a	7.89 a	5.89 a
	Nux	2.54 b	2.75 b	3.53 b
	MT	2.11 c	2.50 b	2.82 b
	CV	15.3	18.2	30.1
SOR8	RP _s	2.11 ab	0.00 b	0.00 a
	Nux	2.23 a	0.00 b	0.46 a
	MT	2.08 b	1.40 a	0.87 a
	CV	6.1	14.4	39.9

^zMain effect means followed by the same letter (in columns) are not significantly different at $P \leq 0.05$ according to Duncan's multiple comparison test.

^yCulture condition.

^xAccession.

^wAnalysis of variance's coefficient variation.

^yMS, control treatment (Santana-Buzzy et al., 2006); N50, 50% bellow the control medium nitrate concentration; N150, 50% over the control medium nitrate concentration; SUC6, sucrose 6%; SUC8, sucrose 8%; MAN2, mannitol 2%; MAN4, mannitol 4%; MAN8, mannitol 8%; SOR2, sorbitol 2%; SOR4, sorbitol 4%; SOR8, sorbitol 8%.

(MAN8 and SOR8), but showed less effect on number of leaves and root length.

These results suggest that low and mid osmoregulator concentrations (mannitol and sorbitol) in the culture media could reduce the growth of the Habanero pepper plantlets while maintaining an adequate physiological stage when stored in vitro without subculture for 8 months.

Selection of the in vitro germplasm storage condition. Two multivariate analyses were performed to settle the better in vitro germplasm storage condition. The UPGMA cluster analysis based on the Euclidian distance coefficient allowed identifying the groups of treatments that induced similar growth behavior. The Hotelling's pseudosta-

tistic T^2 test suggested six similarity groups; this clustering was confirmed in the CDA analysis.

The six groups were settled at an Euclidian distance of 0.38 (Fig. 2A); because of the singular effect of the MS, N50, and SUC6 culture media, each one performed a group by itself; the fourth group (LOP) was built up by N150, SOR2, and SUC8; the fifth group was performed by MAN2, MAN4, and SOR4 (MOP) culture media, whereas the MAN8 and SOR8 set up the sixth group (HOP).

The CDA as a classification analysis allowed verifying the suitability of groups and the real distances among them were estimated. Based on six-group formation, a canonical correlation over 0.95 was obtained

for the three linear canonical functions (Table 5). The first two eigenvalues gathered almost all variability (98.6%) with a $P \geq 0.0019$ (Table 6), so groups were represented in a biplot (Fig. 2B). The most important variables to group classification were plant height and root length in canonicals 1 and 2, respectively. When observations were reclassified, groups matched 100% and agreed with UPGMA cluster analysis (Fig. 2A).

The better in vitro conservation condition was promoted by the MOP group (medium osmotic potential) because it inhibited the plantlet shoot elongation (2.33 cm) and leaf formation (3.6 leaves) and considerably reduced root elongation (3.53 cm) (Table 6). The MOP group was characterized by low values of plant height and intermediate root length. Inside the MOP group, the MAN2 condition showed better plant quality, the plantlets looked like field plants but small in size, and the shoot height and leaf development were proportional and had straight stems, bright green leaf color, and slow but constant growth rate (Fig. 1D).

The groups with one element, MS and SUC6, promoted the higher plantlet height (5.3 and 4.21 cm, respectively) and had higher values for both canonical functions (Fig. 2B; Table 6). The N50 group was characterized by its intermediate shoot growth (3 cm) and number of leaves (6.5) inhibition, but promoted root elongation (6.2 cm) like in the control condition and severely inhibited chlorophyll formation during the culture time (Fig. 1B).

The low osmotic potential (LOP) group had a low effect on plantlets' growth (Table 6); these plantlets showed intermediate values, with the root (3.8 cm length) being the less affected organ. The high osmotic potential (HOP) group strongly affected the growth of plantlets with the lowest values of shoot (2 cm), root length (0.4 cm), and showed less than one leaf (Table 6; Fig. 2B). The HOP group established such a stress condition that plants had bad quality.

In all other culture conditions than MAN2, after 35 weeks of storage, the leaves turned grayish green and became less shiny and turgent, probably as a result of the osmotic stress incited by concentrations and the added compounds.

Discussion

There are no in vitro conservation antecedents on the *Capsicum* genus, although in vitro germplasm banks do exist for other Solanaceae such as potato (*Solanum tuberosum* L.) (Lopez-Delgado et al., 1998) and minimal shoot growth is recognized as a major method for their germplasm conservation (Sarkar et al., 1999).

In vitro germplasm conservation involves particular procedures that make it a suitable alternative for intermediate and recalcitrant species like Habanero pepper. Pepper is still considered as a recalcitrant species, even in vitro (Kumar et al., 2005; Ochoa-Alejo and Ramirez-Malagón, 2001).

Fig. 1. In vitro conservation of *Capsicum chinense* Jacq. (A) Plantlets in micropropagation culture (control). (B) Physiological damage of nitrogen at 50% on plantlets. (C) Effect of mannitol 2% on three different genotypes of Habanero pepper. (D) Plantlets on in vitro conservation in mannitol 2% (bars = 1 cm).

Table 4. Minimal growth of in vitro conservation culture conditions throughout *Capsicum chinense* Jacq. accessions.²

TRT ³	Plant ht (cm)		Number of leaves		Root length (cm)	
MS ^w	5.30	a	12.03	a	6.59	a
N50	3.03	c	6.52	c	6.16	ab
N150	3.22	c	5.80	c	3.60	de
SUC6	4.21	b	8.80	b	5.43	bc
SUC8	3.32	c	7.07	c	3.84	de
MAN2	2.32	de	3.83	d	3.87	d
MAN4	2.17	e	2.57	e	2.58	e
MAN8	2.05	e	0.53	f	0.43	f
SOR2	3.13	c	5.88	c	3.51	de
SOR4	2.53	d	4.37	d	4.05	dc
SOR8	2.14	e	0.50	f	0.46	f
CV ^x	21.7		22.1		29.0	

²Main effect means followed by the same letter (in columns) are not significantly different at $P \leq 0.05$ according to Duncan's multiple comparison test.

³Culture condition.

^xAnalysis of variance's coefficient variation.

^wMS, control treatment (Santana-Buzzy et al., 2006); N50, 50% below the control medium nitrate concentration; N150, 50% over the control medium nitrate concentration; SUC6, sucrose 6%; SUC8, sucrose 8%; MAN2, mannitol 2%; MAN4, mannitol 4%; MAN8, mannitol 8%; SOR2, sorbitol 2%; SOR4, sorbitol 4%; SOR8, sorbitol 8%.

C. chinense Jacq in vitro organogenesis was recently reported (Santana-Buzzy et al., 2005) and improved (Santana-Buzzy et al., 2006). Some modifications of a tissue culture

methodology can lead to a reduced growth rate of shoots in culture, switching from micropropagation to a germplasm conservation goal. Lopez-Delgado et al. (1998)

Table 5. Canonical discriminant analysis: correlations of the linear canonical functions.

Canonical function	Canonical correlation	Approximate standard error	Squared canonical correlation
1	0.9977	0.0014	0.9954
2	0.9797	0.0127	0.9597
3	0.9583	0.0258	0.9183

reported the conservation of shoots of 100 potato genotypes in MS medium; the original micropropagation method was modified adding 100 μM of acetylsalicylic acid, and the temperature was lowered to 8 $^{\circ}\text{C}$ at a 16-h light photoperiod for 6 to 18 months without subculture. Romano and Martins-Loução (1999) modified its cork oak (*Quercus suber* L.) micropropagation system for conserving the germplasm; they tested light/dark and low temperatures for different periods of time and finally established 5 $^{\circ}\text{C}$ and a 16-h photoperiod (30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 2 years storing cultures.

Other factors than photoperiod and temperature like light intensity, growth inhibitors, or culture atmosphere can allow growth reduction (Romano and Martins-Loução, 1999). When atmosphere and climatic environmental conditions cannot be manipulated (like light and temperature), the culture medium turns the target environmental factor to be modified, like in our case.

After 35 weeks of storage in a modified micropropagation system (Santana-Buzzy et al., 2006), the growth of Habanero pepper apexes was reduced when osmoregulators were added to the culture medium at intermediate (MOP group) or higher (HOP group) concentrations. The MOP group of cultures (mannitol 2% and 4% and sorbitol 4%) did not damage the plant physiology, but mannitol 2% (MAN2) was selected as the better storage culture because of a marked plantlet growth reduction and better plant quality (Fig. 2D). Similar effects were observed by Golmirzaie and Toledo (1998) with shoots of *Ipomea batata* L. stored in a Murashige and Skoog (1962) medium supplemented with sorbitol (2%) and mannitol (2%) over a period of 16 months.

The modified culture conditions had similar effect on accessions' growth (Table 3), although statistical differences were observed among them (Tables 2 and 3), so genotype effect is inferred from it. The accession RPs was less sensitive to treatments with higher growth, whereas the MT genotype was the most sensitive. This genotype effect has been observed in other micropropagation and in vitro conservation works. Sarkar and Naik (1998) treated seven potato (*Solanum tuberosum* L.) genotypes (wide genetic base) with silver thiosulfate during prolonged in vitro storage and obtained contrasting responses to minimal growth.

The Habanero pepper germplasm did not show differences in the micropropagation system becoming the perfect control treatment (Table 3; Fig. 1A). The culture modifications generated differences among

Table 6. Minimal growth of groups performed in the clustering and discriminant analysis of in vitro conservation culture conditions of *Capsicum chinense* Jacq.²

Groups	Plant ht (cm)	Number of leaves	Root length (cm)
MS ^a	5.30 a	12.03 a	6.59 a
N50	3.03 c	6.52 c	6.16 a
SUC6	4.21 b	8.80 b	5.43 a
LOP	3.23 c	6.37 c	3.77 b
MOP	2.33 d	3.60 d	3.53 b
HOP	2.05 d	0.50 e	0.40 c
CV ³	4.0	7.4	7.8

²Main effect means followed by the same letter (in columns) are not significantly different at $P \leq 0.05$ according to Duncan's multiple comparison test.

³Analysis of variance's coefficient variation.

^aMS, control treatment (Santana-Buzzy et al., 2006); N50, 50% below the control medium nitrate concentration; N150, 50% over the control medium nitrate concentration; SUC6, sucrose 6%; SUC8, sucrose 8%; MAN2, mannitol 2%; MAN4, mannitol 4%; MAN8, mannitol 8%; SOR2, sorbitol 2%; SOR4, sorbitol 4%; SOR8, sorbitol 8%.

germplasm responses on minimal growth but with the same tendency in behavior (Tables 4, 5, and 6; Figs. 2A and 2B). We found a minimal growth condition (MAN2) that allowed the same growth behavior response in different genotypes (Tables 3 and 4; Fig. 1C).

The MAN2 culture condition will let us use it as a generalized in vitro conservation system that spans the subculture time to several months; it permits the storage of large quantities of germplasm and reduces conservation costs (compared with micropropagation) as a product of less chemical and material consumption, less hand labor demanded, and requires a small storage space in the culture room.

The effect of nitrogen reduction in the culture medium (N50) had intermediate reduction on growth, but slightly higher than SUC6, specifically in the accessions Nux and MT, and variable response in root length (Tables 3 and 4). Nitrogen stress (50% or 150%) did not provide good culture conditions for Habanero pepper conservation; this agrees with Bonnier and Van Tuyl (1997) who found different responses in 10 genotypes of oriental lily (Asiatic hybrids), *Lilium longiflorum* L. during 28 months of in vitro conservation as Murashige and Skoog (1962) nutrients were reduced to one-fourth strength and sucrose increased to 6% and 9% at 25 °C in a 16-h photoperiod.

SUC6 had intermediate growth for all genotypes (Tables 3, 4, and 6); the low impact could be explained by the fact that sucrose is used by plant cells to feed. Sucrose is the substrate of the invertase enzyme and the osmoregulator effect diminishes with time. The higher sucrose concentration (8%) had low osmotic potential effect and grouped with cultures: sorbitol 2% and nitrogen 150%. Hddier and Desjardins (1994) found that with an increase of sucrose concentration to 30 or 50 g·L⁻¹ in *Fragaria* × *Ananassa* Duch. cv. Kent micropropagation, the rate of CO₂ assimilation was reduced up to 50%. Similar effects have been reported for *Rossa multiflora* L cv. Montse (Capellades et al., 1991). Sucrose as a carbon source has proved not to be deleterious for pepper (*C. annum* L.) tissue in vitro, even if it is essential for the somatic embryogenesis process from young mature leaves at 8% concentration (Kintzios et al., 2001).

The addition of osmoregulators based on carbon-containing molecules that are not degraded by plant cells, like sorbitol and mannitol, induced a strong effect on minimal growth at high concentrations (HOP group, Fig. 2B), but anatomic and physiological alterations were observed: leaf chlorosis, rolling and distorted leaves, and out-of-proportion shoot-root relationships. Intermediate osmoregulator (MOP group) concentrations induced a suitable growth reduction, although mannitol 2% (MAN2) showed clear differences in plant quality as described previously. This suggests that long time exposure to high osmotic potential could damage the physiology of some tissues or organs of Habanero pepper.

Fig. 2. Group performance according to the behavior of *Capsicum chinense* Jacq. germplasm in response to the in vitro conservation culture conditions. (A) Cluster analysis based on UPGMA procedure. (B) Class formation of canonical discriminant analysis.

Mannitol 2% promoted minimal growth without physiological damage; this agrees with Rech et al. (2005); they observed that the addition of mannitol also significantly improved shoot survival, although no difference was observed between 1% and 2% concentrations.

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