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Osmotic Regulation and Viability during Storage of Potato Microtubers Obtained *in Vitro*

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Abstract: Dehydration, viability, proline, sucrose and glucose concentrations were evaluated on green and white microtubers during storage at two different temperatures. After 10 months at 4 °C, the green and white microtubers showed shrinkage with a dry weight loss of 3.91% and 3.15%. Both, the green and white microtubers at 4 °C presented an enhanced sprouting after storage. At 23 °C, the green microtubers lost the lowest quantity in dry weight (0.8%) and white microtubers lost 2.2%. This behavior is possibly related to the increase in the thickness of the peridermis observed in green microtubers or to the osmotic regulation mediated principally by the observed concentrations of proline and glucose but not sucrose. The best storage conditions for potato microtubers obtained *in vitro* were at 4 °C for green or white microtubers for up to 10 months with little loss of viability.

Key words: Osmolite, peridermis, *Solanum tuberosum*, suberization, tuberization.

1. Introduction

Microplants maintenance in slow growth during *in vitro* culture constitutes an important method for potato genetic resources conservation [1]. Nevertheless, inhibition of gas exchange into the *in vitro* culture flasks causes growth abnormalities, which may not warranty an adequate genetic stability of the micropropagated plantlets [2].

However, miniature tubers (microtubers) produced by potato plantlets growth *in vitro* are convenient for seeds production [3], their maintenance and handling of disease-free material [4]. These tubers may be stored during several months in cold temperature or for short periods at room temperature, in both cases, during this storage period the tubers lose water gradually and they adapt naturally to drying [5].

On the other hand, Naik and Sarkar [6] showed that microtubers may become green when stored under light, presenting less shrinking, lower biomass loss and better sprouting after a 4-month storage. Their results suggest that these conditions may be due to greening since it seems to allow periderm thickening by the suberization of tubers. Furthermore, a more resistant periderm is associated with a higher tolerance to water loss [7].

During storage, due to hydric stress, osmoactive substances can be synthesized and metabolic and physiologic changes can alter the tuber survival [8].

The objective of the present study was to evaluate the storage influence on osmo regulation and viability of green or white potato tubers obtained *in vitro*.

2. Material and Methods

This study was carried out in the Plant Tissue Culture Laboratory of the Morphology and Function Unit at the Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Disease-free Potato plantlets (*Solanum tuberosum* L.) cv. Tollocan growing *in vitro* were obtained from Programa Mexicano de Papa (INIFAP, Campo Experimental Valle de Toluca, Metepec, Edo. Méx). Plantlets were

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multiplied through uninodal cutting subculture, every 30 days (throughout 4 months), they were maintained in glass test tubes of 25 × 150 mm, containing 15 mL of semisolid Murashige and Skoog [9] media, supplemented with 100 mg/L inositol, 0.4 mg/L thiamine, 2% sucrose and 8 g/L agar. Media pH was adjusted to 5.7 before autoclave sterilization for 15 min at 121 °C and 1.05 kg/cm^2. Cultures were maintained at 22±2 °C and photoperiod of 16 h (100 μmol/m^2·s photonic flux of cold white fluorescent light).

For microtuber induction culture media at the same pH was used, but with 80 g/Lsucrose and 5 μM 6-BA (Benciladenine), cultures were maintained at 20 °C and in darkness per 30 days. Tubers were harvested and divided into two groups: (1) A group of tubers that were placed in light (30 μmol/m^2·s photonic flux of cold white fluorescent light) for green microtuber induction (modified from Naik and Sarkar [6], (2) another group was kept in darkness (white microtubers). Both groups were maintained at room temperature of 23 ± 1 °C and 38%-43% RH (Relative Humidity) for 10 days.

After getting the induced tubers, the experiment was performed in a completely randomized factorial design with green and white microtubers that were stored in refrigeration (4 °C with 58%-65% RH, in darkness), or at room temperature (23 °C with 38%-43% RH, in darkness), getting 4 treatment groups with 5 repetitions (unsealed Petri dishes with 10 tubers each).

During the storage period (10 months), biomass loss and sprouting were determined, as well as glucose, sucrose, and proline levels according to Lorenzen and Ewing [10], Stepan-Sarkissian and Grey [11], and Bates, et al. [12], respectively.

Microtubers were fixed with FAA (Formaldehyde-Acetic Acid-Ethyl Alcohol 5:5:90), for 12 h. Then they were dehydrated in ethyl alcohol (50%, 75%, 85%, 96% and 100%) for 2 h in each solution, the later (100%) was repeated three times. Thereafter they were placed in a mixture of ethanol: xylol, 75/25, 50/50, 25/75, and finally xylol, for 6 h in each solution. Microtubers were then embedded in paraffin blocks at 57 °C and sections of 10 μm thickness were obtained in a vertical sliding microtome. The sections were stained with the safranine-Fast Green double staining technique [13].

Data were analyzed using two-way ANOVA, when appropriate, with the Tukey's Test and statistical significance was accepted when $p < 0.05$. Percentage data were transformed using arcsine transformation before analysis. Results shown are in a non-transformed way.

3. Results and Discussion

The tuberization period of cuttings at each node was uniform, in 7 days the 50% of all tubers appeared, as reported by Simko [4] (tuberization uniformity is expressed as the time between the appearance of 25% to 75% from all microtubers). The plantlets formed sessile tubers, situated next to the stems. Some tubers were formed at the end of short stolons. Between 2 to 3 days in light conditions, the tubers obtained began to turn green, according to Naik and Sarkar [6].

After 10 months of storage at 4 °C, green and white microtubers showed tissue contraction and dry weight loss between 3.15% and 3.91%. Both green and white tubers maintained at 4 °C, had higher viability than those maintained at room temperature, measured as germination after the storage period (Table 1). Some of these tubers sprouted during storage, likely due to the high humidity.

On the other hand, green tubers stored at 23 °C had the lowest dry weight loss (0.83%) among all treatments, although most of these tubers died (50%) (Table 1), as suggested by Naik and Sarkar [6], they reported that greening improved tuber storage in terms of contraction, biomass loss, and sprouting.

Furthermore, white tubers lost less dry weight at 23 °C (2.23%) than at 4 °C (3.15%), nevertheless white tubers maintained in refrigeration or room temperature consumed less glucose than green ones (Fig. 1a,Table 2).

Table 1 Effect of storage conditions on dry weight and microtuber sprouting after 10 months of storage.

Treatment	Dry weight			Characteristics of microtubers during storage (10 months)			Microtubers sprouting after storage (%)
	Initial (mg)	Final (mg)	I – F (mg)	No sprouted (%)	Sprouted (%)	Dead (%)	
Refrigeration green microtubers	11.41 ± 3	7.5 ± 2	3.91a	60.71a	28.57	10.71	60.71a
Refrigeration white microtubers	10.97 ± 3	7.8 ± 3	3.15a	66a	18	16	60a
Room conditions green microtubers	11.59 ± 4	10.7 ± 3	0.83b	50b	0	50	43b
Room conditions white microtubers	10.77 ± 2	8.5 ± 2	2.23a	49.27b	24.63	26.08	45b

Data shown are combined from three experiments with 50 microtubers per treatment. Data in each column containing the same letter have no significant difference among each other ($p < 0.05$, Tukey's Test).

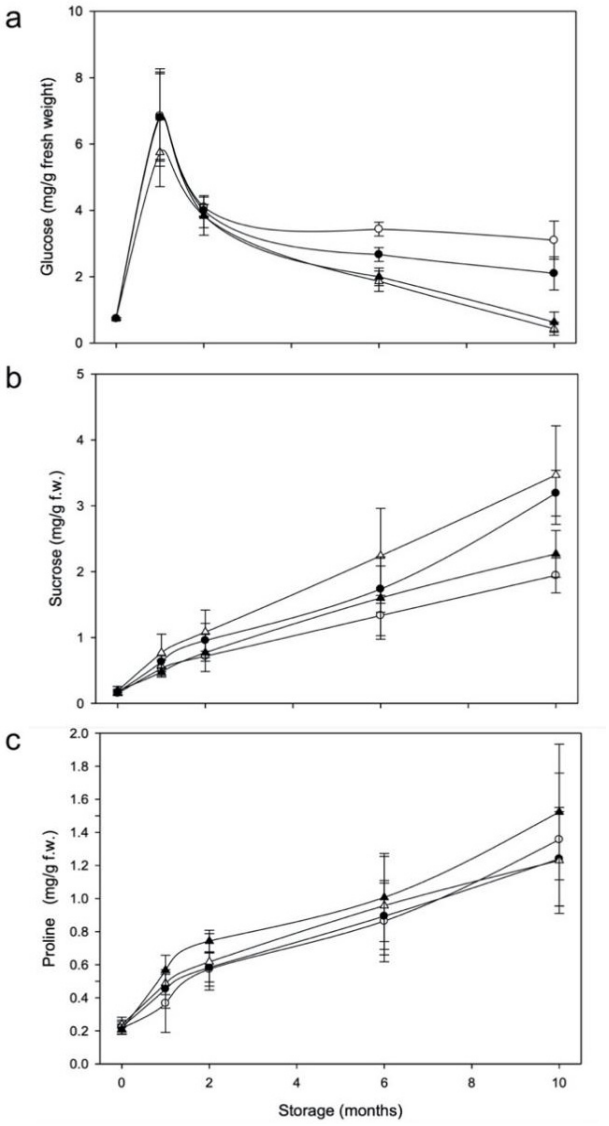
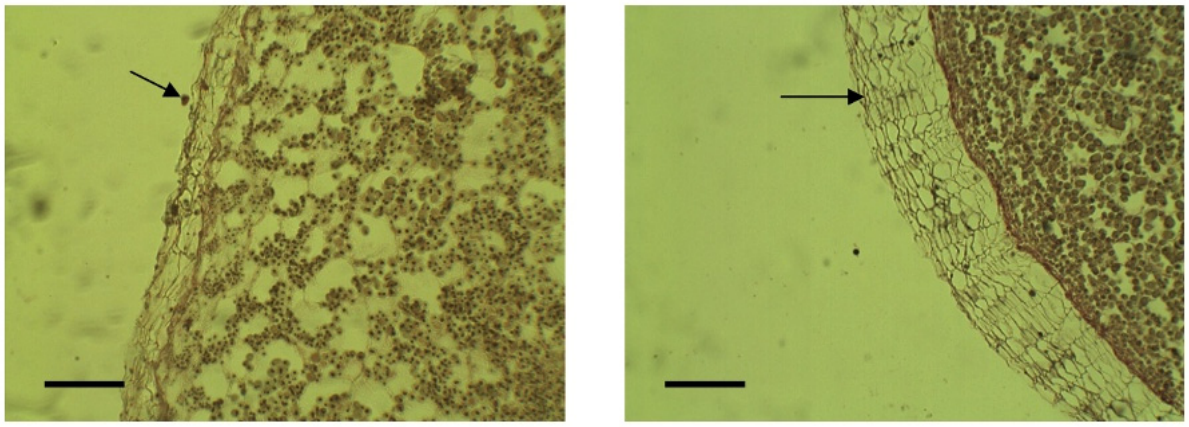


Fig. 1 Storage effect over osmolytes concentration in potato microtubers for 10 months storing. (a) Glucose concentration (mg/g of fresh weight). (b) Sucrose concentration (mg/g of fresh weight). (c) Proline concentration (mg/g of fresh weight).
White microtubers stored at 4 °C (open circles); white microtubers stored at 23 °C (closed circles); green microtubers stored at 4 °C (open triangles), and green microtubers stored at 23 °C (closed triangles). Data shown are the mean ±standard deviation of 5 replicates for each treatment.

Table 2 Osmolytes concentration in potato microtubers after 10 months storing.

Osmolytes	White microtubers			Green microtubers		
	Initial	10 months		Initial	10 months	
		4 °C	23 °C		4 °C	23 °C
Glucose (mg/g f.w.)	0.74 ± 0.3	3.15 ± 0.6[b]	2.1 ± 0.5[a]	0.81 ± 0.3	0.43 ± 0.2	0.63 ± 0.3
Sucrose (mg/g f.w.)	0.53 ± 0.13	1.92 ± 0.31	3.38 ± 0.6	0.62 ± 0.21	4.35 ± 0.91	2.28 ± 0.34
Proline (mg/g f.w.)	0.39 ± 0.29	1.31 ± 0.47	1.22 ± 0.32	0.63 ± 0.21	1.21 ± 0.35	1.53 ± 0.41

The same letter in a column indicates no significant difference as determined by a Tukey's test with a 95% level of confidence ($p < 0.05$).



(a) (b)

Fig. 1 Histological sections of white (a) and green microtubers (b), showing strong differences in the thickness of epidermis in the latter. The bar represents 250 µm.

This behavior in all microtubers might be related to osmotic regulation, mediated by the high proline and sucrose concentrations found (Figs. 1b and 1c; Table 2).

Regulation of water loss mediated by glucose and proline as principal components has also been reported in tomato and sweet potato cells in suspension [14, 15].

The gradual decrease in the metabolic activity of the isolated microtubers and a progressive increase in their weight loss can be attributed to their immature state in which, natural senescence occurs due to respiration and other metabolic processes in their tissues during storage [1]. High glucose consumption of green microtubers may be due to a high respiratory activity in room conditions, which produces a decrease in viability.

The result was in agreement with varietal difference in fresh weight loss as in Diamant, Asterix and Granola cultivar [16, 17] or microtubers of the Kennebec cultivar which only had a decrease in fresh weight of approximately 10%, even after 4 months of storage in room temperature, as recorded by Park, et al. [18]. In microtubers of Kufri cultivar stored for 8 months at 4 °C or under environmental conditions, weight loss was higher in tubers of 4 mm as compared to 6 and 8 mm [19].

The size, storage conditions and containers influence the storability of potato microtubers. Small microtubers are more vulnerable to storage damage [6]. Hossain, et al. [17], reported that decay of microtubers is also a very serious problem during storage. More than 45% of the total number of small microtubers was lost in 4 months of storage.

The different weight loss among tubers might be related to the presence of a thin peridermis and less suberization of 4 to 5 cell layers (Fig. 2a), likely having a different chemical composition of suberin

and waxes less resistant to water loss [20], than the thick peridermis of 12 to 15 cell layers observed in green microtubers (Fig. 2b). This way we corroborate the hypothesis of Naik and Sarkar [6], which states that tuber greening likely thickens or suberizes the microtuber peridermis, this process makes them more tolerant to water loss, explaining the lower weight loss at 23 °C, but not the loss of viability.

4. Conclusions

The effect of green and white microtubers storage on their water loss and osmolytic dynamics, under refrigeration or environmental conditions, was investigated.

Microtuber greening induced by light produced a thick peridermis in tubers, which might be useful for diminishing weight loss at 23 °C, nevertheless, the viability of these tubers decreased, possibly due to a higher respiratory activity. White tubers stored at 23°C lost less weight and had less viability than those stored in refrigeration.

Both, green and white microtubers stored in refrigeration presented maximal weight loss but also the highest viability rates. Best conditions for microtubers storage were either green or white microtubers at 4 °C, to keep these propagules in good condition for longer periods.

Acknowledgments

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