Tissue Osmotic Potential and Membranes Permeability of Nopalitos (*Opuntia* spp.) Affected by Changes in Soil Water Potential♦

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ABSTRACT

The effects of environmental factors on physiological characteristics of nopalito are scarcely known in nature and farms. The effect of the soil water potential (Ψ_W) on osmotic potential (Ψ_π) and membranes permeability (damage index, I_D) under greenhouse conditions were evaluated in five cultivars and one wild accession of nopalitos. A completely randomized design, with one plant as an experimental unit and six replications, per treatment, were used. Following a 60-day drought treatment, the osmotic potential diminished exponentially, on average from -0.24 up to -1.33 MPa in the wild accession *O. streptacantha* while cv. Copena V1 reached the maximum reduction, from -0.30 to -1.48 MPa, as soil Ψ_W decreased from -0.39 up to -3.27 MPa. At -3.27 MPa of soil Ψ_W, the I_D ranged among cultivars from 3.5% in cv. Solferino up to 29.0% in cv. Moradaza. It is concluded that changes in Ψ_π of nopalitos may be an intrinsic physiological characteristic to distinguish *Opuntia* plants. The high tolerance of *Opuntia* spp. to soil water deficit, for a relatively long time (60 days), is defined, among several metabolic modifications, by small changes of Ψ_π and minimum cellular membranes damage.

Keywords: Cellular osmotic potential, *Opuntia* spp., water potential, water restriction.

INTRODUCTION

Cactus of the genus *Opuntia* belongs to a xerophyte group of plants, mainly growing in the wild of arid and semiarid regions with erratic rain and poor soils affected by erosion (Reyes-Agüero *et al*. 2005a). Because their high ecological adaptability, *Opuntia* plants are widely cultivated as commercial plantations in Mexico, Brazil, Chile, Argentina, Italy, Spain, and U.S.A. (Reyes-Agüero *et al*. 2005b). "Nopalitos" are young cladodes or vegetative young buds of adult plants (*Opuntia* spp.) eaten in Mexico as vegetable (Reyes-Agüero *et al*. 2005a, b).

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The quality parameters of nopalitos are defined by consumers, farmers, and merchants; but, up to now, they are not formally nor totally described. However, it could be said that succulence of nopalitos, partially determined by water content and chemical composition of their tissue, is one of the most important characteristics for consumers (Corrales-Garcia *et al*. 2004). The effects of natural and cultivation environments on the physiological characteristics and quality of nopalitos are partially known.

Due to their physiological and biochemical characteristics, such as their photosynthetic crassulacean acid metabolism type (CAM), and their exceptional resistance to drought, *Opuntia* plants can be a model for studying the metabolic changes that lead to plant tissue survival, even when the plant remains without water supplies for several weeks (Pimienta *et al*. 2003; Aguilar and Peña-Valdivia 2006).

Among the responses of some plants to water deficit, is the active accumulation of solutes, named osmotic adjustment, contributing to maintain turgor and, therefore, growth during water stress (Loveys *et al*. 2004). Also, as a result of these adjustments, plants can maintain water uptake and their physiologic processes.

Cellular membranes have been recognized as a target for several stresses caused by both biotic (pathogenic parasites) and abiotic (water and nutriment deficit, heat, cold, salinity) factors (Shewfelt 1992). Changes in membrane lipid composition and activity of enzymes, depending on the membrane integrity, can affect the cellular integrity, regulation of the cellular content leakage, and physiochemical properties of the membrane itself (Shewfelt 1992; Prášil and Zámečník 1998). The electric conductivity of the medium where the tissue is suspended has been used to evaluate the integrity and functionality of the cell membranes (Abraham *et al*. 2004; Sanchez-Urdaneta *et al*. 2003 and 2004). Major effects in cellular membranes of relatively short-time water-stressed plants have been quantified in cultivated plants. After 48 hours of water deficit, polyethylenglycol (PEG) caused between 18 and 28% of membrane damage in barley leaves (Kocheva and Georgiev 2003). Also, maize shoots membranes were affected between 20 and 65% when they reached water deficit equivalent to -0.15 and -1.76 MPa (10 and 40% PEG6000 added to the substrate, respectively) (Mohammadkhani and Heidari, 2007). Similarly, 13-day-old maize plants exposed for 24 hours to -1.4 MPa of substrate water potential resulted in cellular leaves membranes significantly damaged (10%) (Valentovič *et al*., 2006).

The aim of this work was to describe the effect of soil water potential (Ψ_W), by withholding water for 60 days, on the osmotic potential (Ψ_π) and the cell membranes properties of nopalitos (*Opuntia* spp.) of Atlixco, Copena V1, Milpa Alta, Moradaza, and Solferino cultivars, and a wild accession of *O. streptacantha* grown under greenhouse conditions.

MATERIALS AND METHODS

Five Mexican cultivars, Atlixco, Copena V1, Milpa Alta, Moradaza, and Solferino of nopal (*Opuntia ficus-indica*), and a wild accession of *O. streptacantha* were used (Table 1). From each cultivar, 12 one-year-old cladodes were collected and planted in pots with 25 kg of soil (a mixture of two parts of forest soil and one part of fine sand, with a sandy clay loam texture). Plants were watered twice per week until vegetative buds (nopalitos) developed and reached an average length of 8 to 10 cm. At this moment plants were divided into two groups, one continued to be watered through duration of the experiment and, to the other one, water was withheld for two months. One nopalito was harvested from each experimental unit every 10 days.
Cellular juice was extracted from a nopalito sample using a hypodermic syringe and the Ψ_π was then quantified in a vapor pressure osmometer (Wescor 5520, USA). Membrane permeability and damage

index were quantified following the technique reported by Prášil and Zámečník (1998) and Sanchez-Urdaneta *et al*. (2003 and 2004). In this case two tissue cylinders (3 cm in diameter) obtained from apical, central and basal zones of the nopalito, were weighted and submerged in 25 ml of deionized water, which was continuously and gently shaken; then, the electric conductivity of media was quantified every 15 minutes.

Table 1. Cultivars and wild accession of *Opuntia* spp. used in this study

Cultivars and Wild Accession of *Opuntia* spp.	**Locality of Recollection or Production**
Atlixco (*Opuntia ficus-indica*)	Tlaxcalcingo, Puebla State
Copena V1 (*Opuntia ficus-indica*)	Chapingo, Mexico State
Milpa Alta (*Opuntia ficus-indica*)	Distrito Federal
Moradaza (*Opuntia ficus-indica*)	Tecamac, Mexico State
O. streptacantha	Tamazunchale, San Luís Potosí State
Solferino (*Opuntia ficus-indica*)	Tecamac, Mexico State

According to Prášil and Zámečník (1998) leaked electrolytes into the media is directly proportional to membranes damage of stressed tissues; then the damage index could be estimated using the following equation:

$$I_D\,(\%) = ((R_t - R_o)/(R_f - R_o))\ 100$$

where

I_D is the damage index

R_t is the electrical conductivity of the media of stressed tissue

R_o is the electrical conductivity of the media of nonstressed tissue (control)

R_f is the electrical conductivity of the media of nonstressed tissue after having been frozen (to ensure total liberation of tissue's electrolytes).

To calculate I_D, the values of electric conductivity were adjusted to one gram of tissue, data from the three nopalito zones sampled were averaged and it represented one repetition. During each sampling (every 10 days), a soil sample was taken 10 cm from the pot surface and placed in cups of commercial thermocouple-psychrometers (Wescon C-52, Inc. Utah, USA) connected to a dew-point microvolmeter (Wescon HR-33T, Inc, Utah, USA). After 6 h of thermal and water-vapor equilibrium the water potential (Ψ_W) was determined using the dew-point mode.

A completely randomized design, with one plant as an experimental unit and six replications per treatment were used. The results were analyzed using a variance analysis among treatments, means were grouped with Tukey's Honestly Significant Difference (HSD) tests with $P<0.05$ using SAS statistical software. Graphic representation of the data was done with the Jandel Scientific SigmaPlot (version 9) software for personal computers.

RESULTS AND DISCUSSION

Soil water potential (Ψ_W)

Watered pots maintained soil humidity around -0.39 MPa (38% moisture). In contrast, withholding water gradually reduced soil Ψ_W, from -0.39 up to -3.27 MPa after 60 days (Figure 1).

Osmotic potential of nopalitos (Ψ_Π)

Osmotic potential in nopalitos from watered plants averaged -0.24 MPa, including together *Opuntia* cultivars and wild accession. Withholding water decreased soil Ψ_W and Ψ_Π in all six treatments, this reduction followed an exponential trend, and statistic differences among some treatments were observed. Among the highest Ψ_Π in watering treatments were *O. streptacantha* and cv. Copena V1 with -0.20 and -0.29 MPa, respectively. A drastic droop in Ψ_Π occurred when soil Ψ_W changed from -0.39 (watered condition) to about -1.46 MPa, after the first 10-30 days of withholding water; but, the Ψ_Π droop in cv. Atlixco, Copena V1, and Moradaza at this soil Ψ_W was conspicuous. Thereafter, for the next 30-50 days, changes of Ψ_Π were smaller, but it continued until soil Ψ_W reached -3.27 MPa, after withholding water for 60 days. At this moment, the Ψ_Π reached the lowest values in all six cultivars and in the wild accession. In this soil, Ψ_W *O. streptacantha* and the cv. Copena V1 maintained the wider significant difference, -1.34 and -1.48 MPa, respectively (Figure 2). These results indicated that nopalito reacts as other tolerant-to-dehydration species because they develop some kind of metabolic regulation from the beginning of water stress. Thus, they survive drought, by maintaining tissue turgor, and their stomata may remain partially open (during the night in the case of the nopal plants), even when water deficit remains (Loveys *et al*. 2004).

Electrolytes leakage and damage index (I_D)

It has to be mentioned that in the wild accession, vegetative buds were inhibited almost totally after withholding water, probably due to an infection of the *Dactylopius coccus* (cochinilla) on nopal cladodes. Therefore, electrolytes leakage and I_D were evaluated only in the cultivars. Similarly, in cv. Copena V1 vegetative buds exposition were partially inhibited, so electrolyte leakage evaluations in this cultivar were done every 20 days.

Electrolytes leakage from nonfrozen tissue, in all treatments, showed two phases; the first one was fast and brief, and took about 10 min. The second one was relatively slow and longer, and it took from 20 to 60 min (Figure 3). However, the electrolytes leakage was variable among cultivars and, in general, affected by the soil Ψ_W (Figure 3). The differences in the rate of electrolytes leakage among watered treatments were small, but statistically significant (Figure 3, $P<0.05$ and Table 2). This result suggests differences in electrolyte composition, electrolytes leakage regulation, or both, among the nopalitos of *Opuntia ficus-indica* cultivars. Electrical conductivity of the media depends on both electrolyte type and concentration (Prášil and Zámečník 1998).

The two-phased electrolyte leakage observed in this work seems to be typical for plant tissues of many species. Similar electrolyte-leakage trend found in nopalito has been documented in several plant organs of bean (*Phaseolus vulgaris* L.), maize (*Zea mays* L.), maguey (*Agave salmiana* Otto ex Salm-Dyck), turnip (*Brasica napus* L.), wheat (*Triticum aestivum* L.), fir (*Abies* sp.), and onion (*Allium cepa* L.) (Prášil and Zámečník 1990 and 1998; Sánchez-Urdaneta *et al*. 2003 and 2004; Tsougkryanis *et al*. 2007).

Freezing caused a fast electrolyte leakage from nopalito tissues, which reached maximum electrical conductivity 30 minutes after starting incubation (less than a half time spent for nonfrozen samples) and was approximately four times higher than in all nonfrozen nopalitos (compare Figures 3 and 4). In addition, clear differences ($P<0.05$) in the maximum electrical conductivity among cultivars was observed when the tissue was frozen (Figures 3 and 4). These results indicate that the type and proportion of electrolytes, or both, are different among *Opuntia* cultivars. Mean maximum electrical conductivity of frozen nopalitos grouped in two, one group with a mean value of 239 (s.e. ±5.41) mS/cm/g tissue, which included Atlixco and Solferino cultivars, while the other group reached a mean electrical conductivity of 198 mS/cm/g (s.e. ±4.27) tissue, and included Copena V1, Milpa Alta, and Moradaza cultivars (Figure 4).

Table 2. Maximum electric conductivity ($mS/cm^2/g$ fresh tissue) of the suspension media of nopalitos (*Opuntia ficus-indica*) of five cultivars. Plants were kept during 60 days without watering.

Time Without Watering (d)	**Atlixco**	**Copena**	**Milpa Alta**	**Moradaza**	**Solferino**
	Electric Conductivity ($mS/cm^2/g$ nonfrozen tissue)				
0	50.85 lm	54.85 k-m	47.16 m	52.14 lm	48.65 m
10	74.45 e-i	-	61.08 j-l	69.75 h-j	55.03 k-m
20	89.30 a-d	72.62 g-i	57.17 k-m	78.39 d-h	60.49 j-l
30	78.85 d-h		65.75 i-k	78.91 d-h	64.22 i-k
40	78.65 d-h	92.02 ab	73.24 f-i	79.40 c-h	84.96 a-e
50	92.68 ab	-	90.40 a-c	90.58 ab	95.16 a
60	88.20 a-d	81.79 b-g	83.93 b-f	95.64 a	88.65 a-d
Cultivar df	4				
Treatment df	27				
c.v.	6.71				
P	0.0001				

Values followed by the same letter indicate similarity of electric conductivity on the Tukey test of mean comparison ($P \leq 0.05$).

Diminishing soil Ψ_W increased significantly the electrical conductivity of resuspension media of nopalitos, and it was almost duplicated in some cultivars when soil $\square_W$ reached the lowest levels, -2.46 and -3.27 MPa, after 50 and 60 days of withheld water, respectively (Figure 3). This response is generally due to metabolic changes that include biochemical and physiological modification, such as composition or reorganization of cellular membranes and cellular osmolytes content (Gigon *et al*. 2004; Tsougkrianis *et al*. 2007). In connection with this increase of electrical conductivity in this study, Ψ_Π dropped with an exponential trend in response to a decrease of soil Ψ_W (Figure 2). Changes at the biochemical level, like composition and functionality of the cellular membranes, have been documented as a drought response of different plant species (Gigon *et al*., 2004). Electrolyte leakage from drought-stressed tissues, both root and leaves, has been used as an indicator of drought tolerance. Moderate and high water deficits induced after 48 hours with 6.25 to 25% polyethylenglycol (PEG) caused great injury -- between 18 and 28% -- in membranes of barley leaves (Kocheva and Georgiev, 2003). Water deficit, equivalent to -0.15 and -1.76 MPa, induced with 10 and 40% PEG6000 added to the substrate, in six-day-old seedlings of two maize cultivars indicated greater electrolyte leakage in roots than in shoots because membrane damage reached between 20 up to nearly 100%, while in shoots membrane damage fluctuated between 20 and 65% (Mohammadkhani and Heidari, 2007). Similarly, in 13-day-old maize plants exposed for 24 hours to -1.4 MPa of substrate water potential, cellular root membranes were significantly more damaged (20-53%) than leaves (5-10%) (Valentovič *et al*., 2006). Beneragama and Yamamoto (2006) observed that leaves of 20-day-old plants of turf-type bermudagrass (*Cynodon dactylon* (L.) Pers.) and bahiagrass (*Paspalum notatum* Flugge) showed a 5-fold increase in electrolyte leakage (between 33 and 41%) as affected by simulated terminal drought (20 days by withholding water), equivalent to a decrease of relative water content from 92 to 69%.

However, cultivars Copena V1, Milpa Alta, and Solferino reached a maximum electrical conductivity before 60 days of withheld water, when soil Ψ_W was only -1.72 or -2.46 MPa, and remained almost constant with a lower soil Ψ_W (Figures 3 B, C, and E; Table 2). This result could be interpreted as a partial recovery of nopal plants from the condition generated by the soil dryness, after 40-50 days of withheld water. In addition, all the cultivars showed the smallest changes of Ψ_Π after 50 days of withheld water, and cv. Moradaza reached the minimum Ψ_Π since 50 days (Figure 3). This reaction could be interpreted as a partial recovery and may be a result of changes at biochemical, physiological, and morphological levels, like modification of carbon assimilation (storage and utilization), osmolites accumulation (*i.e.* proline, oligosarcarides), and antioxidant system, among others (Blum, 1996; Josep, 2005). In addition, it has been experimentally demonstrated that condensed humidity is incorporated, during night, through areoles and hydatodes, to nopal plants; similarly, water incorporation to nopal plants could also happen through the stomata and epidermis (Aguilar and Peña-Valdivia, 2006). In the present study, a similar phenomenon could have occurred because dew point temperatures were reached almost every day during the experiment and sometimes condensed humidity was observed on the nopal plants early in the morning. However, it seems nonconspicuous to all *Opuntia* cultivars.

A lag in reaching the maximum electric conductivity of the media with soil Ψ_W reduction was also observed; tissue of nopalitos from watered plants spent an average of 75 min to reach maximum electric conductivity, while those from plants without watering spent between 10 to 40 min more (Figure 3). This result can be taken as evidence that cellular membranes of nopalitos are altered due to decrease of soil humidity because the electrolyte's leakage was accelerated.

Prášil and Zámecník (1998) suggested expressing alterations in the stressed tissue with the "damage index" (I_D) proposed by Flint *et al*. (1967) because electric conductivity of the media (absolute conductivity) can be affected by the concentration and type of electrolytes in the tissue. In the present study, the I_D of nopalitos increased as soil Ψ_W decreased. But, the soil Ψ_W affected differently the *Opuntia* cultivars because the I_D changes in Atlixco and Moradaza, depending on soil Ψ_W, exhibited a linear trend, while in Milpa Alta and Solferino the trend was sigmoid, and Copena V1, with a lower number of samples, exhibited a Gaussian-like trend (Figure 5). Another difference among cultivars was the maximum I_D at the soil Ψ_W of -3.27 Mpa because I_D fluctuated from 19.21% (s.e. ±1.36), in cv. Atlixco, up to 29.03% (s.e. ±1.38), in Moradaza (Figure 5). Results of I_D confirmed the tolerance of

Opuntia ficus-indica plants to the soil's water deficit because an I_D between 35 and 36% has been calculated for roots of domesticated and wild common bean seedlings, as well as maguey, after remaining only 24 h in a substrate at Ψ_W of -2.35 MPa (Sanchez-Urdaneta *et al*. 2003 and 2004). Also, an I_D between 18 and 28% in leaves of barley, after 48 h of water deficit, has been reported (Kocheva and Georgiev 2003). Related to this aspect, wild variant *O. streptacantha* possibly reached the lowest I_D, among all nopal variants studied, but the experimental growth conditions, mainly soil dryness, RH and temperature, facilitated the *Dactylopius coccus* (cochinilla) infection on nopal cladodes, which is used for production of natural red pigment (carmin). As a result, plants of *O. streptacantha* did promote low vegetative buds.

The high water content per unit leaf area indicates the succulent character of the species. According with Lambers *et al*. (1998) common adaptation of plants tolerant to drought stress, like nopal, is a highly negative tissue osmotic potential. Modification of both succulence and tissue composition on nopalitos as a result of changes of soil water availability will decrease quality.

CONCLUSIONS

Decrease of soil Ψ_W, between -0.39 and -3.27 MPa, affects similarly, but with a different intensity, the Ψ_π in nopalitos (*Opuntia ficus-indica*) of the cultivars Atlixco, Copena V1, Milpa Alta, Moradaza, and Solferino, and between them and a wild accession of *Opuntia streptacantha*. Changes in Ψ_π of nopalitos may be an intrinsic physiological characteristic to distinguish *Opuntia* plants. The high tolerance of *Opuntia* spp. to the soil's water deficit for relatively long periods, up to 60 days, is expressed as moderate changes in the Ψ_π and minimum alterations in the cell membranes permeability.

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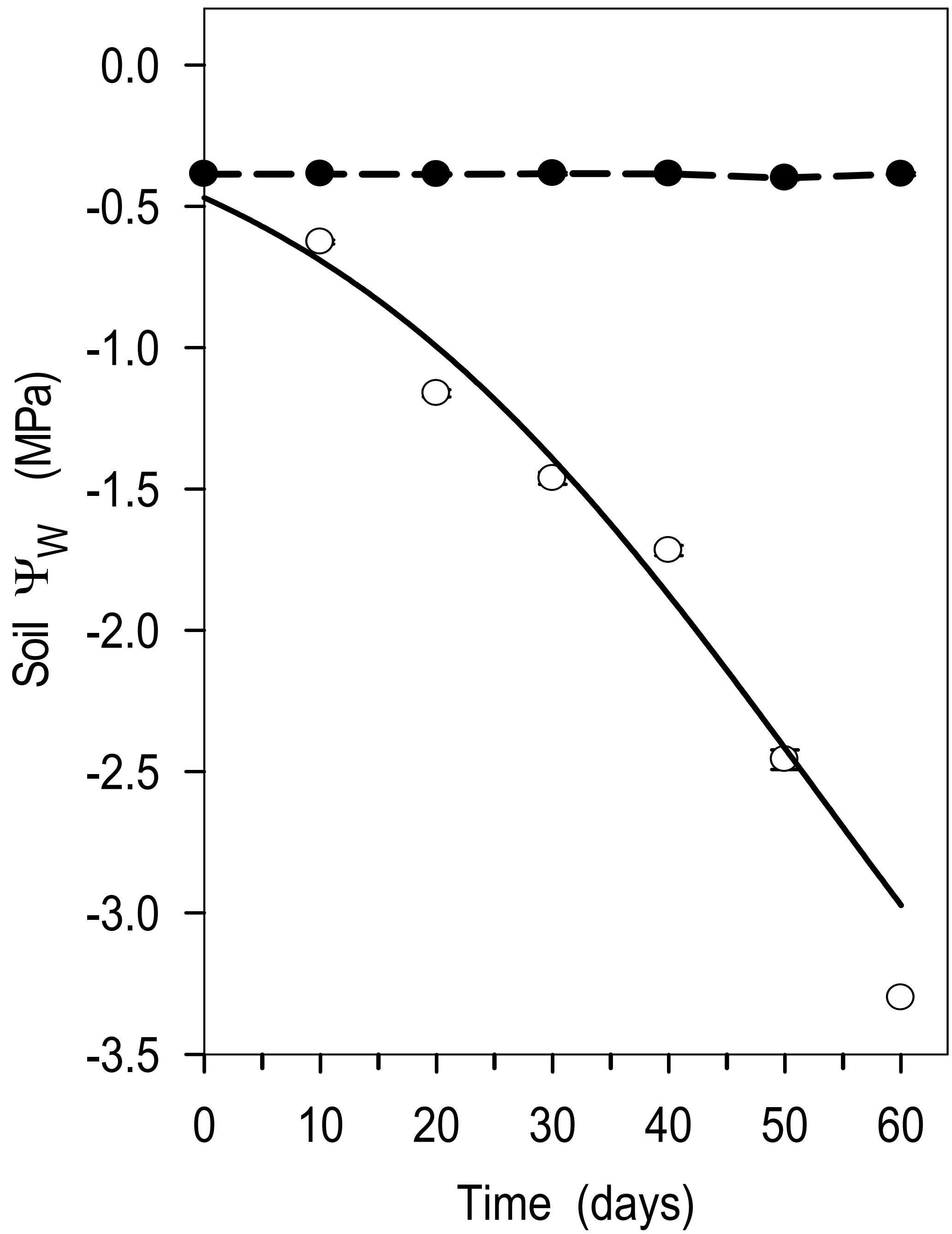

Figure 1. Soil water potential (MPa) in pots where one-year-old plants of Opuntia spp. were grown for 60 days, watering twice a week (●) or withholding watering (○) under greenhouse conditions.
Each data represents the mean value + the standard error (n=6).

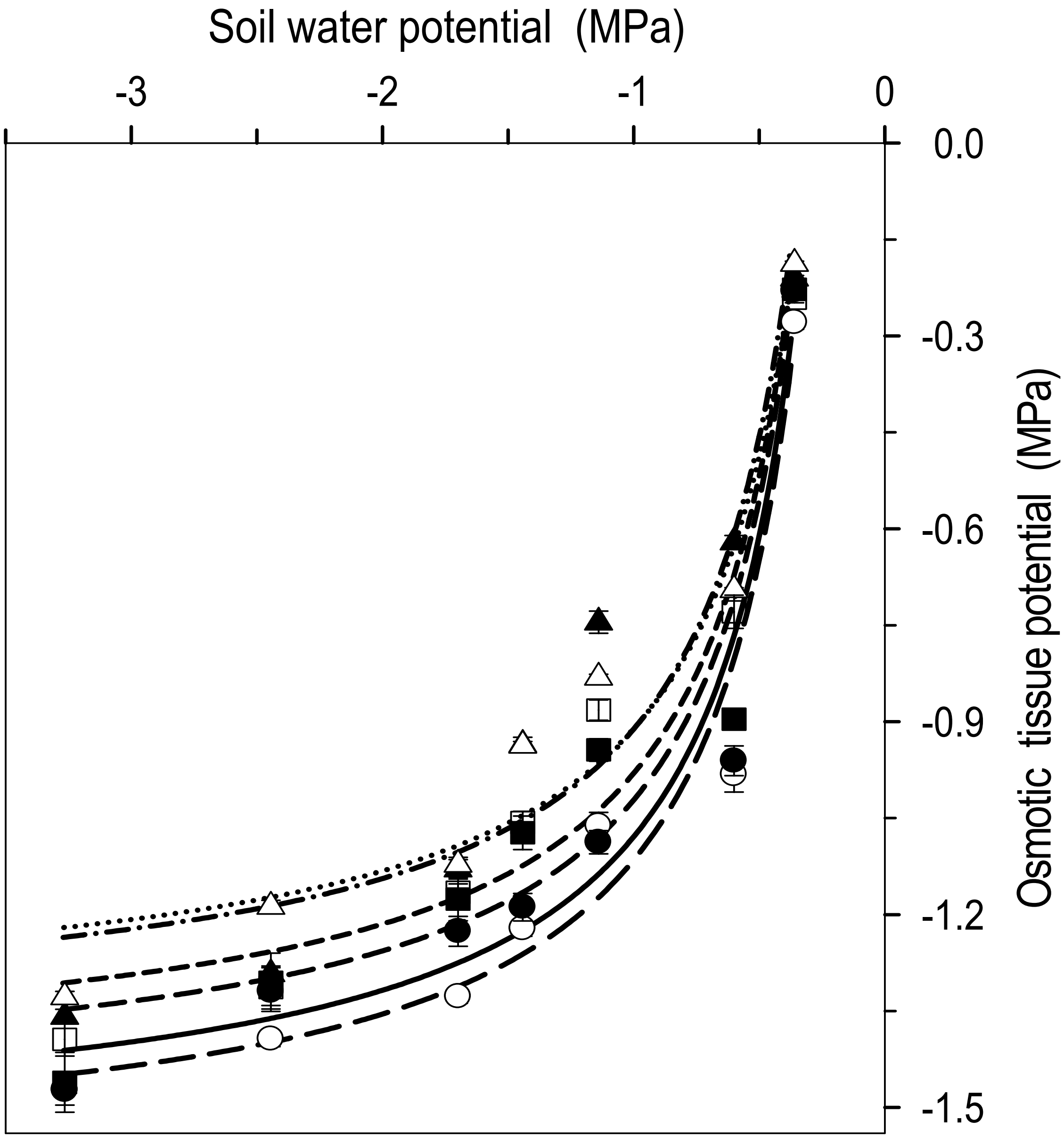

Figure 2. Effect of soil Ψ_W in the osmotic potential of nopalitos *Opuntia* spp.
(●: cv. Atlixco, ■: cv. Copena V1, □: cv. Milpa Alta, ▲: cv. Moradaza,
○: cv. Solferino, and △: wild *Streptacantha* sp. accession).
Each data represents the mean value + the standard error (n=6).

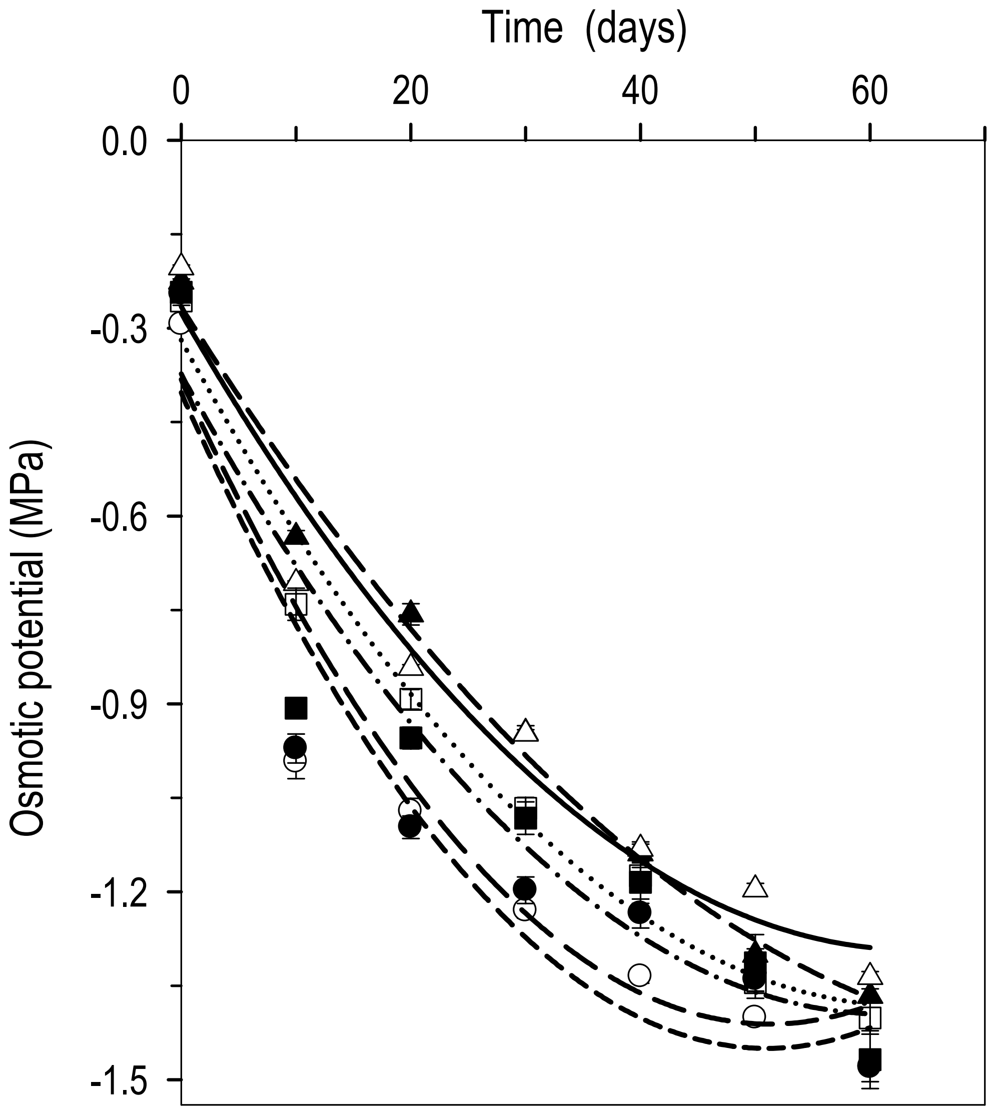

Figure 3. Changes in the osmotic potential of nopalitos *Opuntia* spp. (●: cv. Atlixco, ■: cv. Copena V1, □: cv. Milpa Alta, ▲: cv. Moradaza, O: cv. Solferino, and △: wild *Streptacantha* sp. accession) during 60 day without watering. Each data represents the mean value + the standard error (n=6).

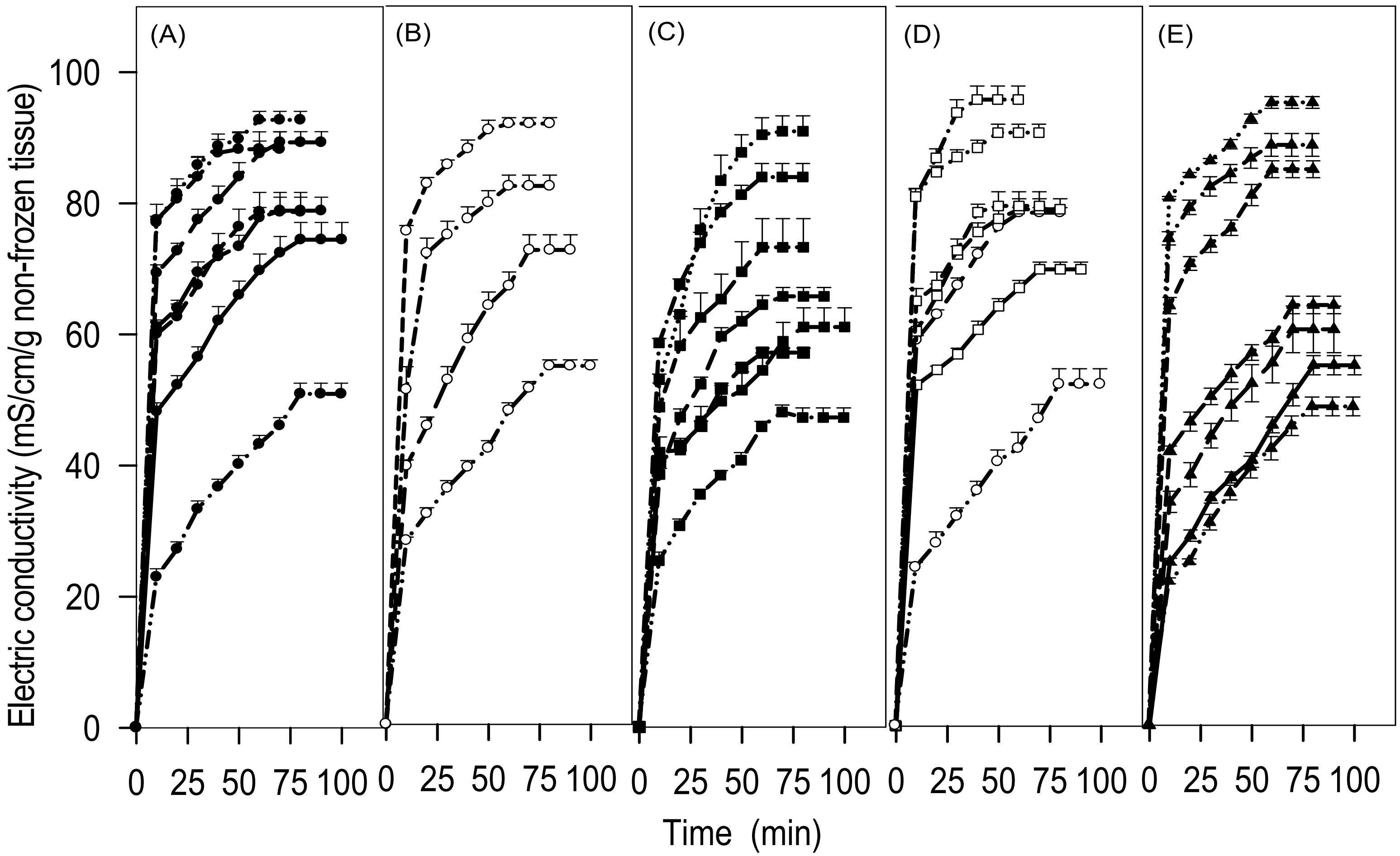

Figure 4. Electric conductivity of resuspension media of nopalito (*Opuntia ficus-indica*) segments, throughout the time. Cultivars Atlixco (A), Copena V1 (B), Milpa Alta (C), Moradaza (D) and Solferino (E) were grown under greenhouse conditions and watering, soil water potential of -0.39 MPa (★), or withhold watering for 10 (-0.63 MPa, ●), 20 (-1.16 MPa, ○), 30 (-1.46 MPa, ■), 40 (-1.72 MPa, □), 50 (-2.46 MPa, ▲) and 60 days (-3.27 MPa, △).
Each data represents the mean value + the standard error (n=6).

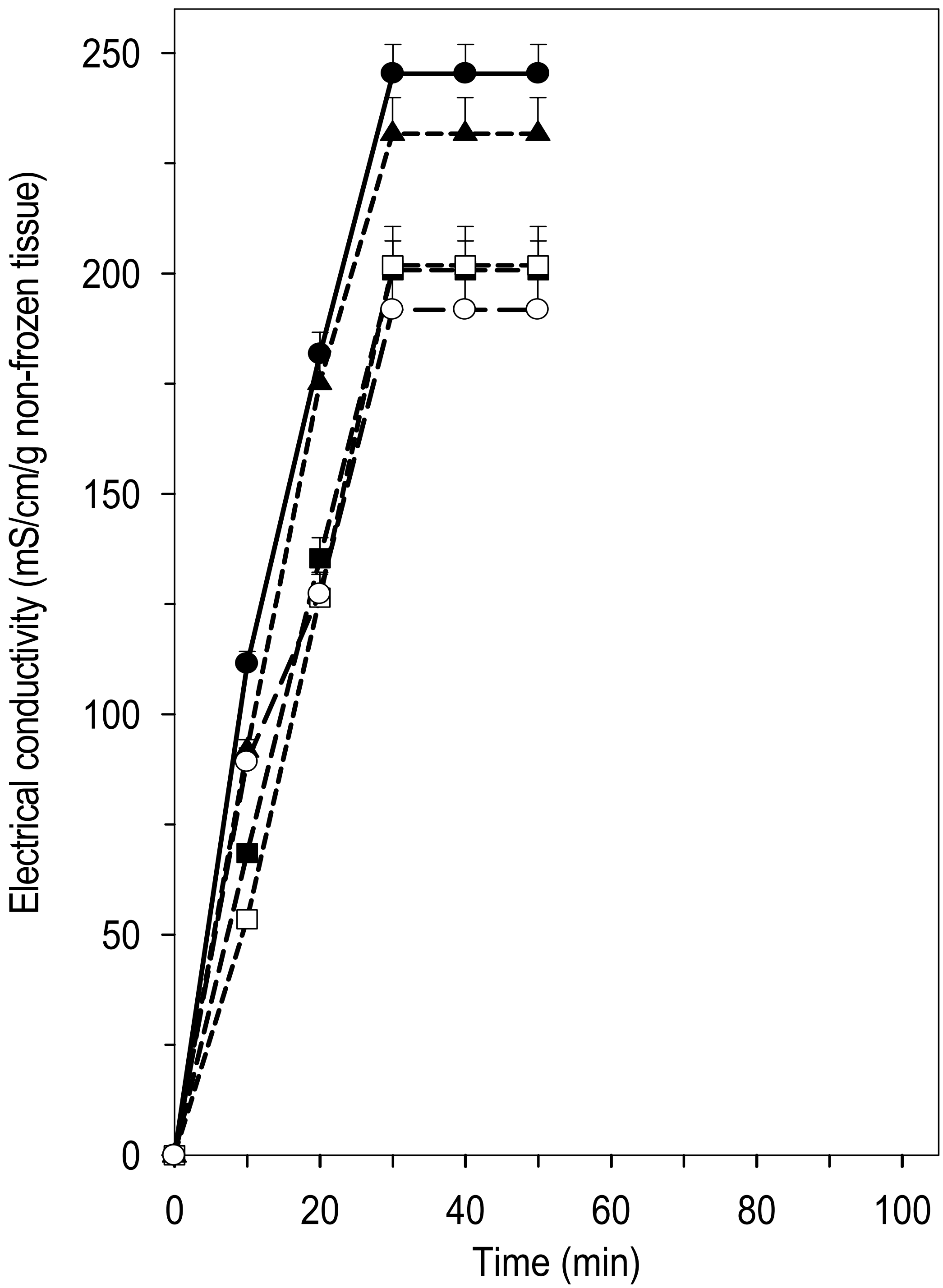

Figure 5. Electric conductivity of resuspension media of frozen segments of Mexican cultivars of nopalito (*Opuntia ficus-indica*). Plants were cultivated under greenhouse conditions, at soil Ψ_A of -0.39 MPa. Cultivars: Atlixco (●), Copena V1 (■), Milpa Alta (□), Moradaza (▲), and Solferino (○). Each data represents the mean value + the standard error (n=6).

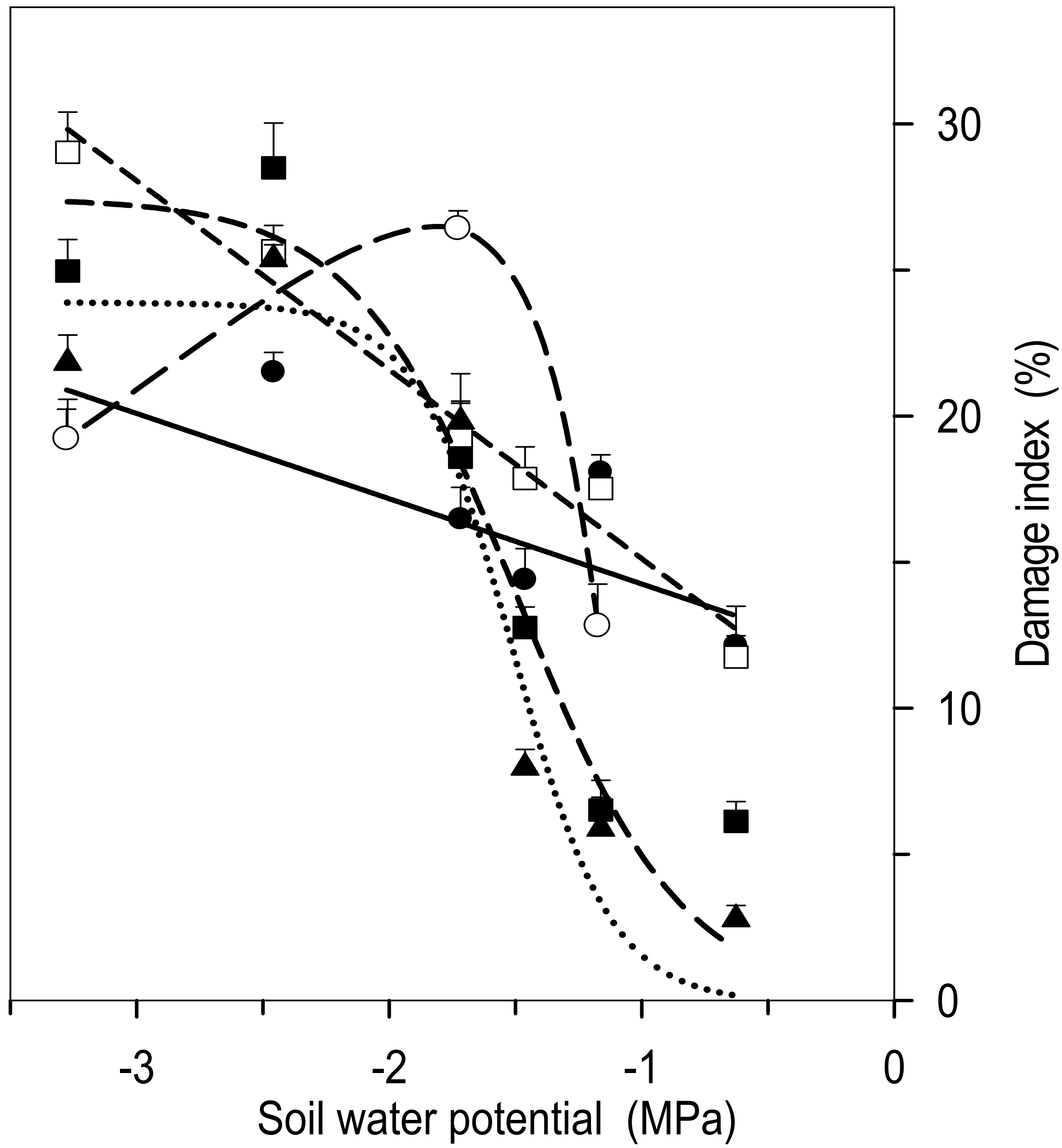

Figura 6. Effect of soil water potential in the index damage of nopalito (Opuntia ficus-indica) cell membranes. Plants of the cultivars Atlixco (●), Copena V1 (■), Milpa Alta (□), Moradaza (▲), and Solferino (O) were cultivated under greenhouse conditions and watering (soil Ψ_A of -0.39 MPa) and withholding watering. Each data represents the mean value + the standard error (n=6).