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Mineral Nutrition and Chlorophylls of *Cucumis melo* L. Grown under Different Saline Conditions in a Protected Wetland Area

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Authors' contributions

This study was carried out in collaboration between the authors. All authors managed the literature searches, read and approved the final manuscript.

Research Article

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ABSTRACT

A field experiment was carried out to evaluate the responses of melon plants (*Cucumis melo* L.) under soil salinity (S1: 0.5 dS m^{-1}, S2: 1.0 dS m^{-1} and S3: 2.5 dS m^{-1}). The irrigation water was from a drainage water channel system following the traditional and sustainable irrigation system. Mineral nutrition, water content and chlorophyll in leaves were studied at flowering and harvesting periods. Nutrient and water content parameters were measured in leaf blades and petioles. Results evidenced significant differences in N, K and Na content. N significantly decreased in response to soil salinity in leaf blades. However, salinity significantly increased K and Na uptake. Macronutrients and micronutrients showed higher concentrations in leaf blades than petioles, except for Kand Na. K/Na ratio was higher in response to soil salinity. Higher K/Na ratio in leaf blades might indicate selectivity for K instead of Na as a strategy to combat salt stress. Significant differences were observed for the chlorophyll content with salinity, decreasing values at higher soil salinity.

Keywords: Plant nutrition; salt stress; K/Na ratio; water content.

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1. INTRODUCTION

Agriculture with high water requirements under increased occurrence of extreme drought events have forced irrigation with poor quality water from both irrigation drainage and groundwater sources, causing processes of degradation, reduction of the production capacity, and soil salinization [1,2]. Salinization affects about 30% of the irrigated land at world level [3]. In Europe, about 1-3 million hectares of the land are affected by salinization and most of the salt-affected soils are situated in the Mediterranean basin [4].

Excessive salinity is one of the most important environmental stress factors that greatly affect the nutrition of plant in arid and semiarid regions. Salinity disturbs the mineral-nutrient relations in plants through their effects on nutrient availability, transportation, and partitioning [5,6,7]. Additionally, salt stress also induces ion deficiency or imbalance of nutrients since the accumulation of high Na concentrations in tissues of plants growing in saline media restricts the uptake of essential nutrients in many species [8,9]. Thus, salt induced injuries can occur due to nutrient deficiency [10], which might cause growth inhibition, leading to cell death [11]. However, the negative effect of soil salinity depends on the plant tolerance aptitude and the salt level, since there are differences among species in the capacity to maintain nutrient concentrations for growth under salt stress [12,13,14].

Plants need an internal water potential below than of the soil to maintain turgor for growth. High salt tolerance is based mainly on the absorption of salts and their use in turgor maintenance. Therefore, the ability of plant cells to reduce sodium concentrations in the cytosol is a key process associated with the ability of plants to grow under high salt regimes [6,15]. K is an essential factor in plants grown under salinity stress since is an osmotic mediating cell expansion and turgor-driven movements and a competitor of Na under saline conditions [16]. Other mineral nutrients also play a vital role in regulating processes influencing growth and responses to environmental stresses.

Agriculture in arid and semi-arid zones characterized by salinity is limited by the salt tolerance of the crop. Muskmelon (*Cucumis melo* L.) is an important irrigated crop in such regions, increasingly being cultivated using low-quality saline waters [5,17,18]. Previous studies reported that salt tolerance of melon plants could be different according to varieties of melon and growing stages [5,17]. Most studies about muskmelon nutrition were carried out in controlled conditions such as greenhouses and controlled environment growth chambers [5,6,17,19].

The objective of the present study was to assess mineral nutrition, water content and chlorophyll content in leaves (leaf blades and petioles) of melon plants (*Cucumis melo* L. cv. saccharinus) at different growth stages: flowering and harvest when they were cultivated under different salinity levels in field conditions.

2. MATERIALS AND METHODS

A field experiment was conducted at Carrizales, situated in Elche (Alicante), in the Southeast of Spain from early April to end of July 2011. Carrizales is an agricultural area included in the Valencian Community List of Wetlands. The area is environmentally suitable because is anecological connection between the Natural Park of El Hondo de Crevillente-Elche and the Natural Park of Salinas de Santa Pola, both of them are included in the Ramsar List of Wetlands of International Importance (Ramsar Convention, URL: http://www.ramsar.org) (Fig. 1).

The climate is arid to semiarid Mediterranean with an average annual rainfall of 250 mm and a thermal regime of warm temperature with an average annual temperature of 19ºC. Along the experiment, received rainfall during planting season was 36 mm, and evapotranspiration recording 608 mm using Penman-Monteith formula [20]. The global radiation during the main stages of melon development was over 600 Wm-2 (Elche Meteorological Station).

The soil in this area was classified as Fluvisols [21] according to FAO/ISRIC/IUS and the nomenclature revision [22], with a clay loam-texture based on the Bouyoucos method [23]. The main characteristics of the soils were determined using standard methods (pH and electrical conductivity (EC) in deionized water 1:2.5 and 1:5 w/v, respectively; N-Kjeldahl [24]; available phosphorus (P) by the Burriel-Hernando method [25]; exchangeable Ca, Mg, K and Na in ammonium acetate extraction 1N and Fe, Mn, Cu and Zn using Lindsay and Norwell extraction [26], and measured by AAS-ES; and available boron (B) in hot water extraction). Table 1 shows the characteristics of the soils in the area of our study.

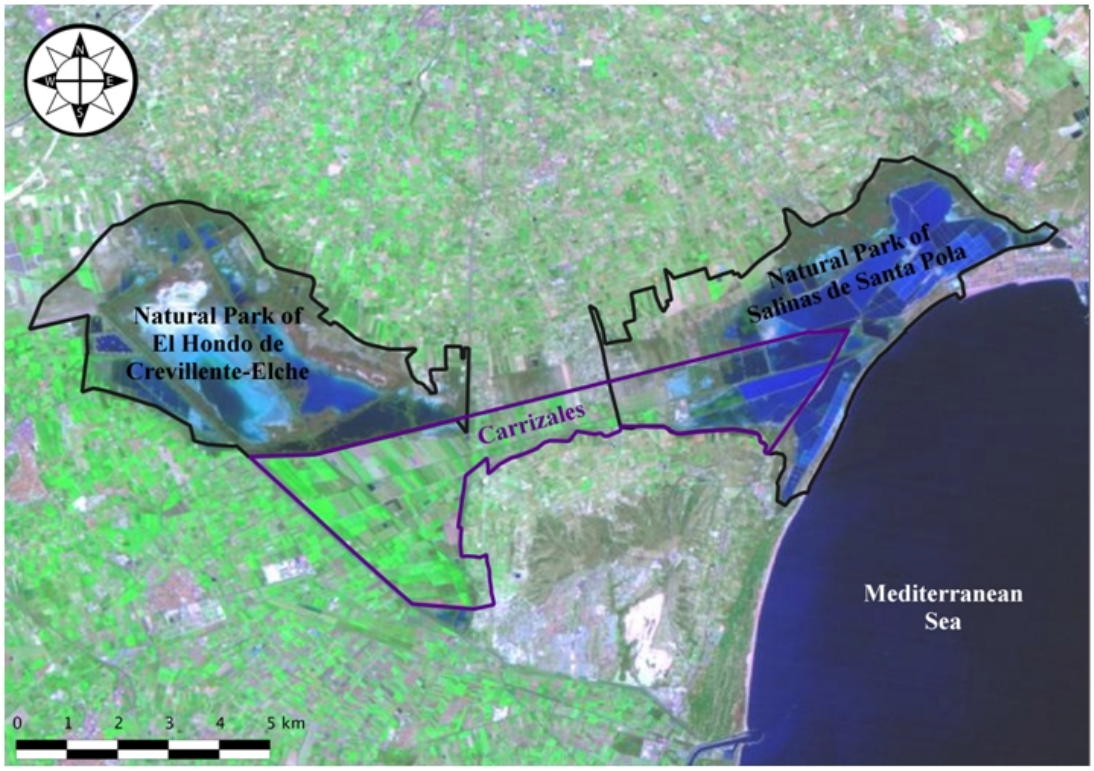

Fig. 1. Location map of *Carrizales* study area and surrounding Natural Parks

Table 1. Different soils characteristics of the study. Data are the means ± standard deviation

Parameters	Units (d.m.)	S1	S2	S3
pH		8.5 ± 0.3	8.5 ± 0.4	7.9 ± 0.1
EC	dS m-1	0.5 ± 0.1	1.0 ± 0.0	2.5 ± 0.1
CO32-	%	51.1 ± 3.6	43.5 ± 4.6	47.4 ± 2.3
N	g kg-1	1.3 ± 0.0	1.5 ± 0.2	1.7 ± 0.3
P	mg kg-1	59.1 ± 7.0	53.5 ± 8.0	39.0 ± 5.1
Ca	g kg-1	3.8 ± 0.3	4.3 ± 0.2	4.5 ± 1.1
Mg	g kg-1	0.9 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Na	g kg-1	0.3 ± 0.0	0.8 ± 0.1	1.3 ± 0.2
K	g kg-1	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.0
Fe	mg kg-1	1.2 ± 0.3	1.3 ± 0.2	1.1 ± 0.1
Cu	mg kg-1	1.1 ± 0.1	1.3 ± 0.1	1.8 ± 0.1
Mn	mg kg-1	2.1 ± 0.6	3.0 ± 0.3	2.1 ± 1.0
Zn	mg kg-1	1.4 ± 0.4	1.2 ± 0.3	1.3 ± 0.6
B	mg kg-1	2.0 ± 1.1	1.2 ± 0.3	1.5 ± 0.0

The study area is a sustainable agricultural system, where irrigation water is from a drainage water channel system following the traditional irrigation of *Carrizales* (Fig. 2). The plots were flood irrigated until field capacity (every 15 days approximately).

Fig. 2. Drainage channel associated with the traditional irrigation system of *Carrizales*

The measurements of the water quality parameters were determined following the Standard Methods for the Examination of Water and Wastewater [27]. Table 2 shows the main characteristics of the irrigation water used in the present study.

Table 2. Irrigation water characteristics

Parameters	Units	Average	Standard deviation	Maximum	Minimum
pH		7.7			
.1	0.1	7.9	7.6		
EC	dS m-1	4.1	0.9	5.3	2.4
HCO3	mg l-1	526.8	18.1	559.5	506.1
NO3-	mg l-1	17.6	8.4	30.7	10.0
P	mg l-1	5.4	0.7	6.4	4.6
NH4+	mg l-1	0.7	0.1	0.9	0.5
Ca	mg l-1	189.9	10.5	206.0	179.9
Mg	mg l-1	146.4	32.8	176.9	85.0
Na	mg l-1	825.0	77.1	930.8	720.9
K	mg l-1	12.4	0.9	14.2	11.6
Cl	mg l-1	1318.7	246.1	1645.4	1032.4
B	mg l-1	0.9	0.2	1.2	0.4

Melon plants were grown under three soil saline conditions (S1: 0.5 dS m^{-1}, S2: 1.0 dS m^{-1} and S3: 2.5 dS m^{-1}; Table 1). Each treatment included two plots. In each one, plants were planted every 0.5 m, and the separation between lines was 1.5 m (Fig. 3). Standard organic (20 t ha^{-1}) and inorganic fertilization (90 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5 and 180 kg ha^{-1} K_2O) for muskmelon was previously applied.

Twenty randomly selected plants per plot were chosen for the measurements at flowering (60 days after transplanting) and harvest (90 days after transplanting). Two fully mature leaves were taken from each plant for chemical analyses.

The collected leaves were immediately enclosed in plastic bags with wet filter paper and transported to the laboratory for further analyses. In the laboratory, leaf blades and petioles were separated and weighed to obtain fresh mass. Then, the samples were placed in a pre-heated oven at 65ºC for at least 48 h until constant weight in order to obtain dry mass (d.m.).

Fig. 3. Melon plants growing in an experimental plot

Chemical analyses to determinate elemental concentrations in each leaf part were carried out on a dry weight basis. N concentration in the leaf tissue was determined using the Kjeldahl method [28]. Leaf samples were calcined in a muffle furnace at 550ºC for 4 h, dissolved with 4 ml of HCl 1:1 in a sand-bath for 2 h, filtered and lead to a final volume of 25 ml with deionized water. Ca, Mg, K, Na, Fe, Cu, Mn, Zn were determined in these solutions directly by inductively coupled plasma mass spectrometry (ICP-MS, VG PQ Excel, Thermo Elemental, Winsford, UK) and B was analyzed in the solution following the azomethine-H method [29].

Pigment content was determined in 80% acetone extract. Chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (total Chl) were calculated according to Lichtenthaler [30].

The statistical analysis was achieved using IBM SPSS 20.0 software. Data were subjected to two-way analysis of variance (ANOVA) test to find the differences in the measured parameters among soil salinity and growth stages. Means (in each sampling period) were compared using Duncan's Multiple Range test at 5% significance level.

3. RESULTS AND DISCUSSION

Mineral nutrient concentration measured in leaves of *Cucumis melo* L. plants was significantly different between leaf compartments (petioles and blades) and was affected by saline stress conditions. N content significantly decreased during the growth period, with lower values at harvest period than flowering stage for both leaf parts: blades and petioles (Table 3 and 4).

Soil salinity had a significant effect on N content in leaf blades, showing lower values at high salinity levels (Table 3 and 5). A continuous decrease in N concentration for the growth period has been reported for several horticultural crops [31,32] and this trend of N can be linked to the effect of N on leaf photosynthesis and growth [6,31,33]. In their study, Carvajalet al. [17] suggested that once the older leaves start to die due to the long term exposure to salt, muskmelon plants can no longer support continued growth.

Leaf blades showed significantly higher P concentration at harvest period, due to an accumulation along the plant development. P is required for processes including the storage and transfer of energy, the regulation of some enzymes, and the transport of carbohydrates [16], processes that are of great importance in fruit development.

Ca and Mg content in leaves showed an opposite trend with respect to N, increasing at the end of growth in both leaf parts (Table 3, 4 and 5). Ca and Mg accumulation could ameliorate the inhibitory salt effects on growth since they regulate many physiological processes that influence both growth and responses to environmental stresses [34,35].

The maintenance of these elements absorption and transport under salt stress is an important determinant of plant salt tolerance [36]. The leaf concentration may be explained by the important availability of these elements in the calcareous soils.

Table 3. Nutrient concentrations, water content and dry to fresh mass ratio in leaves of *Cucumis melo* L. growth in the different soils in two growth stages: flowering and harvest (average ± standard deviation). Different letters indicate significant differences at $P \leq .05$ between soil salt levels for each growth stage

Parameters	Flowering			Harvest		
	S1	S2	S3	S1	S2	S3
N (g kg^{-1})	39.5±0.4 a	32.9±5.1 ab	30.9±0.8 b	28.0±0.1 a	25.9±1.0 b	23.0±0.5 c
P (g kg^{-1})	2.3±0.1 a	2.1±0.1 a	2.3±0.2 a	3.2±0.2 a	2.9±0.2 a	2.5±0.2 a
Ca (g kg^{-1})	51.0±1.4 a	41.4±2.0 a	54.3±0.4 a	59.0±1.4 a	60.4±2.7 a	63.8±1.4 a
Mg (g kg^{-1})	14.1±0.3 a	16.3±3.8 a	17.0±3.1 a	18.2±1.3 a	19.6±1.5 a	18.2±1.9 a
K (g kg^{-1})	21.3±1.4 a	26.6±0.5 b	30.0±1.8 c	17.7±0.3 a	22.6±0.1 b	29.3±0.1 c
Na (g kg^{-1})	2.7±0.1 a	3.6±0.3 b	3.9±0.1 b	3.3±0.3 a	3.5±0.2 a	3.9±0.0 b
K/Na	6.4±0.3 a	6.9±0.2 ab	7.8±0.6 b	5.4±0.4 a	6.5±0.3 b	7.5±0.1 c
Fe (mg kg^{-1})	81.5±11.3 a	115.0±31.4ab	143.0±12.9 b	223.0±5.3 a	235.0±6.9 a	278.0±7.4 b
Cu (mg kg^{-1})	9.8±0.9 a	9.1±2.3 a	10.4±1.6 a	8.9±0.9 a	10.3±0.8 a	10.3±0.9 a
Mn (mg kg^{-1})	47.3±0.7 a	46.0±1.3 a	32.3±1.4 b	21.8±0.2 a	46.1±3.5 b	24.2±5.1 a
Zn (mg kg^{-1})	33.3±3.5 a	23.1±2.6 b	22.3±1.6 b	24.0±0.1 a	24.0±2.7 a	24.1±2.9 a
B (mg kg^{-1})	120.5±9.9 a	88.3±11.4 b	71.2±3.0 c	191.0±8.4 a	143.0±9.2 b	133.2±9.6 b
Water content (%)	84.4±0.1 a	82.3±2.5 a	82.7±0.4a	82.4±0.1 a	82.4±1.6 a	86.6±0.4 a
Dry to fresh mass ratio(g g^{-1})	0.16±0.0 a	0.18±0.0 a	0.17±0.0 a	0.17±0.0 a	0.17±0.0 a	0.16±0.0 a

Table 4. Mineral nutrient concentrations, water content and dry to fresh mass ratio in petiolesof *Cucumis melo* L. growth in the different soils in two growth stages: flowering and harvest (average ± standard deviation). Different letters indicate significant differences at $P \leq .05$ between soil salt levels for each growth stage

Parameters	Flowering			Harvest		
	S1	S2	S3	S1	S2	S3
N (g kg^{-1})	20.7 ± 1.2 a	21.9 ± 1.0 a	22.4 ± 1.3 a	17.7 ± 0.8 a	12.4 ± 2.8 b	11.4 ± 0.1 b
P (g kg^{-1})	1.6 ± 0.1 a	2.6 ± 0.1 a	1.8 ± 0.2 a	1.5 ± 0.1 a	1.8 ± 0.4 a	1.6 ± 0.4 a
Ca (g kg^{-1})	14.0 ± 1.2 a	16.0 ± 1.0 a	17.6 ± 1.0 a	22.2 ± 1.7 a	19.2 ± 0.5 a	20.6 ± 1.7 a
Mg (g kg^{-1})	6.1 ± 0.2 a	6.4 ± 0.5 a	5.6 ± 1.4 a	9.6 ± 0.1 a	9.5 ± 0.6 a	9.2 ± 0.8 a
K (g kg^{-1})	52.5 ± 0.7 a	72.1 ± 3.8 b	75.0 ± 1.4 b	56.0 ± 0.1 a	58.1 ± 3.5 a	79.8 ± 3.9 b
Na (g kg^{-1})	23.7 ± 0.2 a	15.1 ± 1.6 a	15.6 ± 3.6 a	21.8 ± 1.7 a	23.1 ± 0.9 b	28.4 ± 1.2 c
K/Na	2.2 ± 0.1 a	4.8 ± 0.4 b	5.0 ± 1.0 b	2.6 ± 0.2 a	3.3 ± 0.2 b	3.6 ± 0.3 b
Fe (mg kg^{-1})	73.3 ± 0.4 a	84.9 ± 4.2 a	34.9 ± 10.3 b	44.9 ± 4.2 a	46.1 ± 2.5 a	40.4 ± 9.4 a
Cu (mg kg^{-1})	3.6 ± 0.9 a	4.6 ± 0.9 a	5.2 ± 0.6 a	6.7 ± 2.0 a	3.8 ± 0.4 a	4.1 ± 1.3 a
Mn (mg kg^{-1})	8.2 ± 0.7 a	6.0 ± 1.0 ab	4.0 ± 0.8 b	11.4 ± 0.1a	6.4 ± 2.8 a	10.4 ± 1.1 a
Zn (mg kg^{-1})	15.6 ± 1.3 a	14.6 ± 3.4 a	15.7 ± 4.0 a	14.5 ± 1.1 a	15.2 ± 1.8 a	13.3 ± 0.5 a
B (mg kg^{-1})	17.2 ± 4.1 a	14.4 ± 3.5 ab	10.0 ± 6.0 b	23.2 ± 0.2 a	21.4 ± 1.0 a	25.4 ± 5.5 a
Water content (%)	95.2 ± 0.1 a	95.4 ± 0.4 a	94.8 ± 0.6 a	94.7 ± 0.1 a	94.6 ± 0.7 a	94.7 ± 0.6 a
Dry to fresh mass ratio (g g^{-1})	0.05 ± 0.1 a	0.04 ± 0.1 a	0.05 ± 0.1 a	0.05 ± 0.1 a	0.05 ± 0.1 a	0.05 ± 0.1 a

Soil salinity significantly increased K and Na in leaf blades and petioles (Table 3, 4 and 5). In general, leaf blades had higher nutrient concentrations than petioles, except for K and Na (Table 3 and 4). K and Na content significantly increased in petioles at harvest stage. These results suggest a preferential transportation of K to melon leaves as a mechanism of salt tolerance. K is responsible for stomatal regulation, which is a principal mechanism controlling water balance and nutrient transportation in plants [37]. Salt-tolerant cultivars have a mechanism to select high K contents under salt stress. Na content significantly increased in petioles at the end of growth, indicating that petioles retain more Na, which prevent an excessive salt accumulation and ion toxicity in leaf blades. According to Botia et al. [5], mechanisms of salt tolerance may be associated with partitioning processes. K/Na ratio showed significantly higher values in response to soil salinity in the two studied stages (Table 3-5). Previous works found increments of K/Na selectivity at high salinity in melon plants grown in greenhouse conditions [38,39]. High K/Na ratio leads to a favorable ionic balance with increased K uptake. Selectivity for K instead of Na could play an important role in salt tolerance because a high K/Na ratio is much more important than a low Na concentration in many species [13,16,40,41].

The micronutrients concentration showed higher values in leaf blades than petioles which may be related to their physiological functions. Leaf Fe content significantly increased with high salt doses in leaf blades at two growth stages. However, B decreased in response to salinity, but increased at harvest compared to flowering stage. These findings agree with those reported in previous studies, where B concentration was measured in leaves of other melon varieties [42,43]. Lower B absorption under high salinity could be mainly due to lower transpiration.

Water content and dry to fresh mass ratio did not show statistically significant differences among salt levels and growth stages. Petioles showed higher water content values than leaf blades. However, dry to fresh mass ratio of leaf blades was higher than petioles. These results are consistent with previous observations, suggesting that lower dry to fresh mass ratio of support structures is indicative of large water-filled vessels in petioles [44,45]. An adjustment of plants to the soil water potential was found due to the maintenance of water content in leaves even though the saline treatments and no visual symptoms of saline injuries were observed.

Significant differences were obtained for the chlorophyll content (Chl a, Chl b and total Chl) with salinity, decreasing values at higher soil salinity content (Fig. 4, Table 6). Chlorophyll concentration was reduced from S1 to S2 and S3 about 15-20% at flowering stage and in the harvest period this reduction was also increased (over 40%). This significant reduction could be associated with the higher accumulation of salts in S2 and S3 than S1 (Table 6). A reduction in leaf chlorophyll under salinity was also reported in salt-resistant tomato species [34].

High salinity degrades chlorophyll [34], thus concentrations of chlorophyll components of the photosynthetic apparatus are normally used to quantify leaf senescence in salt-stressed plants. The diminution of chlorophylls followed the same trend of N when increasing salinity.

Stepien and Klbus [46] suggested that the reduced photosynthesis at high salinity levels could be attributed in part to the reduced content of K, which is key in maintaining the steady-state photosynthetic rate and contributed to better regulation of stomata opening. K was accumulated in melon leaves with salinity in this field experiment and could be used as a mechanism to prevent salinity injuries by this plant.

Table 5. Statistical results of one-way and two-way analysis of variance (ANOVA): *P* ≤ .001 (***), *P* ≤ .01 (**), *P* ≤ .05 (*) and ns (not significant differences)

	Growth stages	Salinity	Growth stages x Salinity
Leaf blades			
N	***	**	ns
P	***	ns	ns
Ca2+	***	ns	ns
Mg2+	*	ns	ns
K+	***	***	**
Na+	ns	***	ns
K+/Na+	***	***	ns
Fe	***	***	ns
Cu	ns	ns	ns
Mn	***	***	***
Zn	ns	*	*
B	***	***	ns
Water content	ns	ns	ns
Dry to fresh mass ratio	ns	ns	ns
Petioles			
N	***	ns	**
P	ns	ns	ns
Ca2+	***	ns	ns
Mg2+	***	ns	ns
K+	***	***	***
Na+	***	**	***
K+/Na+	*	***	ns
Fe	***	***	***
Cu	ns	ns	ns
Mn	**	*	*
Zn	ns	ns	ns
B	**	ns	*
Water content	ns	ns	ns
Dry to fresh mass ratio	ns	ns	ns

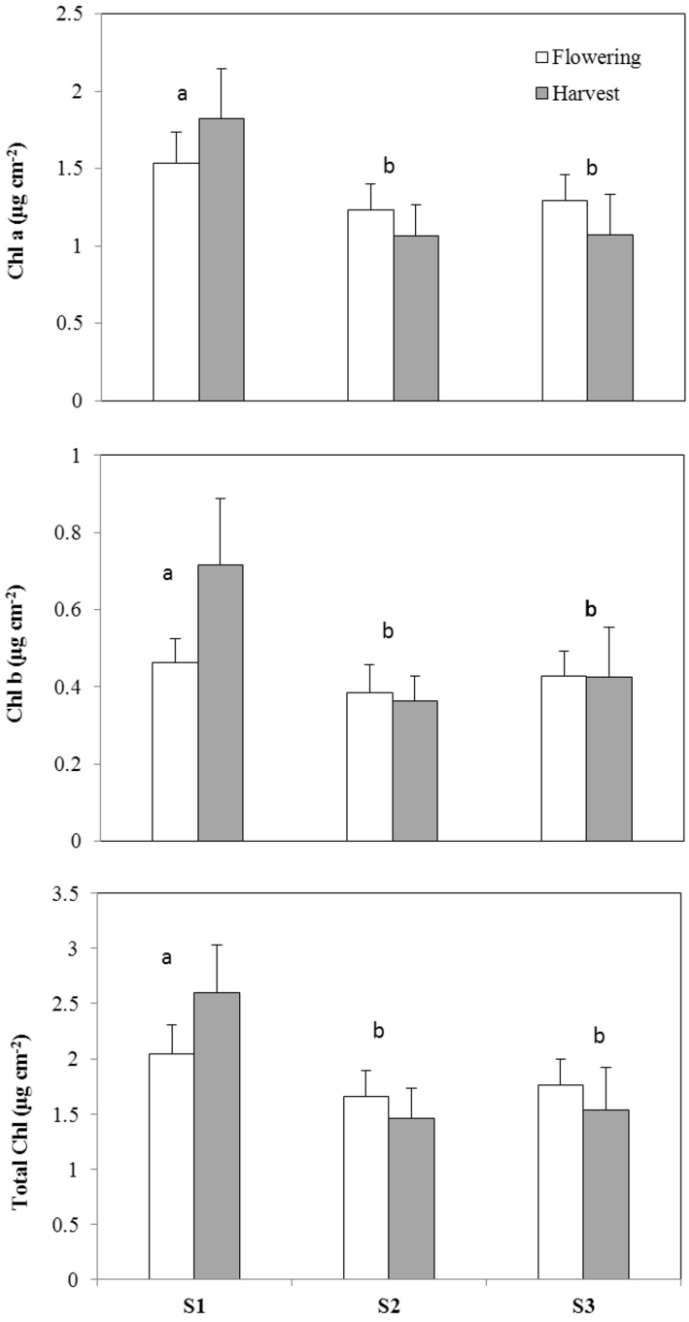

Fig. 4. Effect of salinity on Chl a, Chl b and Total Chl (µg cm^{-2}) of *Cucumis melo* L. plants at two growth stages (Flowering and Harvest). Values are means of ten replicates (*n*=10), error bars indicate standard deviation, different letters are significantly differences with salinity levels at $P \leq .05$ according to Duncan's multiple range test

Table 6. Results of p-values, *P* ≤ .001 (***), *P* ≤ .01 (**), *P* ≤ .05 (*) and ns (not significant), of two-way analysis of variance (ANOVA) for chlorophyll a (Chl a), chlorophyll b (Chl b) and Total Chlorophyll (Total Chl) at different growth stages (flowering and harvest) and salinity levels

	Growth stages	Salinity	Growth stages x Salinity
Chl a	ns	***	*
Chl b	*	***	ns
Total Chl	ns	***	*

4. CONCLUSION

Nutrient concentration in leaf parts (petioles and blades) of melon plants cultivated under different soil salinity conditions was affected. These field observations mostly agree with those indicated by other authors working under controlled conditions as greenhouses and controlled environmental growth chambers. Although no visual symptoms of salt injuries were observed, a decrease in leaf N during the growth stages (from flowering to harvest) was determined and photosynthetic pigments followed the same trend. Petioles showed higher values of Na compared to leaf blades. These results highlight the role of petioles in salt tolerance of melon plants. Additionally, leaf blades showed a high selectivity for K instead of Na. A high K/Na ratio is related to the resistance of melon plants to salinity.

In this sense, it may be possible to determine the salinity tolerance of melon plants analyzing the ratio K/Na in petioles and could be used as an indicator of salinity tolerance of the melon cultivars.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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