



## Salinity tolerance determination in four sunflower (*Helianthus annuus* L.) hybrids using yield parameters and principal components analysis model

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### ABSTRACT

Globally, around 800 million hectares are affected by salinity. This abiotic stress causes plant growth inhibition, disruptions in physiological processes in plant cells, and yield losses in many crops. Sunflower is the third-most oilseed crop globally produced, and it is considered moderately tolerant to salinity. There are few studies about the genotypic variability existing in sunflower for responses to salinity, especially the changes in yield and oil content and quality under salinity. The present work aimed to study the effects of salinity on achene yield in four sunflower genotypes and their components and on the oil content and quality and their relationships. Four sunflower hybrids (ACA885, TRITON MAX, SRM769 and SRM779) were grown at 130 mM NaCl irrigation solution under controlled environmental conditions. The achene yield, the yield components, and the content and oil quality were determined. Based on the four studied genotypes, salinity decreased achene yield by 75.1%. SRM779CL had a lower achene yield reduction by salinity. Yield component that most explained this tolerance was the number of achenes per plant. SRM779CL was the hybrid with the highest oil percentage loss. Contrarily, salinity increased the oil content in ACA885. Salinity decreased the ratio between oleic (18:1) and linoleic acid (18:2) in all genotypes. Therefore, salt stress increased the percentage of unsaturations in the four genotypes examined in this work. Finally, considering the 12 parameters measured, principal components analysis could determine that SRM779CL showed the best performance under control conditions while ACA885 was the most tolerant under salinity.

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### 1. Introduction

The world faces many environmental issues regarding agriculture, such as soil salinity, which cause low yield, mainly in marginal agricultural regions (El-Bially et al., 2022; Hamid et al., 2021; Saudy et al., 2021). Among all abiotic stresses, water deficit and salinity are most

widespread in crops (Hamid et al., 2021). Moreover, salinity is widely spread in arid or semi-arid regions (Abd El-Mageed et al., 2022; Hu et al., 2018), and it is a gigantic issue for agricultural crops (Aslam et al., 2021). Punctually, around 800 million land hectares (~50% of world irrigated lands) are affected by salt (Syed et al., 2021). Other authors mentioned that salinity is the leading abiotic stress besides the other ones, such as a water deficit and low or high temperatures (El-Bially et al., 2018, 2022; Thakur et al., 2010). Some authors indicated that agricultural losses caused by salinity had been estimated at 6% of worldwide cultivated land (Nisha Nandhini et al., 2021). Furthermore, Shrivastava and Kumar (2015) estimated that 20% of the total cultivated and 33% of irrigated agricultural lands worldwide are affected by high salinity. Around 85% area worldwide is only slightly to moderately

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affected by high salt concentrations, while the remainder 15 % suffers from severe to extreme limitations for crop cultivation (Wicke et al., 2011). Considering the literature cited, there is a global need to have crops with high levels of salinity tolerance (Temme et al., 2019).

At a soil level, the presence of salt reduces the ability of the plant to take up soil water, producing a water deficit. In other words, the accumulation of soluble salts in the soil decreases water potential causing physiological water deficit or osmotic stress (Egamberdieva et al., 2019; Niamat et al., 2019). Another effect of soil salinity is the reduction of hydraulic conductivity (Klopp and Daigh, 2020). The presence of salts causes dispersion of soil particles reducing their permeability and hydraulic conductivity (Zabala et al., 2020). A high concentration of various soluble salts characterizes saline soils (Han et al., 2022; Li et al., 2022), which impose water deficit, limiting water uptake by plants, as outlined by Chiacchiera et al. (2016).

Numerous studies have demonstrated that plant changes under salt stress and its tolerance mechanisms are mostly accompanied by several morphological changes (Acosta-Motos et al., 2017; Ma et al., 2021).

At a plant level, salinity promotes osmotic and toxic effects (Li et al., 2022). Osmotic effects of salinity produce stomatal closure, a decrease in the photosynthetic rate, and in the last instance, a reduction in growth (Zhou et al., 2018). The excessive amounts of salt in the transpiration stream cause injury to cells in the leaves (James et al., 2011). Sodium and chloride salts are commonly associated with salinity conditions, with most plants being sensitive to excess concentrations of those ions (Han et al., 2022; Li et al., 2022). Sodium is one of the most injurious ions in saline soils due to its toxicity (Abbas et al., 2021), reducing hydraulic conductivity even in the xylem vessels of plants (Zabala et al., 2020). Consequently, many studies about salt stress employ NaCl as a salinizing agent, as was performed in this work.

At the cellular level, the major toxic ions in salinized soils are Na<sup>+</sup> and Cl<sup>-</sup>, although SO<sub>4</sub><sup>2-</sup>, HCO<sub>3</sub><sup>-</sup>, Mg<sup>2+</sup>, and other chemical species also contribute to the harmful salinity effects among higher plants (Ma et al., 2021). High concentrations of Na<sup>+</sup> and Cl<sup>-</sup> induce plants to synthesize reactive oxygen species (ROS), a secondary effect of salinity (Hosseinfard et al., 2022). The ROS level increase under various environmental stresses, which would disturb normal cellular metabolism through oxidative damage to nucleic acid, proteins, and lipids (Zentgraf et al., 2022). The increased level of ROS causes the oxidation of biological membranes and destroys cellular structures (Zentgraf et al., 2022). Moreover, the toxic impact of ROS is lipid peroxidation, membrane deterioration, as well as DNA and protein damage (Arif et al., 2020).

Salinity tolerance of crop plants needs to be improved to enable them to grow in marginal areas already affected by salinity (Farhadi Machekposhti et al., 2017). Use of both genetic manipulation and traditional breeding approaches will be required to develop salt-tolerant cultivars better able to cope with the increasing soil salinity constraints (C ccoli et al., 2015). Plants have evolved three main mechanisms to enable them to tolerate salinity stress (Zhang et al., 2022). Osmotic tolerance involves the plant's ability to tolerate the drought aspect of salinity stress and to maintain leaf expansion and stomatal conductance. It can be demonstrated that the responses of plants to osmotic stress are independent of nutrient levels in the growth medium (Nisha Nandhini et al., 2021). The second two mechanisms of tolerance involve the ability to reduce the ionic stress on the plant by minimizing the amount of Na<sup>+</sup> that accumulates in the cytosol of cells, particularly those in the transpiring leaves (Kumari and Bhatla, 2021). The first mechanism works by excluding Na<sup>+</sup> from leaves (Kumari and Bhatla, 2021), the second by efficiently compartmentalizing Na<sup>+</sup> in the vacuole or in particular leaves cell types where the damage to metabolism is kept to a minimum (Li et al., 2022; Zhang et al., 2022). Both processes involve up- and downregulation of the expression of specific ion channels and transporters, allowing the control of Na<sup>+</sup> transport throughout the plant (Kumari and Bhatla, 2021; Li et al., 2022). Symplastic pathway for salt (NaCl) transport needs transporters and channels. Non-

selective cation channels (NSCCs) and Na<sup>+</sup>/H<sup>+</sup> antiporter (NHA) or SOS1 are needed for the influx of Na<sup>+</sup> (Arif et al., 2020).

NSCCs are mediated by two gene families that are glutamate receptor-like channels (GLRs) and cyclic nucleotide-gated channels (CNGCs). Different isoforms of high-affinity K<sup>+</sup> transporters (HKTs) and cation/H<sup>+</sup> exchanger (CHX) participates in the long-distance transport of ion (Na<sup>+</sup>) through the xylem and phloem. HKTs promote the uptake of Na<sup>+</sup> to a large extent and enhance Na<sup>+</sup> accumulation in the plant. Aquaporin, like plasma membrane intrinsic protein (PIP) isoforms, also promotes Na<sup>+</sup> uptake inside the plant. Low-affinity cation transporters (LCT1) and AKT1 are indirectly involved in the influx of Na<sup>+</sup>. Therefore for the uptake of Na<sup>+</sup> transporters like NSCCs, NHA, PIP2, LCT1, and AKT1 are associated, whereas for long-distance transport and distribution of toxic Na<sup>+</sup> transporters associated are HKTs, NSCCs, NHA, and CHX (Isayenkov and Maathuis, 2019).

Plants have mechanisms to reverse salinity cell damage, such as the gene expression for superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), and the non-enzymatic antioxidants such as ascorbate, phenolic compounds (Gill and Tuteja, 2010), and glutathione (Kamińska et al., 2022).

Plant adaptation to saline conditions includes genetic variability and mechanisms that contribute to access restriction of these and other potentially deleterious ions to metabolically active sites, both at the organ and subcellular levels (C ccoli et al., 2015; Li et al., 2022). Other physiological mechanisms to improve salinity tolerance are the generation of organic or inorganic molecules to provide an osmotic balance (Zhang et al., 2022) and ROS detoxifying mechanisms (Mittova et al., 2009; Pittaro et al., 2016).

The plant also responds to salinity by upregulating the level of endogenous phytohormones, i.e., abscisic acid (ABA), auxins, cytokinins, salicylic acid (SA), jasmonic acid (JA), polyamines, gibberellins, and brassinosteroids (BRs) (Arif et al., 2020). Plant upregulates many genes, transcription factors, proteins, and metabolites to provide plant tolerance against salinity. Omics approaches like genomic, transcriptomic, proteomic, and metabolomic are essential responses of the plant in adapting to salinity and enhancing crop productivity (Benjamin et al., 2019).

Sunflower, belonging to the Asteraceae family, is a Native American annual crop (Flagella et al., 2002). This species has a large flowering head (inflorescence), and it grows in a wide range of soils (Alaboudi et al., 2018; Castro and Campos Leite, 2018; de Aquino et al., 2019). In terms of production, it is the third-most oilseed globally produced, the fourth-most relevant culture for vegetable oil production, and the third-most important culture in oilseed meal production (Pilorg , 2020). The United States of America, Ukraine, and Argentina are the leading world producers of sunflower (de Oliveira Filho and Egea, 2021). Moreover, global sunflower production has increased more than twice since 2000 (Hryvusevich et al., 2021). Furthermore, sunflower achenes account for approximately 10 % of the world's edible plant-derived oil (de Oliveira Filho and Egea, 2021; Pilorg , 2020). All of the above statements demonstrate the importance of sunflower cultivation at a global level.

Sunflower is moderately tolerant to salinity with no significant yield reduction by soil salinity up to 4.8 dS m<sup>-1</sup> (Francois, 1996; Farhadi Machekposhti et al., 2017). The studies on sunflower yield and its changes under salinity are controversial.

Han et al. (2022) explained that the threshold (to have not significant yield reduction by salinity) for oleic sunflower is 1.3 dS m<sup>-1</sup> instead the 4.8 dS m<sup>-1</sup> reported by Francois, (1996). These authors also reported that to ensure a 75 % seed yield, the soil salinity should be <6.4 dS m<sup>-1</sup>. Therefore, studies on the behavior of sunflower crops under salinity are needed. Although the effects of salt stress on the sunflower yield are sufficiently known, data concerning the salinity effect on oil quality parameters are still few. In the literature, the salt stress effect on the oil yield of sunflower under drip irrigation with saline water was reported (Han et al., 2022), revealing that increasing salinity

promotes an increase in oleic acid content and a decrease in linoleic acid content (Han et al., 2022). However, in that work, only one genotype was studied. Therefore, more studies are required to clarify the changes in sunflower oil quality and genotypic variability under salinity.

Other authors have stated that a reasonable solution to develop and utilize salt-affected farmland is planting industrial and salt-tolerant crops using proper irrigation and fertilizer management (Zeng et al., 2014). In this context, the present work aimed to study the effects of salinity on achene yield in sunflower genotypes and their components. The present research also seeks to bring clarity over the changes in oil content and quality and their relationships under salt stress. These effects were examined using a multiparametric model, the principal component analysis, highlighting the tolerance to salinity and its underlying mechanisms.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Three successive growth experiments were carried out as described by Cécconi et al. (2012, 2015). The experiments were performed with a completely randomized experimental design.

Briefly, four sunflower hybrids genotypes were studied: SRM779CL (from Sursem company), ACA885 (provided by Association of Argentine Cooperatives, ACA), TRITON MAX (TM, from SURSEM company), and SRM769 (provided by Sursem company). All experiments were performed in a growth chamber (walk-in type). The average temperatures were  $25.5 \pm 1.0$  °C (day) and  $19.0 \pm 1.1$  °C (night), and the mean irradiance was  $14.52 \pm 0.44$  mol m<sup>-2</sup> d<sup>-1</sup> (600 μE of total photon flux). The growth chamber was adjusted to a 16 h photoperiod. The seeds were soaked in a 30 % commercial bleach solution for 20 min, washed four times with distilled water for 5 min, laid on moist tissue paper in Petri dishes, and kept at 28 °C in darkness in the culture chamber. The germinated seeds were transferred to 7 L pots (35 cm in high × 15 cm in diameter) containing washed sand only. After 2 days of acclimation, plants were well watered with half-strength Hoagland solution (Matuszak-Slamani et al., 2022). Two treatments were applied: control plants irrigated only with Hoagland solution (control plants, C) and salinized plants irrigated with a 130 mM NaCl solution (salinized plants, S). The salinization was started as soon as the first pair of leaves were 1 cm in length, from plant emergence at 150 °C–200 °C d<sup>-1</sup> (the time in which leaf 1 and 2 reached this size varied among plants but was distributed uniformly among genotypes). Gradual salinization was provided by daily increments of 35 mM NaCl in the nutrient solution until reaching 130 mM final concentration maintained up to the end of the trial by periodical irrigation with a salinized solution, being this provided at 1.5 times the void volume to prevent salt build-up. The salinity levels in the experiments were monitored by measuring the electrical conductivity of the drainage solutions. The NaCl concentration in the three experiments provided a water-potential value in the root medium of -0.65 MPa (130 mM NaCl), which was similar to the -0.65 MPa value used in several studies of sunflower under water deficit (Pereyra-Irujo et al., 2008). Air temperature and incident photosynthetically active radiation were measured and registered every 15 min with a digital data logger (Cavadevices SATM). Thermal time (°C d<sup>-1</sup>) from seedling emergence was calculated as the daily integral of the difference between average day temperature and the base temperature for sunflower growth and development of 4.8 °C (Granier and Tardieu, 1998).

### 2.2. Determination of the achenes yield and their components

To achieve the correct pollination, flowers of each capitulum were brushed manually from the moment the first tubular flowers began to open until the end of the anthesis. Once the plants reached physiological maturity, heads were harvested manually. The following determinations were performed: (i) number of achenes per plant/head using a

Cavadevices SATM brand grain counter, (ii) weight of grains per plant/capitulum using a Pioneer series balance (210 G × 0.1 MG) PA214 OHAUS®, and (iii) average weight of each grain per plant, being this one obtained as the ratio between the weight of all achenes of the plant and the number of achenes per plant. Before the weight registration, the grains were placed in an oven at 30 °C for 4 days to equilibrate their moisture content and to compare the weights between genotypes.

### 2.3. Determination of oil content and the fatty acid profile

Oil content of the sunflower achenes was determined according to the IRAM 5544 standard for oilseeds and their products. The sample preparation for oil extraction was performed by grinding the whole achene to reach a proper particle size estimated at 1 mm (according to the IRAM sieve) and avoiding altering the original moisture content. An amount of 3–5 g ground achene was transferred to a cartridge, this was placed into the extractor equipment, and hexane was used as extraction solvent. Then, the refrigerant was connected, and the system was heated up to hexane boiling point over the time foreseen for the ground seeds under analysis. Subsequently, hexane contained in the extracted oil was evaporated and placed in an oven with forced air circulation at a temperature of 130 °C ± 2 °C for 1 h. The extracted oil was placed in a desiccator, allowed to cool and weighed. Oil content (%) was obtained as:

$$\text{Oil content (\%)} = (m_3 - m_2)/m_1 \times 100 \quad (1)$$

where  $m_1$  was the initial mass of ground achene (g),  $m_2$  was the mass of the extraction vessel (g), and  $m_3$  was the mass of the extraction vessel and the extracted oil (g).

The fatty acid profile of the samples of sunflower oil extracted from the seeds was determined according to IRAM 5652 standard norm (preparation of methyl esters of fatty acids for animal and vegetable oils and fats). Moreover, IRAM 5651 norm (determination of fatty acids and their methyl esters by gas chromatography for oils and fats from vegetables and animals) were applied. The sample preparation for its subsequent analysis consisted of weighing 4 g of sample (extracted oil) in a balloon. Subsequently, 40 mL of methanol, 0.5 mL of methanolic solution of potassium hydroxide (5.6 g of potassium hydroxide in 100 mL of methanol), and the boiling regulator were added. The refrigerant was connected and brought to a boil for 10 min. The balloon was cooled, and its content was transferred to a separating funnel. Two successive washes were performed with equal volumes of water and heptane (20 mL). Furthermore, the phase rich in heptane, which contains the methyl esters, was dried with anhydrous sodium sulfate, filtered through cotton and the solution was evaporated to approximately 20 mL in a boiling water bath under a nitrogen stream. Thus, the sample was obtained for fatty acid profile analysis by gas chromatography. Determinations were performed in a gas chromatograph, and the following characteristics and analysis conditions were used: Clarus 500 model (Perkin Elmer®), FID detector, ZB-WAX column (15 m long, 0.32 mm diameter, 0.10 μm film), carrier gas: helium at 1 mL min<sup>-1</sup> flow rate with a Split ratio of 50:1, injector temperature of 150 °C, temperature detector of 250 °C and oven temperature from 150 °C up to 250 °C, at 10 °C min<sup>-1</sup> for 15 min.

### 2.4. Statistical analysis

Data were analyzed by ANOVA using the Fisher least significant difference (LSD) test using a 5 % significance level. The adjustment of errors to normal distribution was verified using the Shapiro-Wilks test, and homoscedasticity was verified through the Levene test. In this study, as twelve parameters were measured on the same experimental units, a multivariate analysis was performed to obtain an appropriate interpretation of the variable correlation and its relative weight on the final results. Thus, Principal Components Analysis, Biplot and Minimum

Spanning Tree of the parameters were run to get a summary of the effects of salinity as a whole. Principal Components Analysis reduces the multivariate space to only two variables (principal component 1 and principal component 2) to discriminate those that influence the statistical results. It is a simplified way to explain the total variability among samples. Minimum Spanning Tree was plotted in the Biplot to show Principal Component Analysis or dimension reduction accuracy. In the Biplot, lines joining the experimental units without crossing each other indicate a better dimension reduction, therefore a good explanation of the multivariate reality. In the cophenetic correlation, the coefficient quantifies how better the dimension reduction through Principal Component Analysis was. Statistical analyses were run using the InfoStat Professional software (Universidad Nacional de Córdoba).

### 3. Results

#### 3.1. SRM779CL hybrid tolerated salinity, maintaining the number of achenes per plant (NAP)

The effect of salinity on the yield values in achenes per plant ( $\text{g plant}^{-1}$ ) for the four studied sunflower hybrids (SRM779CL, ACA885, TM, and SRM769) is described in Fig. 1. The average achene yield for each genotype under non-saline conditions is shown as dark grey columns. Light grey columns represent the average achene yield for the four genotypes under salinity (Fig. 1). The lines above the bars represent standard errors.

Under both controlled and salinity conditions, all the grain yield reductions were statistically significant (Fig. 1). As described, the SRM779CL and ACA885 genotypes showed decreases of 66.4 % and 67.9 %, respectively, while TM and SRM769 genotypes had decreases of 82.3 % and 76.3 %, respectively. So, the most affected genotype was TM (89.6 %), while the most tolerant hybrid to salinity was SRM779CL (66.4 %).

From Table 1, it can be deduced that salinity decreased the number of achenes per plant by 51.4 % (on average, considering all genotypes). Variability was found in the NAP parameter, i.e., treatment at 130 mM NaCl decreased the NAP values in 43.6 %, 47.0 %, 59.1 %, and 55.8 %, for the SRM779CL, ACA885, TM and SRM769 genotypes, respectively. More tolerant and more sensitive hybrids could be determined, being the SRM 779 CL hybrid the least affected by salinity (having 56.4 % achenes per plant regarding the control plants). Conversely, the TM hybrid was the most affected (having 40.9 % achenes per capitulum compared to the control plants).

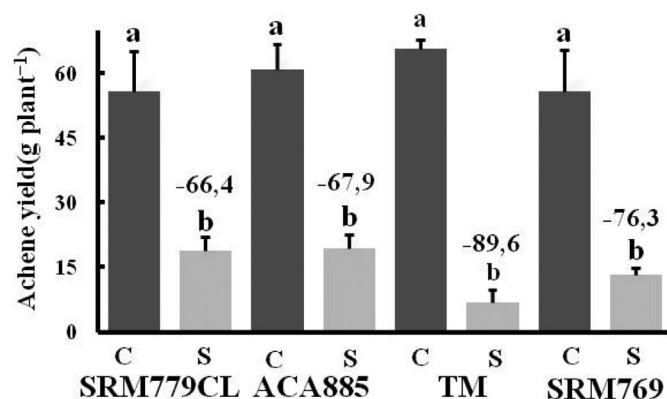


Fig. 1. Effect of salinity (130 mM NaCl) on achene yield ( $\text{g plant}^{-1}$ ) in four sunflower hybrids genotypes. In each column, the same letters indicate no significant differences among means at  $P < 5\%$  (0.05). C: Plant growth under control conditions (no salinized, irrigated only with half-strength Hoagland's solution), S: salinized plants,  $n \geq 5-9$  plants per line and conditions. TM: TRITON MAX genotype. Standard errors are plotted at the top of each column.

Table 1

Effect of salinity (130 mM NaCl) on the number of achenes per plant (NAP,  $\text{n}^\circ\text{g plant}^{-1}$ ) and achene weight (AW, g) in four sunflower hybrids. In each row, means followed by a common letter are not significantly different at  $P < 5\%$  (0.05). 0 mM: plants grown under controlled conditions, 130 mM: salinized plants;  $n \geq 5-9$  plants per hybrid and condition.

Genotype	Saline condition	NAP ( $\text{n}^\circ\text{a plant}^{-1}$ )	AW (g)
SRM779CL	0 mM	1576.50 $\pm$ 122.75 cd	35.05 $\pm$ 4.23 c
	130 mM	888.25 $\pm$ 101.04 b	20.97 $\pm$ 2.20 ab
% variation		-43.6 %	-40.2 %
ACA885	0 mM	1402.75 $\pm$ 99.09 c	43.06 $\pm$ 1.99 c
	130 mM	742.50 $\pm$ 80.89 ab	25.90 $\pm$ 3.03 b
% variation		-47.1	-39.9
TRITÓN MAX	0 mM	1841.00 $\pm$ 97.18 d	36.03 $\pm$ 2.76 c
	130 mM	473.25 $\pm$ 176.84 a	13.10 $\pm$ 2.27 a
% variation		-74.3	-63.6
SRM769	0 mM	1401.00 $\pm$ 121.56 c	39.03 $\pm$ 4.33 c
	130 mM	618.5 $\pm$ 82.62 ab	21.63 $\pm$ 0.72 b
% variation		-55.9 %	-44.6
LSD		332.73	8.51

Salinity had a marked effect on the average achene weight (AW, g) (Table 1). Significant differences in the AW parameter were recorded, being this genotype-dependent. The average weight of achenes decreased by 40.2 % for SRM 779 CL, 40.1 % for ACA 885, 63.6 % for TM, and 44.6 % for the SRM 769 hybrid. Under salinity, the average decrease in the weight of achenes was 45 %. The TM hybrid was the most affected (showing 36.4 % of the weight of the achenes regarding the average for control plants). Conversely, the most tolerant hybrid for this parameter was the ACA885 hybrid (60.2 % of the average weight of achenes in comparison with the weight of achenes for control plants).

#### 3.2. Salinity effect on oil content is genotype dependent

Fig. 2 shows the oil contents in achenes for the four studied hybrids (under both control and saline conditions). The average of oil content for each genotype under non-saline condition is shown as dark grey columns. Light grey columns represent the average of oil content for the four genotypes under salinity (Fig. 1). Under control conditions, the average value for this parameter was 38.43 %. Under salinity, the oil content was an average of 25.25 %. Comparing the results among treatments, the SRM779CL, TM, and SRM769 decreased their oil contents by 47.1 %, 57.9 %, and 34.6 %, respectively. In contrast, the ACA885 hybrid showed a 5.5 % increase in oil production.

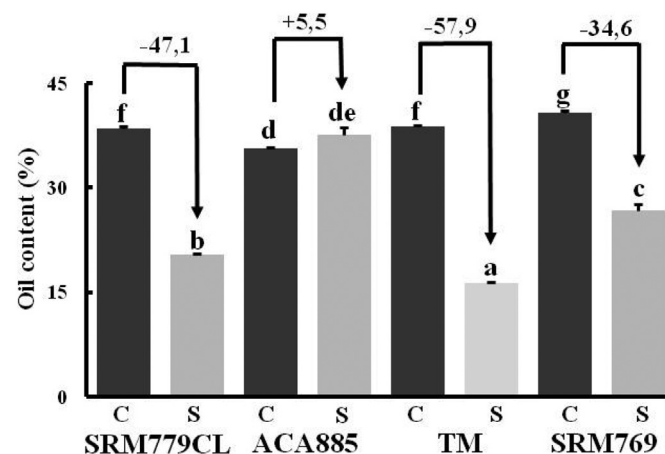


Fig. 2. Effect of salinity (130 mM NaCl) on oil content (%) in four sunflower hybrids. Columns with the same letter are not significantly different based on the LSD at  $p \leq 0.05$ . C: control plants; S: salinized plants;  $n \geq 5-9$  plants per hybrid and salt condition (treatment). TM: TRITON MAX genotype/hybrid. Standard errors are plotted at the top of each column.

### 3.3. Salinity changed the fatty acid profile

The fatty acid profile changed under saline conditions (Table 2). Salinity increased the content of  $\leq 14C$  fatty acids by 380.8 % in SRM779CL and 100.9 % in the TM hybrid. On the other hand, the ACA885 and SRM769 hybrids decreased this parameter by 66.8 % and 20.8 %, respectively. Under salinity, all changes were significant except for the SRM769 hybrid.

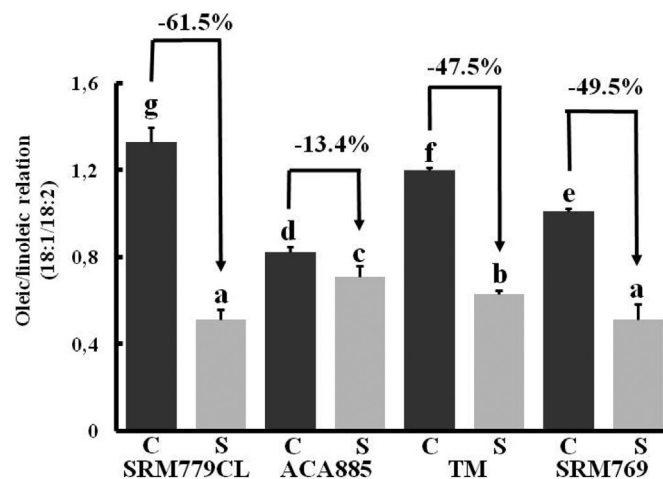
Salinity increased the proportion of palmitic acid (16:0) by 60.6 % in SRM779CL, 27.1 % in TM, 19.9 % in SRM769 hybrid, and 8.4 % in the ACA885 hybrid. Under control conditions, the content of stearic acid (18:0) for SRM779CL, ACA885, TM, and SRM769 hybrids were: 2.42 %, 1.87 %, 3.14 %, and 2.07 %, respectively. The salinized plants presented a content of 3.19 %, 2.11 %, 5.18 %, and 2.49 %, respectively. Thus, salinity caused a significant increase in stearic acid content (18:0) of 31.8 %, 12.8 %, 64.9 %, and 20.3 %, respectively.

Regarding the content of oleic acid (18:1), percentages of 50.94 % for SRM779CL, 39.98 % for ACA885, 48.08 % for TM, and 43.68 % for SRM769 hybrid were obtained. Under saline stress conditions, these hybrids showed a content of 24.9 %, 36.2 %, 31.25 %, and 29.21 %, respectively. Salinity significantly decreased the oleic acid content (18:1) in the four genotypes. The percentages of decreases were 51.1 %, 10.4 %, 35.1 %, and 33.1 %, respectively.

Salinity significantly increased the content of this fatty acid in the four genotypes. Due to salinity, the two genotypes that increased this content were SRM769 and SRM779CL, with increases of 31.8 % and 29.6 %, respectively. Hence, the two genotypes that least increased this content were TM and ACA885, with values of 25.1 % and 6.5 %, respectively. As can be seen, oleic and linoleic acids represent the highest amount of fatty acids in the total oil of sunflower achenes. Under control conditions, the summation of both represented 89.16 %, 88.63 %, 88.1 %, and 86.97 % for the SRM779CL, ACA885, TM, and SRM769 hybrids, respectively. Under salinity conditions, both fatty acids represented 74.43 %, 88.01 %, 81.33 %, and 86.25 % for SRM779CL, ACA885, TM, and SRM769 hybrids. Considering the contents of oleic and linoleic acid, salinity decreased the synthesis of monounsaturated oleic acid and greatly increased the content in the polyunsaturated linoleic acid (this issue is explained in detail in Section 3.4).

Under control conditions, the content values of linolenic acid (18:3) were: 0.24 %, 0.19 %, 0.04 %, and 0.05 % for the four studied hybrids (SRM779CL, ACA885, TM, and SRM769). Under salinity conditions, these hybrids presented contents of 2.53 %, 0.04 %, 0.08 %, and 0.05 %, respectively. Salinity only significantly increased the content of this fatty acid in the SRM779CL hybrid (+954.2 %).

For other long-chain fatty acids ( $\geq 20C$ ), salinity increased this content by 213.6 % (SRM779CL), 169.1 % (ACA885), and 89.8 % (TM). This parameter decreased by 14.1 % in SRM769 genotype.



**Fig. 3.** Effect of salinity (130 mM NaCl) on oleic acid (18:1)/linoleic acid (18:2) relation in four sunflower hybrids. Bars with the same letter are not significantly different based on the LSD at  $p \leq 0.05$ . C: control plants; S: salinized plants;  $n \geq 5$ –9 plants per hybrid and salt condition (treatment). TM: TRITON MAX genotype/hybrid. Standard errors are plotted at the top of each column.

### 3.4. Salinity decreased the C18:1/C18:2 relation

Fig. 3 shows the ratio of 18:1/18:2 fatty acids (percentage of oleic acid/linoleic acid). In control conditions, values of 1.33 for SRM779CL, 0.83 for ACA885, 1.3 for TM, and 1.01 for SRM769 were obtained. Under salinity, ratio values of 0.51, 0.71, 0.63, and 1.01 for SRM779CL, ACA885, TM, and SRM769 hybrids, respectively, were obtained. Considering this parameter, salinity strongly affected SRM779CL, SRM769, and TM hybrids, decreasing the ratios by 61.5 %, 49.5 %, and 47.5 %, respectively. However, the lesser affected hybrid was ACA885 showing a 13.4 % decrease in this parameter.

### 3.5. Multivariate analysis

A high cophenetic correlation coefficient was obtained from principal component analysis (0.99). It indicates a good projection quality of the observations in the plane of the selected components (PC1 and PC2). Principal components 1 and 2 explained 82.3 % of the total variation (considering the 12 parameters evaluated). Principal component 1 explained 64.1 % of the total variation separating the four hybrids and the treatments. Regarding the 12 parameters studied in the present work, the SRM779 hybrid displayed the best behavior under control conditions. Besides, this one was the most sensitive under salinity. ACA885 was the hybrid that had the best performance under salinity, followed by SRM769, TM, and SRM779 (Fig. 4).

**Table 2**

Effect of salinity (130 mM NaCl) on fatty acid composition in four sunflower hybrids. Rows with the same letter are not significantly different based on the LSD at  $p \leq 0.05$ . 0 mM: control plants, 130 mM: salinized plants,  $n \geq 5$ –9 plants per hybrid and salt condition (treatment). TM: TRITON MAX genotype/hybrid.

Genotype	Saline condition	$\leq 14$ (%)	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Other acids ( $\geq 20$ )
SRM779CL	0 mM	1.08 ± 0.12 a	4.97 ± 0.07 a	2.42 ± 0.09 b	50.94 ± 0.25 h	38.22 ± 0.38 a	0.24 ± 0.05 a	2.13 ± 0.08 b
	130 mM	5.19 ± 0.24 c	7.98 ± 0.13 f	3.19 ± 0.18 c	24.9 ± 0.5 a	49.53 ± 0.76 de	2.53 ± 0.1 b	6.68 ± 0.17 f
% variation		+380.6 %	+60.6 %	+31.8 %	-51.1 %	+29.6 %	+954.2 %	+213.6 %
ACA885	0 mM	2.14 ± 0.12 b	6.33 ± 0.07 d	1.87 ± 0.09 a	39.98 ± 0.25 e	48.65 ± 0.38 d	0.19 ± 0.05 a	0.84 ± 0.08 a
	130 mM	0.71 ± 0.3 a	6.86 ± 0.16 e	2.11 ± 0.22 ab	36.2 ± 0.63 d	51.81 ± 0.95 e	0.04 ± 0.13 a	2.26 ± 0.21 b
% variation		-66.8 %	+8.4 %	+12.8 %	-10.4 %	+6.5 %	-78.9 %	+169.1 %
TM	0 mM	1.16 ± 0.13 a	5.21 ± 0.07 b	3.14 ± 0.1 c	48.08 ± 0.28 g	40.02 ± 0.42 b	0.04 ± 0.06 a	2.36 ± 0.09 b
	130 mM	2.33 ± 0.13 b	6.59 ± 0.07 e	5.18 ± 0.09 d	31.25 ± 0.27 c	50.08 ± 0.4 e	0.08 ± 0.06 a	4.48 ± 0.09 e
% variation		+100.9 %	+27.1 %	+64.9 %	-35.1 %	+25.1 %	+100.1 %	+89.8 %
SRM769	0 mM	1.25 ± 0.13 a	5.61 ± 0.07 c	2.07 ± 0.1 a	43.68 ± 0.29 f	43.29 ± 0.43 c	0.05 ± 0.06 a	4.06 ± 0.1 d
	130 mM	0.99 ± 0.15 a	6.73 ± 0.08 e	2.49 ± 0.11 b	29.21 ± 0.32 b	57.04 ± 0.48 f	0.05 ± 0.07 a	3.49 ± 0.11 c
% variation		-20.8 %	+19.9 %	+20.3 %	-33.1 %	+31.8 %	0 %	-14.1 %
LSD		0.49	0.27	0.37	1.04	1.56	0.21	0.35

Concerning main component 1, none of the variables or parameters showed greater weight or leverage than the remaining ones. So, the 12 parameters studied here exercised the same role, discriminating among genotypes and treatments (Fig. 4).

## 4. Discussion

### 4.1. Sunflower achene yield and its changes under salinity

Sunflower is an annual crop moderately tolerant to salinity (Farhadi Machekposhti et al., 2017). In the present work, salinity decreased achene yield per plant and its components (Fig. 1, Table 1). Our results agree with the reported data by Hamam and Negim (2014), in which the increase in salinity (25, 50, 75, and 100 mM NaCl) caused a decrease in wheat plant yield values (14.71 %, 30.02 %, 43.12 %, and 56.08 %, respectively). Therefore, it could deduce that at 100 mM NaCl, the decrease in wheat yield was 60 %. This one could be comparable with the results obtained in this work, in which the plants exposed to 130 mM NaCl showed a decrease in grain yield of 73.2 % (considering the four analyzed genotypes). Other data reported by Farhadi Machekposhti et al. (2017) show the influence of salinity from seawater on the achenes yield in sunflower, informing a decrease of 31.9 g plant<sup>-1</sup> and 26 % g plant<sup>-1</sup> in 2013 and 2014, respectively. In that work, the observed decreases could be explained by a decrease in the number of fruits rather than a decrease in their weight. Similarly, Hamid et al. (2021) reported decreases in sunflower yield between 29 % and 58 % when the salinity/conductivity of the saturation extract increased at 3 and 6 dS m<sup>-1</sup>, respectively. In conclusion, the data reported here highlighted that different salinity levels promote decreases in yield in concordance with previous reports for both wheat and sunflower (Hamam and Negim, 2014; Farhadi Machekposhti et al., 2017; Hamid et al., 2021).

From an integrative perspective, achene yield under salinity could be related to changes in the photosynthetic machinery (Pan et al., 2021). Salinity hampers photosynthetic system, transpiration, and gaseous exchange by decreasing the content of chlorophyll and carotenoids, distorting chloroplast ultrastructure and PSII system, and reducing stomatal conductance (Pan et al., 2021).

### 4.2. Changes in achene yield components under salinity and its physiological implications

Salinity decreased the number of achenes per plant and the average achene weight (Table 1). These results agree with those reported by El Mokh et al. (2022) for the bean crop, in which a decrease of 25.9 % in the number of grains per plant after the application of 58 mM NaCl irrigation solution was observed. They also registered a 32 % decrease in the average grain weight due to salinity. Changes in morphological parameters and yield in sunflower and other species could be caused by a reduction in photosynthesis and an increase in tissue Na<sup>+</sup> concentration (Kumari and Bhatla, 2021). Some new experiments could be performed in order to clarify the relationship between changes in morphological traits and ion homeostasis.

Jamshidi and Javanmard (2018), working with barley under salinity, reported a 57.68 % decrease in the “number of grains/ear” parameter at 140 mM NaCl in the soil. This value is consistent with the average decrease for the NAP parameter (51.4 %) in salinized plants.

Hamam and Negim (2014) evaluated the tolerance to saline stress in 16 wheat genotypes, subjecting the plants to four NaCl concentrations (25, 50, 75, and 100 Mm). The results indicated that the average decreases in the number of grains/ear were 10.31 %, 18.42 %, 32.39 %, and 45.57 %, respectively. The decreases in the average grain weight for the four concentrations were 12.68 %, 23.26 %, 34.39 % and 44.64 %, respectively. The decreased values reported for these two studied parameters (especially at 100 mM NaCl) are in agreement with the ones obtained in our work.

Physiologically, a lower number of achenes per plant may be due to changes in the source–sink relationship produced by salinity (C ccoli et al., 2015). These results could be related to an alteration in the phytohormones relation (Arif et al., 2020). Salinity elicits a quick and continuous change in gene regulation with the response to the hormone. Hormones play a vital role in shaping the plant response to salinity (Arif et al., 2020). For example, ABA acts as an endogenous signaling molecule and maintains plant leaf and soil balance (Arif et al., 2020). A reduced water potential in soil caused by salinity produces water limitation, stomatal closure to maintain water balance, and as a consequence, a reduced capacity to take CO<sub>2</sub> by stomatal closure and then producing a reduction in photosynthetic rate (Pan et al., 2021). Therefore, in the present work, a lower photosynthetic rate could reduce biomass

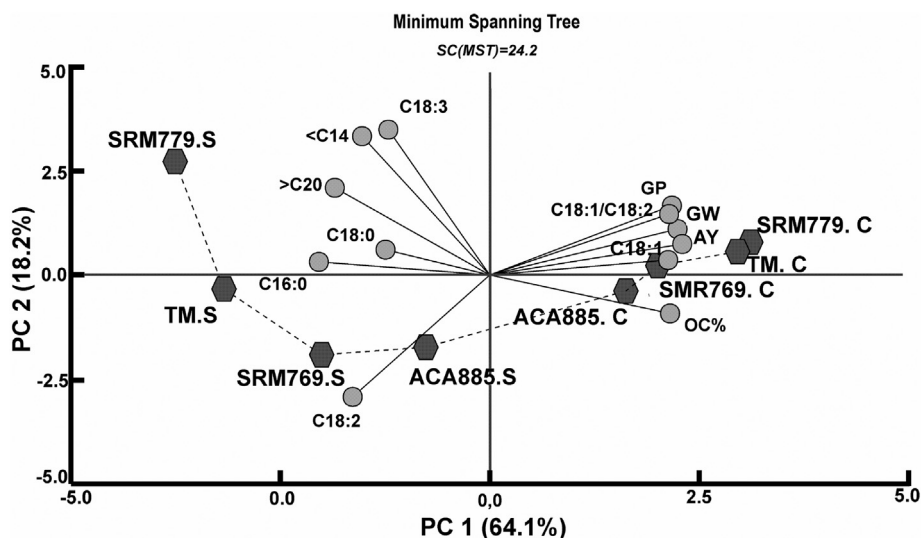


Fig. 4. Principal component analysis in four sunflower hybrids under salinity. Circles represent all parameters measured. Hexagons expressed each genotype and treatment combination. PC1: principal component 1. PC2: principal component 2. SC: sum of squares. MST: minimum spanning tree. GP: achene number. GW: achene weight. AY: achene yield per plant. C18:1: oleic acid content. OC%: total oil content per achene. C18:2: linoleic acid content. C18:1/C18:2: unsaturated relation between oleic acid content and linoleic acid content. C16:0: percentage of palmitic acid. C18:0: percentage of stearic acid. >C20: fatty acid with >20 carbons. <C14: fatty acids with <14 carbons chains. C18:3: content of linolenic acid.

accumulation and the allocation of organic matter in reproductive organs, like the capitula in sunflower and in grains or achenes.

#### 4.3. Understanding the effects of salinity on sunflower oil content and its quality

Salinity effects on achenes' oil content and oil quality are significantly scarce. The results about the effect of salinity on oil content (Fig. 2) agree with the ones reported by Di Caterina et al. (2007), who studied two sunflower hybrids (Carlos and Tenor) subjected to five NaCl treatments. At 120 mM NaCl (similar to 130 mM NaCl used here), the Carlos hybrid showed a decrease in oil content of 36.3 %, while the Tenor hybrid decreased the oil content by 37.9 %. Although the cited literature may be old, it is the closest to what is reported in this work. Farhadi Machekposhti et al. (2017) described that the sunflower crop irrigated with Caspian Sea water decreased the oil content by 34.7 % in 2013 and 35.7 % in 2014 regarding control treatment. The water salinity of the pure Caspian seawater was between 17.9 and 18 dS m<sup>-1</sup>, but it was diluted with "fresh" water, obtaining 13 dS m<sup>-1</sup> C.E., which is equivalent to 130 mM NaCl used in the present research.

On the other hand, a relationship with the data described above could be noted since three of the hybrids studied had an average decrease of 39.35 %, being this similar to the one reported by Farhadi Machekposhti et al. (2017), except for the ACA885 hybrid having a small increase in this parameter (+5 %).

Oil content values reported in the present work under control conditions (between 38 % and 40 %) agree with those reported by Abd El-Hameid and Sadak (2020), who obtained oil percentage yields between 36 % and 39 % for sunflower plants grown in saline conditions. Conversely, these values are superior to the ones reported in our work (between 16 % and 37 %) in plants under 130 mM NaCl. The present work is the first that reports an increase in the percentage of oil content in one genotype due to the effect of salinity (ACA885, Fig. 2). Nevertheless, more experiments are needed to confirm these results.

The physiological traits that underlie changes in achene oil content in sunflower are complex. Salinity creates osmotic stress, increases ROS production, and causes oxidative damage in plant cells, which ultimately decreases crop productivity (Aslam et al., 2021) and achene oil content. In this work, the model of Principal Components Analysis could correlate productivity traits only. More researches that correlate physiological traits (ROS generation, photosynthesis, stomatal conductance changes and ions accumulation in different plant tissues) with oil content in sunflower under salinity are imperatively necessary.

#### 4.4. Changes in fatty acid profile under salinity are genotype-dependent

As it was said, salinity also affected oil content and fatty acid profile in the four genotypes studies (Fig. 2 and Table 2). Flagella et al. (2004) evaluated the effect of saline water irrigation on the fatty acid profile of the sunflower PLATON hybrid. Taking into account the palmitic acid content (16:0), these authors found the opposite behavior to the one observed in our work. Due to salinity, the palmitic acid content decreased by 2.44 %, while in the four hybrids studied here, an average increase of 29 % was obtained. Regarding the content of stearic acid (18:0), our data agree with those reported by Flagella et al. (2004), since salinity increased the content of this fatty acid. In the case of oleic acid content (18:1), the results were opposite to the ones reported in this work (a 4.83 % increase due to salinity in the PLATON hybrid vs. a 32.42 % decrease in the four hybrids studied in this work). Regarding the content of linoleic acid (18:2), the results were notably contrary to those reported by Flagella et al. (2004). The decreasing value was 59.4 %, while our results reported an average increase of 23.25 % (for the four hybrids).

In the present work, salinity caused an increase in the content of polyunsaturated fatty acids (e.g., linoleic acid) concerning

monounsaturated fatty acids (e.g., oleic acid, Table 2). Abd El-Hameid and Sadak (2020) evaluated the effect of salinity through irrigation water at different NaCl concentrations. In this work, salinity decreased the content of palmitic acid (16:0) and stearic acid (18:0) by 10.6 % and 11.50 %, respectively. On the other hand, salinity increased the content of oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) by 0.43 %, 1.05 %, and 6.59 %, respectively. In this case, the reports mentioned a tendency to increase the synthesis of unsaturated fatty acids (oleic, linoleic, and linolenic) due to the salinity effect. Nevertheless, in our work, this increase is characterized by an increase in the proportion of polyunsaturated acids. Yeilaghi et al. (2012) studied the profile of fatty acids of 64 safflower genotypes subjected to salinity (120 mM NaCl). In the Kino-76 genotype, salinity decreased the palmitic acid (16:0) content by 0.4 %. On the other hand, stearic acid (18:0) and oleic acid (18:1) contents increased by 1.26 % and 3.03 %, respectively. The content of linoleic acid (18:2) and linolenic acid (18:3) decreased by 0.53 % and 23.23 %, respectively. The effects of salinity on the content of palmitic acid (16:0), oleic acid (18:1), and linoleic acid (18:2) were opposed to the ones found in the four hybrids sunflower studied here. The increase in the content of stearic acid (18:0) in safflower promoted by salinity (Yeilaghi et al., 2012) agrees with the effect produced by 130 mM NaCl on the fatty acid profile of our sunflower hybrids. Finally, due to salinity, the content of linolenic acid (18:3) decreased in the SRM779CL, TM and SRM769 genotypes, being this effect opposite to the one reported by Yeilaghi et al. (2012).

The effects of salinity on oleic acid (18:1) content and linoleic acid (18:2) content are far from being clear (Flagella et al., 2004; Yeilaghi et al., 2012). The literature on the effects of salinity on the oleic/linoleic ratio in sunflower is practically null. Angeloni et al. (2021) found an increase in grain achene weight and oil content in sunflower when plants were grown under high radiation conditions. Moreover, when sunflower plants were grown under high temperatures, there were changes in oil quality (Angeloni et al., 2021). These results agree with the changes in oil quality detected under salinity in the four hybrids studied in the present work. Trabelsi et al. (2022) reported a decrease in the oleic/linoleic acid ratio of 15.11 % in olive plants grown under salinity. In agreement with this, the ACA885 hybrid got a comparable result (13.4 % decrease under salinity). On the other hand, the remaining three genotypes (SRM779CL, TM, and SRM769 hybrids) showed higher reductions in the ratio of these fatty acids.

Changes in fatty acid content and fatty acid profile could be related to many physiological changes under salinity (Ma et al., 2021). Morphological and physiological changes in sunflower under salinity are produced in roots, stems, leaves, and reproductive organs (Ma et al., 2021). A reduction in sunflower root length and the signaling via ABA could be the reason for the changes and reduction or increases in fatty acid accumulation. These changes are mediated by hormones like ABA, auxins, and gibberellins (Ma et al., 2021). More studies are needed in order to clarify the physiological, biochemistry and hormonal changes in the whole plant produced by salinity.

## 5. Conclusions

Tolerance to salinity, based on the sunflower achene yield and their components under saline stress, was determined in this work. Considering these parameters, the most tolerant hybrid to salinity was SRM779CL, followed by ACA885. The most susceptible hybrids were SRM769 and TM. The yield component that most explained this tolerance was the number of achenes per plant rather than the average weight of each achene. If only oil content was considered, hybrid SRM779CL had the highest oil percentage loss. Contrarily, salinity increased the oil content in ACA885, which is relevant when genotypes are grown in saline soils since those that decrease yield could increase their oil content. Due to the effect of salinity, the fatty acid profile showed ambiguous changes in the four genotypes studied. In all cases, palmitic (16:0) and stearic acids increased their concentration

regardless of the rest of the fatty acids. Salinity decreased the ratio between oleic (18:1) and linoleic acid (18:2). So, salt stress could increase the percentage of unsaturations in fatty acids with 18 Carbons in the four genotypes examined in this work. Finally, considering the 12 parameters measured, PCA could determine that the SRM779CL genotype showed the best performance under control conditions. ACA885 was the most tolerant genotype under salinity, allowing its use in lands under irrigation with saline water or in fields with salinity problems.

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### Declaration of competing interest

The authors declare that the present work was conducted in the absence of any commercial or financial relationship that could be considered as a potential conflict of interest. All authors read and approved the final manuscript.

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