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Changes in Morphological, Physiological and Chemical Characteristics of Sunflower (*Helianthus annuus* L.) Genotypes Induced by Salt Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Authors Parveen and MAH designed the study, wrote the protocol, performed the statistical analysis. Authors SR and AA wrote the first draft of the manuscript, supervised and managed the experimental process throughout. Author JA managed the literature searches and spectroscopy analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Investigations on characterization of various sunflower genotypes regarding their morphological, physiological, chemical parameters, seed oil and its quality through studying fatty acid composition under different salinity levels was carried out at Saline Agriculture Research Centre, University of Agriculture, Faisalabad. Seeds of four sunflower genotypes (FH-385, FH-352, FH-106 and FH-259) were sown in lysimeter and three salinity levels (control, 8dS m^{-1} and 16dSm^{-1}) were developed by using NaCl salt. Results revealed that salinity stress drastically affected the morphological, physiological, chemical parameters and quantity and quality of seed oil in all sunflower genotypes under all levels of salinity stress. Studies further exhibited that sunflower genotype FH- 385 was found leading salt resistant genotype by showing less reduction in all plant

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growth parameters i.e. plant height (25%), shoot fresh weight (30%), SPAD value (13%), relative water content (17%),flower weight (32%), flower diameter (14%), photosynthetic rate (8%), transpiration rate (28%), internal CO_2concentration (24%), stomatal conductance (32%), seed oil (35%), linoleic acid (32%) and K^+/Na^+ ratio (73%) relative to percent of their control at high level of salinity (16dSm^{-1}). The results of our experiment clearly indicated that the sunflower genotypes FH-385 was the most salt tolerant followed by FH-352 and FH-259 while FH-106 was the most salt sensitive genotype.

Keywords: Sunflower; salt stress; gas exchange parameters; seed oil; K^+/Na^+ratio.

1. INTRODUCTION

Around the globe, one of the key factors that contribute towards the degradation of soil is salinization. Poor management practices of soil and water, arid and semi-arid climate (less rainfall, more evapotranspiration rate and high temperature) further speed up the salinization process [1]. About 7% of the soils of the earth are salt affected [2] and by the middle of 21st century this increased salinization will cause the loss of land up to 50% [3]. At present, the high concentration of salts had affected about 77 million hectares (5%) out of the total 1.5 billion hectares of world's cultivated land [4].

Toxicity of ions and inhibition of osmosis are the detrimental effects of salinity. Saline soils having more concentration of salts in soil solution induces osmotic inhibition which reduces water up take ability of plants and ultimately slows down the plant growth. The plant growth is further reduced in saline condition because of ionic toxicity which results due to the presence of excessive salts in rooting medium. Excess of salts causes injures of transpiration stream as well as the cells of transpiring leaves [5]. Among the drastic effects of salinity stress, reduction in plant growth and photosynthetic activities are most noticeable. The key factor responsible for decreased photosynthetic rate is the stomatal closure which is employed to reduce the transpiration rate [6].

The typical characteristic of salt affected soils is the high Na^+/Ca^{2+} and Na^+/K^+ ratios which are due to the presence of surplus amount of exchangeable Na^+ in the soil solution. Plants grown in such soils up take more Na^+ and ultimately reduce the uptake of other cations like Ca^{2+} and K^+. However, these ions are necessary to be available in reasonable amount for the better functioning and stability of cell membranes and enzymes [7]. Under salt stress condition, the adequate K^+ in plant tissues is maintained by various mechanisms like Na^+ and K^+ cellular compartmentation and its distribution in shoots and selective K^+ uptake [8,9]. The strategies that are employed most often by the plants for osmotic adjustment and to keep up the desirable K^+/Na^+ ratios in the cytosol includes prevention of entrance of Na^+, removal of Na^+ from the cellular environment and regulation in the uptake of K^+ [10]. It has been reported that ability of plants for K^+ over Na^+ selective uptake and maintenance of high K^+/Na^+ ratios are considered one of the most important selection criteria for salinity tolerance in plants [11,12].

Sunflower (*Helianthus annuus* L.) is considered as one of the most important oilseed crop because of its high potential for oil seed development and is getting substantial popularity being the short duration. It is considered as moderately salt tolerant crop [13]. The ranges of oil contents in sunflower vary from 40-45%. It contains linoleic acid and oleic acid in abundance and cholesterol in less quantity and in addition it contains 85–95% polyunsaturated fatty acids [14]. It has been reported that saline environment had a great effect on the composition of fatty acids. Increasing salinity level improved the concentration of oleic acid and decreased the concentration of linoleic acid due to salt induced inhibition in enzymes activity, i.e. oleate desaturase [15]. The effects of salinity on fatty acid composition and oil contents had been investigated by different researchers in various oilseed crops like evening primose (*Oenothera biennis*), rapeseed (*Brassica napus* L.) [16] and safflower (*Carthamus tinctorius* L.) [17].

Salt stress is also responsible for significant reduction in oil contents from 38.3 to 3.8 g per head. With increasing salinity, inhibition of oleate desaturase occurred, which is responsible for decreasing oil contents in sunflower seed from 524 to 508 mg oil g^{-1} seed. On the other hand, increased in oleic acid contents was observed from 82.8 to 86.0% and decreased in linoleic acid contents were observed from 6.9 to 2.8% because of salt stress [18]. The possible

mechanism for more oleic/linoleic acid ratio may be because of water stress, buildup of Na^+ and Cl^- ions and peroxidation of lipids under stress occurred due to salinity [19].

Alysimeter study was performed using four sunflower genotypes under three different levels of salinity (control, 8 and 16 dS m^{-1}) with following objectives.

- To find out salt tolerant and salt sensitive sunflower genotypes.
- Studying morphological, physiological and chemical parameters of salt tolerant and salt sensitive genotypes.
- How the quality and contents of seed oil of sunflower are affected under induced salt stress.

2. MATERIALS AND METHODS

2.1 Plant Material, Growth and Treatments

A lysimeter study was conducted at Saline Agriculture Research Centre, University of Agriculture, Faisalabad. Seeds of four sunflower genotypes (FH-385, FH-352, FH-106 and FH-259) were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad. Soil having pH= 7.56,EC_e= 4dS m^{-1} and SAR= 8.075 (mmol $L^{-1})^{1/2}$ was used to fill the lysimeter. Two salinity levels (8 dS m^{-1} and 16 dS m^{-1}) were developed by mixing calculated amount of NaCl salt, whereas no salt were added in control treatment. Twenty seeds of each sunflower genotype were sown in each lysimeter and thinning was done fifteen days after germination to maintain seven plants per lysimeter. The recommended dose of nitrogen, phosphorus and potassium were added in the form of urea, DAP and SOP respectively. Each lysimeter were irrigated with tap water having composition (EC= 0.95 dS m^{-1}, CO_3^{2-}= absent, HCO_3^- = 4.9 me L^{-1}, Cl^-= 1.40 me L^{-1}, Na^+= 2.9 me L^{-1}, Ca^{2+} + Mg^{2+}= 6.4 me L^{-1}, SAR =1.62 ($mmol_c$ $L^{-1})^{1/2}$, TSS= 9.0 me L^{-1}) when required. The experiment was replicated four times using Completely Randomized Design (CRD) in factorial arrangement.

2.2 Growth Analysis

Plant height and shoot fresh weight were measured during harvesting at maturity stage. SPAD value (chlorophyll content) of the leaves was determined by using SPAD instrument (Minolta, Japan), while leaf area and flower diameter were determined by using Vernier Caliper.

2.3 Relative Water Contents (RWC)

The relative water contents of fresh leaves were determined using equation proposed by [20].

2.4 Measurements of Gas Exchange Parameters

Measurements of net photosynthetic rate (A), transpiration rate (E), stomatal conductance (g_s), and sub-stomatal CO_2 concentration (C_i) were made on a fully expanded youngest leaf by using an open system LCA-4 ADC portable infrared gas analyzer.

2.5 Leaf Sap Extraction and Determination of Na^+ and K^+

The youngest fully expanded leaves were separated at harvesting time and stored at freezing temperature to determine K^+ and Na^+ concentration in leaf sap. Frozen leaf samples were thawed and crushed using a stainless steel rod with tapered end. The sap was collected in other Eppendorf tubes by Gilson pipette and centrifuged at 6500 rpm for 10 minutes. The supernatant sap was used for determination of Na^+ and K^+ concentration by using Sherwood 410 Flame photometer.

2.6 Oil Extraction

100g dried seeds from each treatment were crushed and oil was extracted with 0.5 L of n-hexane using a Soxhlet apparatus. Oil contents were determined by evaporating the extractant in a rotary evaporator.

2.7 Fatty Acid Composition

The fatty acid composition (palmitic, linoleic and linolenic acids) was measured by gas chromatography (GC-2014 Standard capillary and packed).

2.8 Statistical Analysis

All data presented in this experiment are means of four replicates. Analysis of variance (ANOVA) was performed by using a statistical package, statistics 8.1®.

3. RESULTS

3.1 Effect of Salinity on Morphological Parameters

Data regarding plant height and shoot fresh weight of sunflower genotypes (Table 1) showed that salinity stress exerted strong negative impact on plants growth. However, the impact differed significantly among sunflower genotypes. The genotype FH-385 was most capable of tolerating salinity as at both salinity levels (8 and 16 dSm^{-1}NaCl) it showed minimum reduction in plant height(16 and 25%) and shoot fresh weight (20 and 30%)respectively when compared with performance of FH-385 grown under control condition and with performance of other genotypes. On the other hand, the FH-106 genotype showed much larger sensitivity to NaCl salinity stress and most drastic reduction was observed in plant height (28 and 50%) and shoot fresh weight (31 and 46%)at both salinity levels as compared with control and all other genotypes.

Salt stress also exerted a drastic effect on flower growth and development by affecting its weight and diameter (Table 1). Both salinity levels caused significant reduction in flower weight and flower diameter of all sunflower genotypes as compared to control. Among the genotypes, FH-106 showed the highest reduction in flower weight (33 and 58%) and diameter (39 and 63%) while minimum reduction in flower weight and diameter were shown by FH-385 (22 and 32%) and FH-352 (11 and 13%) respectively at both levels of salinity stress as compared to control and other genotypes.

Application of NaCl salt stress also resulted in a significant decrease in relative water contents (RWC) and SPAD value of all the sunflower genotypes under investigation (Fig. 1). Among the genotypes, FH-385 was superior in withstanding salinity and thus reduction in RWC (8 and 17%) and chlorophyll contents (6 and 13%) recorded was minimum under both levels of induced salinity (8 and 16 dSm^{-1}NaCl) as compared to control. The genotype FH-106 was most sensitive to salinity stress and showed highest reduction in RWC (13 and 29%) and chlorophyll contents (17 and 27%) at both levels of salinity when compared with control. The results of other genotypes were between the results of these two genotypes.

3.2 Effect of Salinity on Photosynthetic Parameters

Photosynthetic parameters were estimated by studying the photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (*Gs*) and internal CO_2 concentration (*Ci*) of four sunflower genotypes. Results of each parameter are given in Table 2.

Table 1. Effect of salinity on morphological parameters of sunflower genotypes

Morphological parameters		Sunflower genotypes			
		FH-385	FH-352	FH-106	FH-259
Plant height (cm)	S_0	60.5a	59.7a	55.5ab	57.7ab
	S_1	51.0bc (16)	45.2cd (24)	40.0d (28)	41.7d (28)
	S_2	44.2cd (25)	39.7d (34)	28.0e (50)	38.5d (33)
Shoot fresh weight (g)	S_0	137.5a	108.5ab	106.2ab	112.2a
	S_1	109.7bc (20)	86.0cde (21)	73.5ef (31)	87.0cd (22)
	S_2	96.2cde (30)	74.5def (31)	57.2g (46)	69.5fg (38)
Flower weight (g)	S_0	58.0a	54.5a	53.0ab	56.5a
	S_1	45.5bc (22)	42.5cd (22)	35.7de (33)	37.5cde (34)
	S_2	39.2cde (32)	31.5e (42)	22.0f (58)	34.5de (39)
Flower diameter (cm)	S_0	6.9a	5.6abc	5.7abc	6.3abc
	S_1	6.7ab (13)	5.0cd (11)	3.5de (39)	5.2bc (17)
	S_2	5.9abc (14)	4.9cd (13)	2.1e (63)	5.2bc (17)

Effect of salinity on morphological parameters: Ratio (n=4 ± standard deviation). Values with the same letter in each column are not significantly different at P ≤ 0.05. S_0: control, S_1: 8 dS m^{-1}, S_2: 16 dS m^{-1} and values in ( ) are % of their control

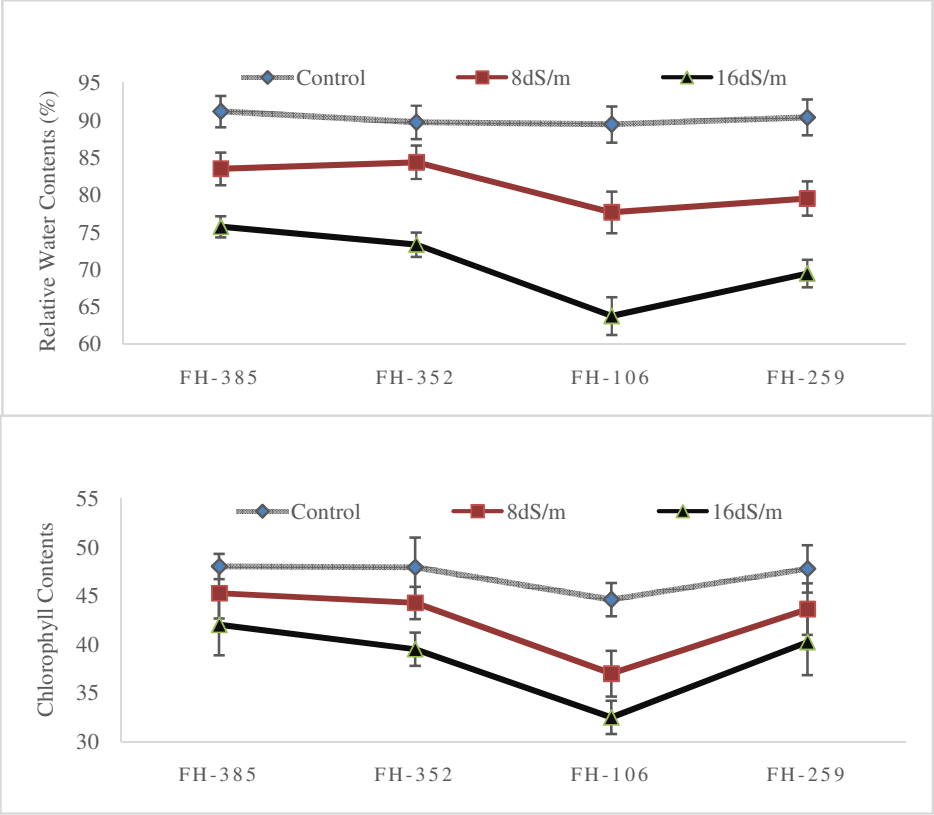

Fig. 1. Effect of salinity on chlorophyll contents (SPAD) and relative water contents (%)

Table 2. Effect of salinity on photosynthetic parameters of sunflower genotypes

Photosynthetic parameters		Sunflower genotypes			
		FH-385	**FH-352**	**FH-106**	**FH-259**
Photosynthetic Rate (*A*) (µmol CO_2 $m^{-2}s^{-1}$)	S_0	27.5a	26.0b	26.3b	25.1d
	S_1	25.8bc (6)	24.1d (7)	23.0f (13)	22.1e (12)
	S_2	25.2cd (8)	22.1e (15)	20.6f (22)	21.9f (13)
Transpiration Rate (*E*) (mmol $H_2Om^{-2}s^{-1}$)	S_0	5.9a	5.1b	4.2cd	4.6bc
	S_1	5.1b (14)	4.0d (22)	3.0ef (29)	3.2ef (30)
	S_2	4.2cd (28)	3.3e (35)	2.0g (52)	2.7f (41)
Stomatal Conductance (*Gs*) ($mmolm^{-2}s^{-1}$)	S_0	2.93a	2.8a	2.75a	2.7a
	S_1	2.41ab (18)	2.11b (25)	1.77c (36)	1.89c (30)
	S_2	2.00b (32)	1.84c (34)	1.30d (53)	1.48d (45)
Internal CO_2 Conc. (*Ci*) (µmol mol^{-1})	S_0	3.19a	2.99a	2.73bc	2.84ab
	S_1	2.76bc (13)	2.01e (33)	1.72fg (37)	1.92ef (32)
	S_2	2.41cd (24)	1.50gh (50)	1.03hi (62)	1.32h (54)

Effect of salinity on photosynthetic parameters: (n=4 ± standard deviation). Values with the same letter in each column are not significantly different at P ≤ 0.05. S_0: control, S_1: 8 dS m^{-1}, S_2: 16 dS m^{-1} and values in ( ) are % of their control

Compared to control, NaCl addition caused significant reduction in photosynthetic rate and transpiration rate in all sunflower genotypes at both levels of salinity. The higher reduction in photosynthetic and transpiration rate was observed in sunflower genotypes FH-106 which was 22 and 52% respectively while minimum reduction recorded in the genotype FH-385 was found to be 8 and 28% respectively with respect to control at higher level of salinity (16 dSm^{-1}NaCl). Other two sunflower genotypes showed reduction in between salt tolerant and salt sensitive genotypes at both levels of salinity.

The effect of stomatal conductance (*Gs*) and internal CO_2 concentration (*Ci*) of four sunflower genotypes (Table 2) under induced salt stress exhibited the same responses. The stomatal conductance and internal CO_2 concentration reduced under the stress, and this reduction was maximum in FH-106 (53 and 62%)and minimum in FH-385 (32 and 24%) respectively at higher level of salinity (16 dSm^{-1}NaCl).

3.3 Effect of Salinity on Oil Contents and Fatty Acid Composition

The significant reduction in the oil contents of seed was observed among all the sunflower genotypes grown under induced levels of salt stress. The extent of reduction was variable and the genotype which showed most reduction in oil contents under both levels of salinity stress(8 and 16 dSm^{-1}NaCl) was FH-106 (22 and 46%) and the minimum reduction was recorded in genotype FH-385 (15 and 35%) with reference to control (Fig. 2).

Salinity also influenced the fatty acids composition of sunflower genotypes. For studying the fatty acids profile, the concentration of palmitic acid, linoleic acid and linolenic acid was determined. However, the impact of salinity differed significantly between sunflower genotypes regarding the composition of fatty acids. The concentration of palmitic acid and linolenic acid showed a significant increment with increasing levels of salinity while the concentration of linoleic acid decreased respectively in all the genotypes (Fig. 3). At both levels of salinity (8 and 16 dSm^{-1} NaCl), the maximum increase in palmitic (65 and 102%) and linolenic (124 and 233%) acids were shown by the genotype FH-385 while the maximum reduction was observed in FH-106 compared with plants grown under controlled conditions. The linoleic acid showed different behavior and decreased with increasing salinity and maximum reduction recorded (33 and 37%) was observed in FH-352 while minimum reduction (17 and 27%) was observed in FH-106 under both levels of induced salinity (Fig. 3).

3.4 Effect of Salinity on Plants Chemical Parameters

Data regarding Na^+, K^+ concentrations and K^+/Na^+ ratio is depicted in (Fig. 4). Significant differences were observed for concentrations of Na^+, K^+ and K^+/ Na^+ ratio in the cell sap of sunflower genotypes. Concentration of Na^+ differed significantly between control and other two levels of salt. By increasing salinity, a significant increase in Na^+ concentration was observed in each sunflower genotype. The lowest Na^+ concentrations were observed in FH-385 genotype and the highest in FH-106 genotype at both salinity levels. The trend in case of potassium was almost reverse, showing decreased K^+ concentration in all sunflower genotypes with increasing salinity. However, this decrease in potassium was more prominent in FH-106 genotype as compared to FH-385, FH-352 and FH-259 sunflower genotypes. The genotype FH-385 was better in maintaining high level of K^+ at both salinity levels. The increasing uptake of Na^+ with increase in the salinity levels resulted in a decrease of K^+/Na^+ ratio. The highest potassium concentration at high salinity level resulted in maintaining higher K^+/Na^+ ratio in FH-385 genotype, showing better performance under saline conditions as compared with other genotypes.

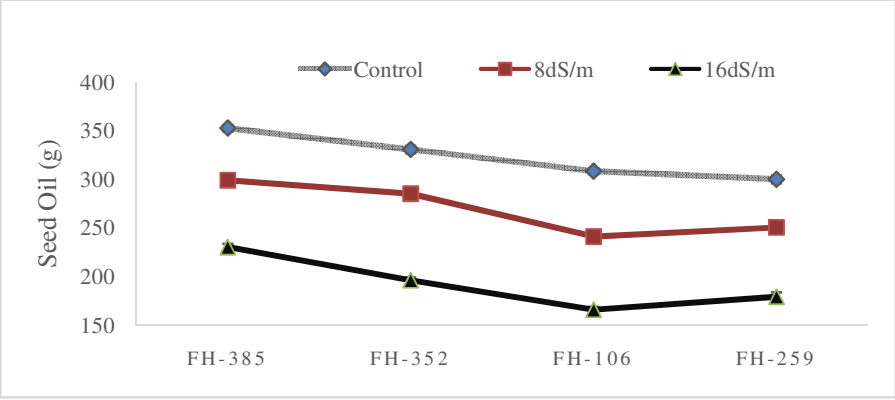

Fig. 2. Effect of salinity on the contents of seed oil (g)

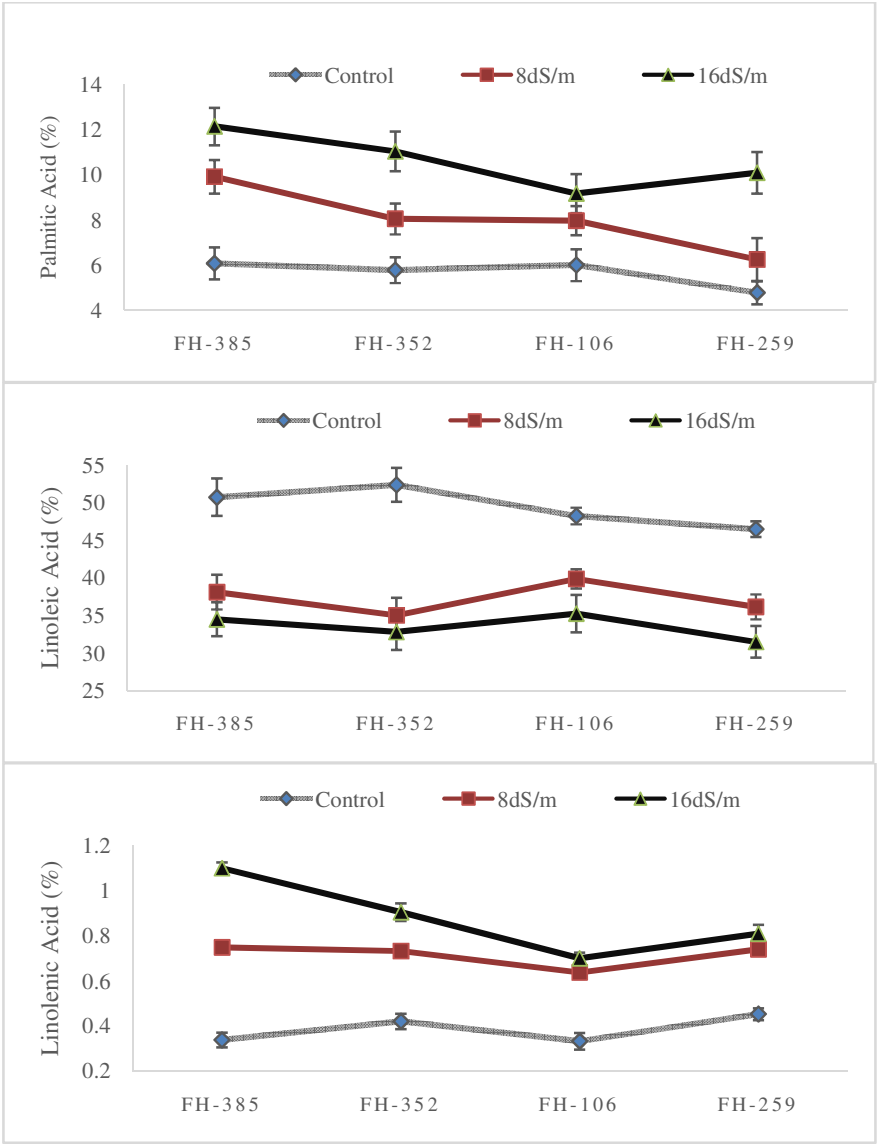

Fig. 3. Effect of salinity on the contents palmitic acid (%), linoleic acid (%) and linolenic acid (%)

4. DISCUSSION

Our results showed that sunflower genotypes responded differentially to different levels of salinity stress. The existence of variation for salinity tolerance could be useful for development of high yielding salt tolerant genotypes [21]. In present study, salinity stress significantly reduced the growth of sunflower by affecting plant morphological (plant height, shoot fresh weight, relative water content, flower weight and diameter), physiological (gas exchange parameters), fatty acid composition (seed oil, palmitic acid, linoleic acid, linolinic acid) and ionic parameters (K^+ concentration, Na^+ concentration, K^+/Na^+ ratio) confirming that salinity caused reduction in plant growth [12,22,23].

Results of current study revealed that salt stress caused a significant reduction in plant height, shoot fresh weight and relative water contents in all sunflower genotypes, but the reduction percentage of all these parameters was noticeable in FH-106 genotype relative to other sunflower genotypes which indicates its sensitivity against different levels of salinity. Production of different crop plants under salt stress versus non stress situation for a longer

period of time is associated with its ability for salt tolerance [24]. High accumulation of Na^+ and Cl^- in leaf sap and non-availability of water due to salinity stress caused the reduction in plant morphological parameters. Salinity stress lead to turn down the osmotic potential of nutrient solution which results in reduction of water uptake and finally plant height, shoot fresh weight and relative water contents [25,26,11].

Flower weight and flower diameter of all sunflower genotypes studied were inhibited by salt stress. Sunflower genotype FH-106 suffered the largest flower weight and flower diameter reduction, while the smallest reduction was observed in genotype FH-385 suggesting that the former is the most salt-sensitive and latter is the most salt-tolerant genotypes. The decreased flower weight and flower diameter with increased salinity was also reported by [27] and [28] in sunflower.

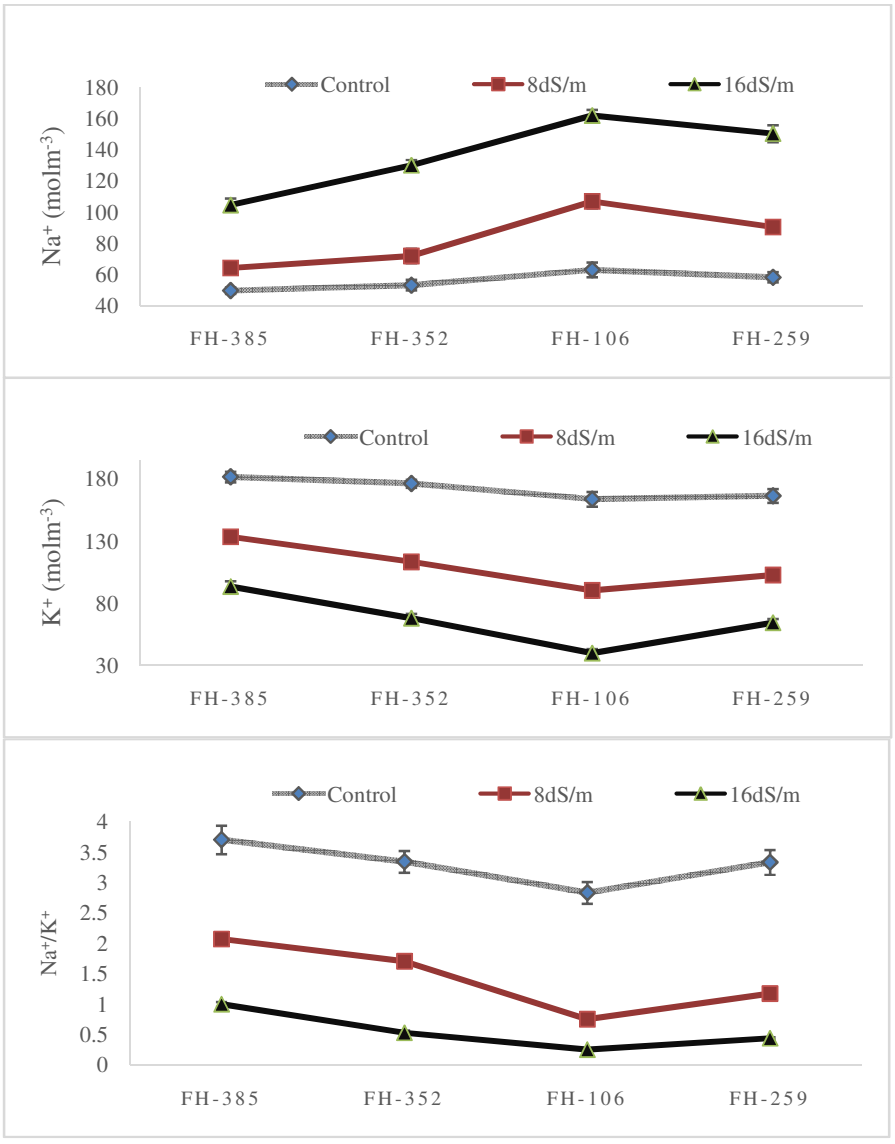

Fig. 4. Effect of salinity on the concentration of Na^+(molm^{-3}), K^+ (molm^{-3}) and Na^+/ K^+ ratio

The decrease in photosynthesis under salt stress condition occurs due to closing of stomata that finally leads not only to decreased leaf transpiration rate and leaf internal CO_2 concentration, but also to non-stomatal factors; For instance reduction in green pigments and leaf area [29]. In the current study, imposition of salinity stress in rooting medium significantly decreased the transpiration rate (*E*), photosynthetic rate (*A*), internal CO_2 concentration and stomatal conductance (*Gs*) in all sunflower genotypes, but the degree of decline in these gas exchange parameters was more prominent in sunflower genotype FH-106. It might be due to the reason that salt stress decreased the availability of water to the plants which is essential for sustainable crop production [30] and plants sensed the water availability around the roots and responded by sending chemical signals via hormone abscisic acid to the shoot to extract adaptive responses, by closing stomata [31]. The sunflower genotypesFH-385 performed superior by maintaining higher transpiration rate, photosynthetic rate, internal CO_2 concentration and stomatal conductance at both levels of salt stress and confirming the preceding research results in which it was reported that salt tolerant genotypes maintain better gas exchange parameters relative to salt sensitive genotypes at high level of salinity [12,32].

Salt stress caused significant reduction in seed oil contents of all sunflower genotypes. However, all sunflower genotypes responded differentially to different level of salinity stress. In present study, sunflower genotypes FH-385 had succeeded in maintaining high level of oil content at all salinity levels. These results are in line with preceding findings in which it was revealed that salinity stress reduced seed oil content in safflower [33] and sunflower [34]. It is broadly acknowledged that the quality of seed oil is closely associated with its fatty acid composition, generally the percentage of palmitic, linolenic, oleic and linoleic acids, but quantity of linolenic and linoleic acids is most important relative to other fatty acids. In the current study, salinity stress significantly increased palmitic and linolenic acid contents in all sunflower genotypes while linoleic acid content decreased but sunflower genotypes FH-385 maintained better fatty acid composition relative to other three sunflower genotypes at both levels of salinity. Thus the reduction in oil quality due to salinity stress in all sunflower genotypes is similar to that observed previously in chia (*Salvia hispanica*), stock (*M. tricuspidata*), evening primrose (*O. biennis*) [16] and *Matthiola incana* [35].

Sodium transport from growth medium to cytoplasm of plant cells depends upon electrochemical potential gradient of Na^+ and presence of Na^+ transport channels in the plasma membranes, which permit Na^+ penetration [36]. This selective uptake of Na^+ ions in plasma membrane may be a key factor in sensitivity or tolerance of sunflower genotypes. High salinity induces an increase in the Na^+ ions concentration which competes with the uptake of other important nutrients ions like K^+ and ultimately leads to K^+ scarcity in plant cells [37,38]. Results of current study depicted that high level of Na^+ ions in leaf sap of salt sensitive sunflower genotypes negatively affects their growth. Our results are in line with previous studies in which the concentration of Na^+ ions increased with increasing salinization [39] that leading to salt injury to plants [40].

On the other hand potassium retaining capability of plant cells is a key factor for salinity tolerance, High retention of K^+ and higher K^+/Na^+ ratio are two major factors that helps the salt-tolerant genotypes to perform well under salt stress condition [41,42]. The salt tolerance ability of sunflower genotypes FH-385 was also due to maintaining high K^+ ion concentration in the cell sap that result in high K^+/Na^+ ratio. More root and shoot fresh weight and higher K^+/Na^+ ratio in salt tolerant genotypes were also recorded by [43]. Present study revealed that sunflower genotypes have variable response to salinity from highly sensitive to highly tolerant ones. This variation in salinity tolerance was due to their ability to maintain high K^+/Na^+ ratio by retaining more K^+ in cell sap.

5. CONCLUSION

Result of present study revealed that salinity reduced plant height, shoot fresh weight, relative water contents, SPAD value, flower weight and diameter, gas exchange parameters, seed oil and fatty acid composition, K^+/Na^+ ratio in all sunflower genotypes at both levels of salinity. However, among all genotypes, FH-385 was found best performing sunflower genotype even in high salinity conditions by showing better morphological, physiological, fatty acid composition and ionic parameters. Therefore, the said promising genotype (FH-385) can be used in future breeding program to develop salt resistant with good quality seed oil sunflower

genotypes and can be recommended for cultivation on salt affected soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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