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Effect of salt stress on leaf photosynthetic and antioxidant traits of *Vicia faba* genotypes

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Salinity is a serious and increasing problem for the agriculture sector in the world due to the expected climate change and water scarcity. This study aimed to screen the tolerance of eleven Egyptian genotypes/ cultivars (Giza716, Giza843, Masr 1, Nubaria 1, 3, 4, and 5, Sakha 1, 3, and 4, and Wadi 1) to salinity stress. The plants were grown under three salinity treatments in addition, to control (T1= 50 mM, T2= 75 mM, and T3= 100 mM NaCl). Biomass, biochemical, and leaf photosynthetic parameters were measured. The results revealed that increasing salinity stress led to a significant increase in soluble proteins, sugars, and phenolic compounds in the leaves of all genotypes compared to the control. Maximum transpiration rate and stomatal conductance values were under control conditions, while the minimum values were under T3 salinity treatment. The photosynthetic rates were generally decreased by increasing salinity stress except for Nubaria 3. The photosynthetic rates of Masr 1, Nubaria 1, and Nubaria 5 genotypes decreased by > 50% of their photosynthetic rate (A) values at T3 compared to their control. The Intrinsic water-use efficiency (iWUE) was remarkably high at T3 salinity treatment for all genotypes. The maximum increment of the antioxidant enzyme CAT activity under salinity stress was recorded in Nubaria 3. We can conclude that the Nubaria 3 genotype was considered the most tolerant genotype to salinity in terms of having better photosynthesis performance and antioxidant activity under salinity stress.

Keywords: Faba bean; salinity stress; photosynthesis; gas exchange; antioxidants; biomass

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INTRODUCTION

Salt stress is considered a destructive abiotic stress that negatively impacts the growth and yield of plants (Neji et al., 2021). High salinity affected about 33% of the world's irrigated agricultural land and 20% of its cultivated land, with a rate of 10% increase annually (Shrivastava et al., 2015). According to some future scenarios, it is estimated that salinity will cause a 50% loss of cultivated land by 2050 in the Mediterranean region and South Asia (Sadak et al., 2015; Alam et al., 2020). Under arid and semi-arid conditions, the impact of salinity stress is more severe and pronounced compared to other regions of the world (Sehrawat et al., 2020). There are two mechanisms by which salinity stress can affect plants: 1) ion toxicity due to the presence of high salt concentrations inside the plant, and 2) osmotic stress that occurs through the deposition of large quantities of soluble salts in the soil. These effects reduce the water supply from soil to plants (Zamin et al., 2017; Safdar et al., 2019). The growth and yield of most crops can be affected negatively by soil salinity, which causes hyper-ionic and hyper-osmotic effects in plants. These impacts lead to the cell membrane's disorganization and more reactive oxygen species (ROS) production, which enhances metabolic toxicity (Joseph et al., 2011). ROS can seriously disturb the normal metabolism of cells through oxidative damage of lipids, proteins, and nucleic acids (Molassiotis et al., 2006). Photosynthetic activity and photoassimilate partitioning are affected by salinity stress and they are associated with damage to chloroplasts and a reduction in chlorophyll pigments and carotenoids (Suwa et al., 2006, Meng et al., 2011). Besides, the expression of Rubisco is suppressed by salinity (Koch, 1996), which reduces plant growth and development (Møller et al., 2010, Ahmad et al., 2018). Moreover, photosynthesis under saline conditions is limited due to the stomatal closure caused mainly by low intercellular intrinsic CO_2 concentrations (Misra et al., 1997). Hence, the generation of salt-tolerant genotypes is an important strategy to cope with the destructive impacts of salinity on crop yield (Gasim et al., 2015).

Faba bean (*Vicia faba* L.) is an important staple crop in the world and the Mediterranean region. Lopez-Bellido et al. (2005) stated that the faba bean is the fourth most important legume after chickpeas, dry beans, and dry peas. About 4.1 million tons in 2014 were the global production of faba bean seeds (FAO (FAOSTAT Database, 2019). Relative to other crops, the faba bean is highly sensitive to salinity stress (Ahmad et al., 2019). The crop becomes non-tolerant to salinity under drought conditions because of the incapability of the plant to efficiently use the available soil moisture (Tavakkoli et al., 2016). Neji et al. (2021) found salt stress influenced all measured parameters in faba bean at 75 mM NaCl, however there was a more noticeable decline of all values at 150 mM NaCl salt concentration. To improve the productivity of *V. faba* under salinity stress, developing more salt

tolerant genotypes is necessary. To achieve this purpose, salinity resistance screening of crop genotypes is the first step in this regard. However, according to Karaköy et al. (2012), and Yadav et al. (2021), very few efforts have been conducted toward screening tolerance of *V. faba* genotypes under saline conditions worldwide. Therefore, the current work aimed to assess the genotypic variation of eleven *V. faba* cultivars in Egypt to salt stress.

METHODS

Plant growth metrics and biomass measurements

Eleven Egyptian *V. faba* genotypes (Giza 716, Giza 843, Masr 1, Nubaria 1, 3, 4, and 5, Sakha 1, 3, and 4, and Wadi 1) were screened for their tolerance to salinity stress. In completely randomized designs, the plants were grown under control and salt stress in the greenhouse (N= 5). In addition to control, three salt stress treatments (T1= 50 mM, T2= 75 mM, and T3= 100 mM NaCl) were used. Thirty days after sowing, the physiological measurements (photosynthetic capacity, biochemical analysis, and enzymology of leaves) were conducted. The following parameters were measured for each cultivar: the leaves number and fresh weight (g), root fresh weight and dry weight after drying it for 72 at 70 ºC, stem length (cm), stem fresh and dry weights.

Photosynthetic activity under salinity stress

Leaf gaseous exchange of each *V. faba* genotype at control and salinity treatments (N=3 leaves, 9 records each) was measured using a portable photosynthesis system (LCpro-SD, ADC Bioscientific, Hoddesdon, UK) equipped with a standard 2 × 3 cm^2 leaf chamber, and a red/blue LED light source. The pots of all replicas were well-watered according to their treatments (0 or T1 or T2 or T3) a day before measurements to a level of the moisture capacity (0.7 L) of a pot. The measurements were carried out on fully expanded leaves from 9:00 am to 2:00 pm. The light was controlled above 1000 µmol m^{-2} s^{-1} using the red/blue LED light source, the flow rate was fixed at 200 µmol s^{-1}, and the leaf block temperature was 23 ºC. Net CO_2 assimilation rate (A), stomatal conductance (g_s) and transpiration rate (E), and internal CO_2 concentration/ambient CO_2 ratio (Ci/ a CO_2) were estimated from gas exchange measurements using the equations developed by von Caemmerer and Farquhar (1981). To eliminate possible effects of air humidity and temperature on transpiration, the intrinsic water use efficiency was calculated as the ratio of A to Gs (Lacono et al., 1998).

Biochemical analysis and enzymology

Total soluble sugars and proteins

Fresh 0.3 g of leaves were extracted with 5 borate buffer (8.63 g boric acid, 29.8 g KCl, 3.5 NaOH dissolved in one Liter distilled Water, pH= 8.5 to 9.5) and then the total soluble proteins were quantified according to Waterborg and Matthews (1994) technique. A sample of the extract was combined with 1 ml of a recently prepared (1:1 v/v) solution of 2 % sodium carbonate in 4 % sodium hydroxide and 0.5 % copper sulphate in 1% sodium tartrate. The mixture was allowed to stand for 10 minutes before adding 0.1 ml Folin and completed to a known volume. The colour density of the mixture was measured after 30 minutes at 700 nm, using Cecil CE. 1010 spectrophotometer. All determinations were performed in triplicate. 0.1 g leaf fresh weight was ground with 5 ml ethanol and centrifuged at 4000 rpm for 10 minutes at 4°C; the supernatant was completed to a known volume with distilled water. Umbriet et al. (1959) method was used for determining total soluble sugars using anthrone. Six ml anthrone solution (2 g l^{-1} H_2SO_4, 0.2%) was added to a three ml sample and heated for 3 minutes in a boiling water bath. After cooling, the generated colour was spectrophotometrically measured at 620 nm with a Cecil CE. 1010 spectrophotometer. All determinations were performed in triplicate.

Phenolic compounds

The plant materials were washed, air-dried, crushed, and ground into fine powder by a grinding machine. In a dark container, 50 ml of 99% methanol was added to 5 g of air-dry plant powder for extraction. The samples were incubated in a shaking incubator at 60℃ for 48 hours. After the incubation period, the samples were filtered through filter paper No. 1. The filtrate was used to determine the total phenolic compound content by the method of Savitree et al. (2004). Half ml of each sample was mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu reagent in a test tube and 2.0 ml of 7.5% sodium carbonate. The covered tubes were allowed to stand for 30 minutes at room temperature. The blue color intensity was read at 760 nm spectrometrically (Cecil CE. 1010 spectrophotometer). All determinations were performed in triplicate. A standard curve of gallic acid was used for the estimation of the total phenol content of the samples.

Antioxidant enzymes

Antioxidant enzymes were represented by peroxidase and catalase enzymes. The guaiacol oxidation method was used to measure peroxidase activity according to Chance and Maehly (1955). In this method, one gram of fresh leaves was homogenized in a pre-cooled mortar and pestle with 3 ml of 0.1 M phosphate buffer pH 7.0. The homogenate was centrifuged for 10 minutes at 6000 rpm at 4°C. The supernatant was used for enzyme assay, and it could be stored on ice within 2-4 h until the assay is conducted. The quartz cuvette was cleaned by using 2.2 ml (10 mM) potassium phosphate buffer, 0.5 ml (8 mM) guaiacol, and 0.2 ml H_2O_2 (30 %). The reaction was started by adding 0.1 ml of enzyme extract to the cuvette and mixing immediately. The cuvette was placed in the spectrophotometer to measure the change in absorbance for 30 seconds up to 3 minutes. Absorbance was measured at 470 nm using Jenway 6405 UV/Vis spectrophotometer. All determinations were performed in triplicate. Catalase was determined using the Góth technique (1991). A known weight of fresh leaves (approximately 0.5 g) was homogenized with 10 ml of cold phosphate buffer (Na/KP) pH 6.8 in a pre-chilled pestle and mortar. The homogenate was filtered and then centrifuged for 10 minutes at 6000 rpm at 4°C. The supernatant was used for enzyme assay. The assaying mixture contained 1 ml of buffered H_2O_2 (Na/K P pH 7.4) and 0.2 of enzyme extract. The reaction was stopped by adding 1 ml of ammonium molybedate (amm. Mol.) (4 g/l) after the incubation period (4 minutes) at 25°C. The formation of a complex between H_2O_2 and amm. Mol. was followed by the decline in absorbance at 405 nm using Jenway 6405 UV/Vis spectrophotometer. All determinations were performed in triplicate.

Statistical analysis

All the experiments were conducted in three replicates. The results in the tables are presented as the means ± SD. One-way analysis of variance (ANOVA I, Tukey post hoc) was performed to assess the variations between control and different treatments of salinity. The data were tested for normality and homogeneity of variance ($p < 0.05$).

RESULTS

Growth metrics

There were significant differences in root length and root fresh and dry weights among all the genotypes at $p<0.05$ (Fig. 1). The obvious response to high salinity was in Giza 843 and Masr 1, while it fluctuated with no definite trend for the other genotypes. The highest length of the root was significant at control conditions for Giza 716, Giza 843, and Nubaria 3. While the highest root length was at T1 and T2 for Nubaria (1 and 4) and Sakaha (1 and 4), respectively (Figure 1). Additionally, some species such as Masr 1 and Sakaha 3 showed no difference in root length between control and T1 and/or T2. The same trend was obtained for root weights with maximum root fresh and dry weights for Nubaria 3. Increasing salinity stress leads to decreased stem lengths comparable with control (Figure 2). The stem lengths of *V. faba* (genotypes) were significantly varied at different salt treatments (T1, T2, and T3) compared to control. Salinity stress leads to significant differences in the stem weights (fresh and dry) but with no definite pattern (Figure 2). The highest values for stem weights were sometimes obtained at control conditions, while in some cases; it was obtained at any degree of salt conditions. Based on dry weight measurement, Nubaria 3-5 and Sakha 1-4 were the most tolerant species under salt conditions. The maximum leaf fresh weights were mostly recorded at control conditions (Figure 3). The leaves number / plant was significantly different among genotypes compared to control with no definite trend (Figure 3). The maximum no. of leaves per plant was recorded for Giza 843 and Nubaria 5 at control conditions, Giza716, Nubaria 1–4 at T1, Sakha 1–4 at T2, Masr 1 and Wadi 1 at T3. The highest number of leaves among all treatments was at T2 for Sakha 4.

Photosynthetic activity under salinity stress

The transpiration rate (T, mmol m^{-2} s^{-1}) of studied genotypes was significantly different, with maximum values for Nubaria 1, 4, and 5 (1.1, 1.3, and 1.7 mmol m^{-2} s^{-1} at control conditions, respectively) (Figure 4). The transpiration rate was lowered compared to control at T1 in Giza 843, Nubaraia 1, Nubaria 5, Sakah 1-4, and Wadi 1. The transpiration was reduced to its lowest rate in Sakha 3 under non-stressful conditions, Giza 843 at T1 and T2 salinity treatment, and Masr1 at T3 salinity treatment, with a range of 0.2-0.7 mmol m^{-2} s^{-1}. Most of the studied cases showed low transpiration rates and stomatal conductance under T3 salinity treatment (Figure 5). The photosynthetic rates (A, µmol m^{-2} s^{-1}) were mainly high at control conditions ranging from 14.09 µmol m^{-2} s^{-1} for Nubaria 5 to 5.39 µmol m^{-2} s^{-1} for Sakha 3 (Figure 6). The highest A rates were observed in Masr 1, Nubaria 1, and Nubaria 5 at control conditions. The photosynthetic rates were decreased by increasing salinity stress for most genotypes except for Nubaria

3, which had its maximum photosynthetic rates at T3 salinity treatment. The photosynthetic rates of Masr 1, Nubaria 1, and Nubaria 5 genotypes decreased by > 50% of their A values at T3 salinity treatment compared to their control. For Giza 716, Sakha 3 and 4, and Nubaria 3 no significant differences were recorded in A under different treatments (Figure 6). The results revealed that the intrinsic water-use efficiency (iWUE) was maximum and remarkably high at T3 salinity treatment for all genotypes followed by the values at T1 salinity treatment except for Masr 1, Nubaria 1, and Wadi 1 genotypes (Fig. 7). The differences in iWUE at control, T1, and T2 treatments were not significantly different for most genotypes.

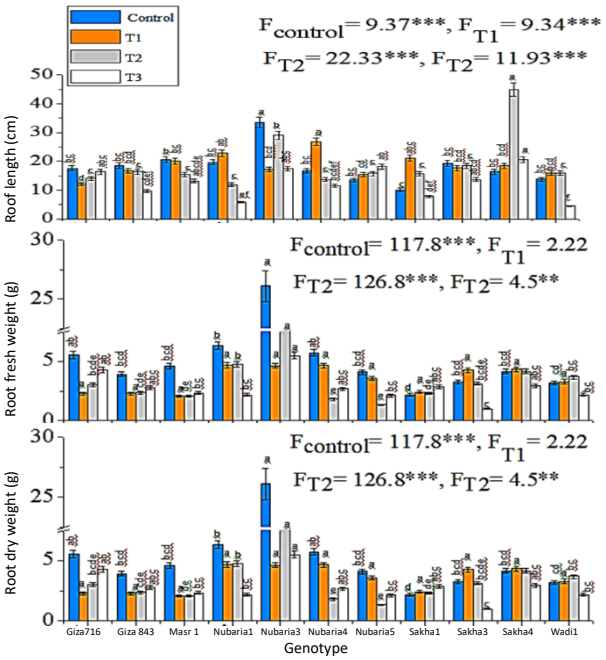

Figure 1. Effect of salinity on the root length, fresh and dry weight of eleven genotypes of *Vicia faba*. Different letters above the column of each genotype indicate significant variations at p< 0.05. Significance levels: * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

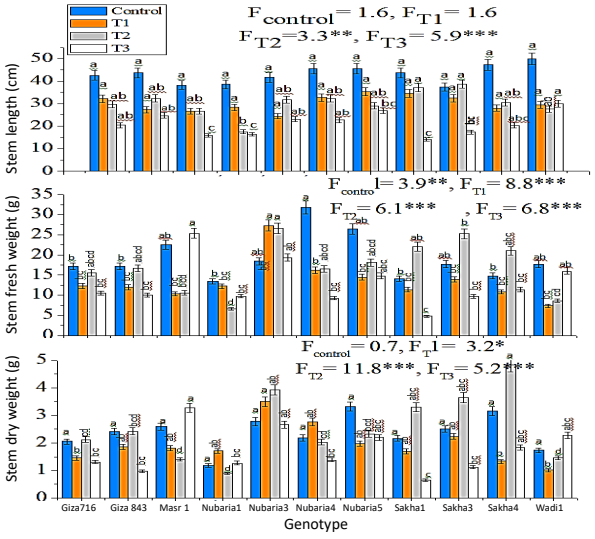

Figure 2. Effect of salinity on the stem length, fresh and dry weight of eleven genotypes of *Vicia faba*. Different letters above the column of each genotype indicate significant variations at p< 0.05. Significance levels: * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

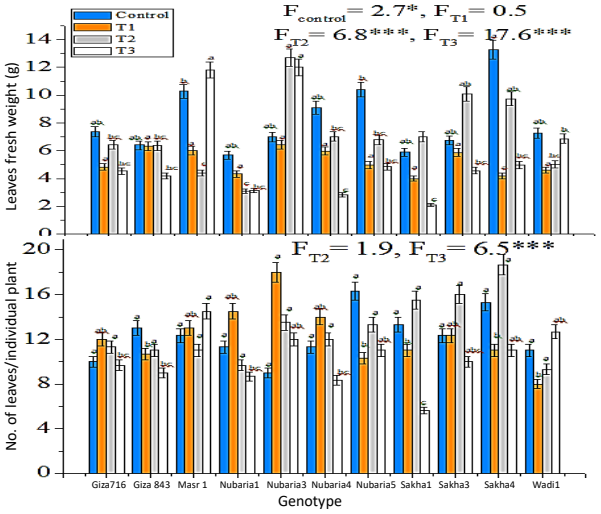

Figure 3. Effect of salinity on the leaves' fresh weight and no. of leaves/individual plant of eleven genotypes of *Vicia faba*. Different letters above the column of each genotype indicate significant variations at p< 0.05 Significance levels: * = P < 0.05, ** = P < 0.01, *** = P < 0.001

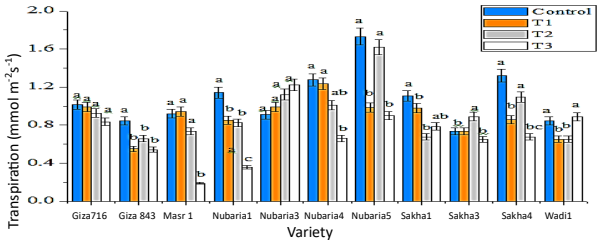

Figure 4. Effect of salinity stress on the transpiration rate of faba bean genotypes in Egypt compared to control. Different letters above the column of each genotype indicate significant variations at p< 0.05. Error bars are shown above columns.

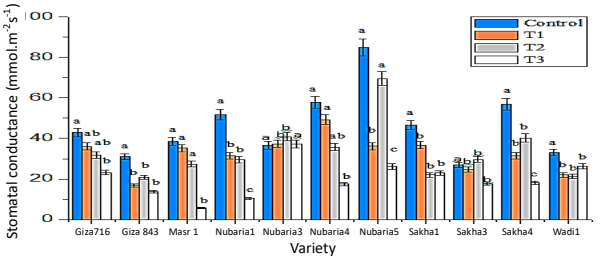

Figure 5. Effect of salinity stress on the stomatal conductance (mmol.m^{-2}. s^{-1}) of faba bean genotypes in Egypt compared to control. Different letters above the column of each genotype indicate significant variations at p< 0.05. Error bars are shown above the columns.

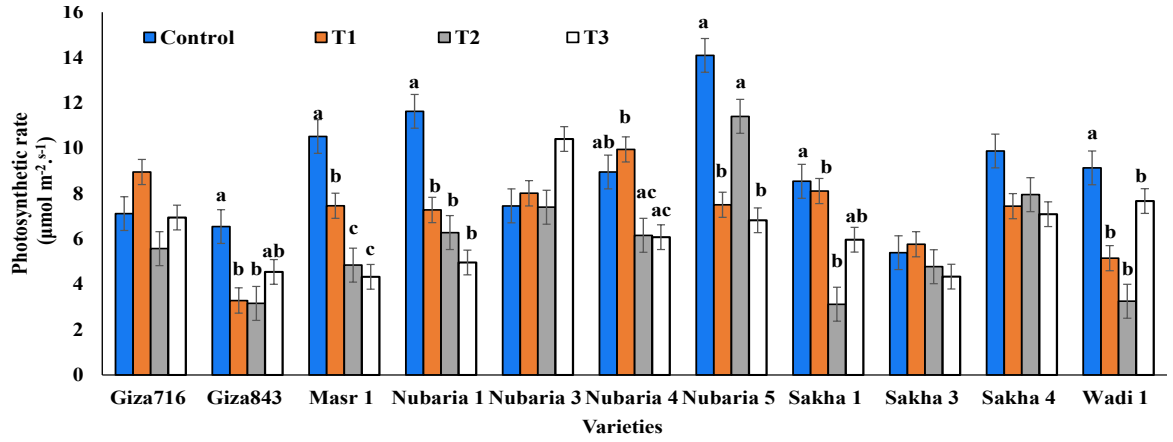

Figure 6. Effect of salinity stress on the photosynthetic rate (µmol.m^{-2}. s^{-1}) of faba bean genotypes in Egypt compared to control. Different letters above the column of each genotype indicate significant variations at p< 0.05. Error bars are shown above columns.

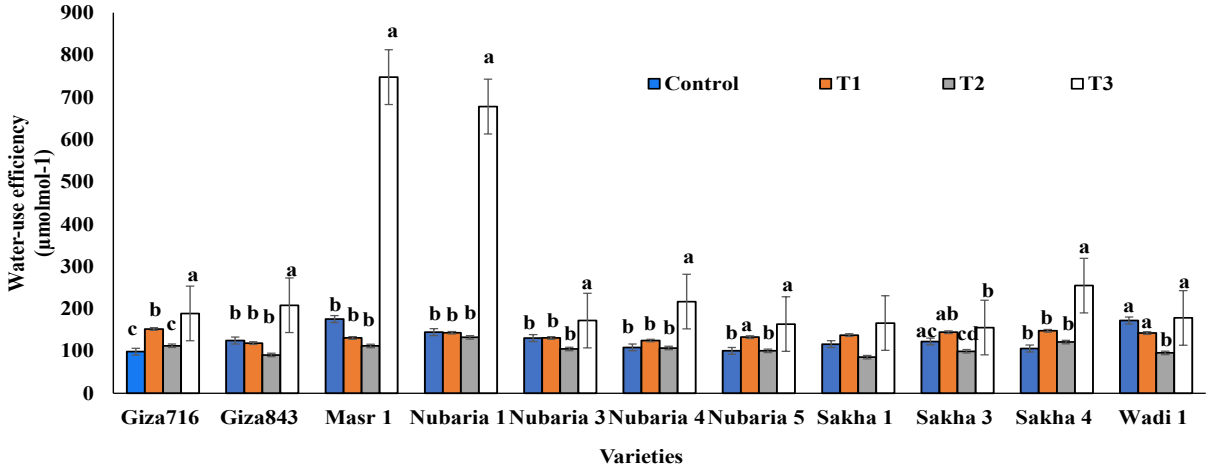

Figure 7. Effect of salinity stress on the intrinsic water-use efficiency (µmol mol^{-1}) of faba bean genotypes in Egypt compared to control. Different letters above the column of each genotype indicate significant variations at p< 0.05. Error bars are shown above the columns.

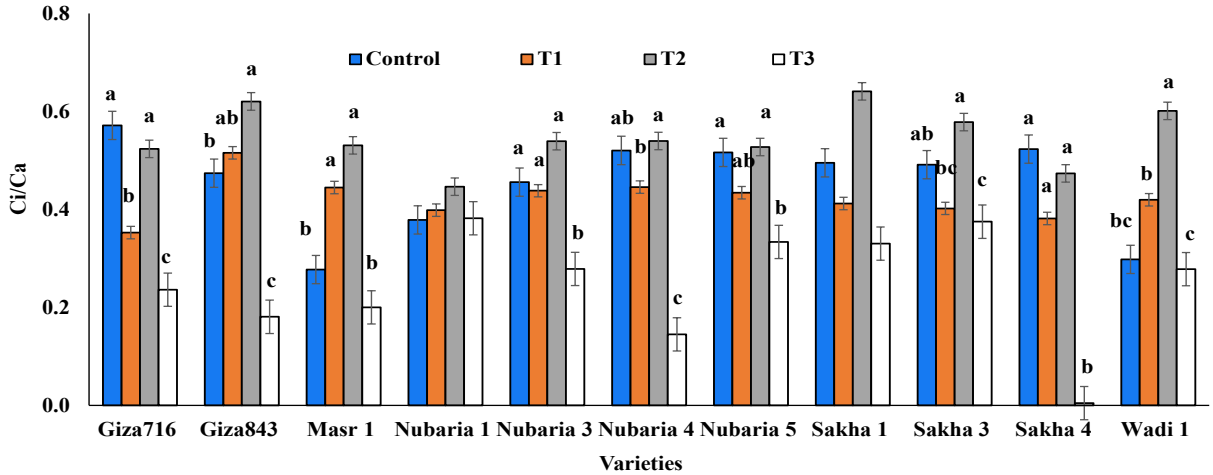

Figure 8. Effect of salinity stress on the Ci/Ca of faba bean genotypes in Egypt compared to control. Different letters above the column of each genotype indicate significant variations at p< 0.05. Error bars are shown above columns.

Moreover, there is no noticeable trend for Ci/Ca ratio except that the lowest ratios were obtained at T3 salinity treatment (Figure 8). The maximum ratio was 0.65 for Sakha 1 at T2 salinity treatment. The relationship between different photosynthetic parameters at different treatments (Supplementary figures, added at the end of this file) showed a significantly high correlation between stomatal conductance total (Gs) and transpiration (T), and iWUE and Ci/Ca under all treatments with R2 > 0.96. On the other hand, A-T, A-iWUE, and iWUE-CO_2 showed slightly higher R2 at T1 and T2 treatment by comparing to stressful one, and lower R2 values at T3 salinity treatment about other salt treatments.

Biochemical analysis and enzymology

Total soluble proteins and sugars

In general, increasing salinity causes soluble sugar and proteins buildup in the leaves of all genotypes of beans (Table 1). Total soluble proteins accumulated to their maximum levels at T3 at in two cultivars Giza 716 and Nubaria 3, while total soluble sugars accumulated in Nubaria 3 only at T3 compared to control. On the other hand, the minimum content of total soluble proteins (74. 95 µg g^{-1} Fwt) and sugars (4.80 mg g^{-1} Fwt) was at non-stressful conditions (control). The values of total soluble sugars level for all bean genotypes were elevated by increasing soil salt concentration compared to the control, except for Masr 1 (Table 1). The increment of total soluble sugar content at T3 salinity treatment was non-significantly compared to the control. The maximum total soluble sugar content was 27.45 mg g^{-1} fresh wt at T3 salinity treatment in Nubaria 3 genotype compared to control. However, the lowest values of total soluble sugar were observed in Nubaria 4, Giza 843, and Sakhah at T3, T2, and T1 salinity treatment, respectively.

Antioxidant compounds and enzymes

Variations in the phenolic compounds for all *V. faba* genotypes were illustrated in Table 2. The phenolic compounds were gradually increased by increasing soil salinity from T1 to T3 salinity treatment. Nubaria 3 showed the greatest phenolic compound concentration compared to other faba bean genotypes under control, T1, and T3 salinity treatments. Genotypes were followed the following order in their phenolics' content: Nubaria 3 > Maser 1 > Giza 716, Nubaria 5 > Sakha 3 > Wadi 1 > Sakha 4 > Giza 843 > Sakaha 1 > Nubaria 1 and Nubaria 4.

Peroxidase (POX) activity of salt-stressed leaves at T3 salinity treatment of all *V. faba* genotypes showed higher values than the control, except for Nubaria 3 and Sakha 3 (Table 3). POX activity decreased in Sakha 4 to reach its minimum activity of 0.23 at T3 salinity treatment. When compared to the control, Nubaria 5 and 4 showed the highest POX activity at T3 salinity treatment. Catalase (CAT) activity in Nubaria 1 genotype was highest under control conditions and T1 salinity treatment when compared to other genotypes (Table 3). While, at T2 and T3 salinity treatment, Nubaria 3 exhibited the maximum activity of CAT. Nubaria 3 was followed by Maser 1 and Sakha 4 for the highly active CAT enzyme at T3 salinity treatment. For five of the genotypes, the increment of the CAT activity in the salt-stressed plants was twofold its activity in control plants. For some genotypes (such as Giza 843, Nubaria 1, Nubaria 4, Sakaha 1, and Wadi 1), CAT activity was non-significantly affected by low salt stress (T1 or/and T2).

DISCUSSION

Salinity stress decreases the growth, biomass, and yield of food crops by up to 70% (Yaseen et al., 2020, Hashmat et al., 2021). In our investigation, increasing salinity stress has a detrimental effect on the *V. faba's* growth and biomass measured parameters when compared to the control. Many researchers found similar findings about the impact of increasing salt stress on *V. faba* genotypes. (e.g., Abdelraouf et al., 2016; Hussein et al., 2017; Ahmad et al., 2018 and Neji et al., 2021).

V. faba as one of legume plants is salt-sensitive (Ahmad et al., 2018). In the present study, the increase in salinity stress leads to an overall decrease in the transpiration rates (E), stomatal conductance (Gs), and photosynthetic rates (A) of all genotypes except Nubaria 3. This may be attributed to the retardation of photosynthesis by stomatal closure produced by a reduction in intracellular CO_2 concentration (Ci) and other variables during salt stress. This trend was reported for two sugar beet (*Beta vulgaris* L.) cultivars and sunflower plants that grow under salinity stress (Dadkhah, 2011; Ma et al., 2022). Salinity stress substantially reduced the leaf photosynthetic and biomass characteristics of *V. faba* cultivars, according to Hussein et al. (2017) and Neji et al. (2021). The relatively stable values of iWUE at control, T1, and T2 salinity treatments compared to T3 treatment may reflect an ability of *V. faba* genotypes to keep relatively high photosynthetic capacity under salt stress.

Table 1. Effect of salinity treatments on the total proteins and total soluble sugars content of faba bean leaves. Different letters in the column of each treatment indicate significant variations at p< 0.05.

	Total soluble proteins (µg g^{-1} F Wt)			
Varieties	0 (Control)	T1	T2	T3
Giza 716	196.27^{abc}±1.10	152.33^{c}±5.6	266.70^{abc}±2.2	878.55^{a} ±39.7
Giza 843	228.61^{a}±10.0	216.66^{b}±0.57	275.72^{ab}±2.2	302.83^{cd} ±17.8
Masr 1	178.66^{cd}±8.00	222.78^{b}±6.7	254.75^{abc}±8.7	262.13^{de}±8.9
Nubaria 1	189.86^{bc}±3.30	220.80^{b}±0.05	239.53^{bc}±5.6	289.97^{cde}±12.3
Nuburia 3	108.50^{f}±13.4	385.53^{a}±16.8	294.48^{a}±6.9	878.60^{a}±39.7
Nuburia 4	149.83^{de}±11.1	168.52^{c}±13.9	113.25^{d}±2.9	234.40^{ef}±29.1
Nuburia 5	74.95^{g}±14.40	168.52^{c}±13.9	292.06^{a}±49.9	324.83^{cd}±11.2
Sakha 1	208.58^{c}±17.9	34.84^{e}±5.70	278.34^{ab}±12.5	396.15^{b}±41.6
Sakha 3	211.83^{ab}±8.9	210.13^{b}±4.80	223.72^{c}±2.8	276.05^{de}±8.7
Sakha 4	138.20^{ef}±7.8	112.35^{d}±3.30	121.47^{d}±13.0	174.30^{f}±0.11
Wadi 1	145.03^{e}±16.1	234.36^{b}±12.7	266.33^{abc}±0.95	347.12^{bc}±6.0
	Total soluble sugars (mg g^{-1}F Wt)			
Giza 716	5.72 ^{fg} ±0.59	13.96^{c}±0.18	15.73^{b}±0.61	18.23^{bc}±1.85
Giza 843	8.20^{cd}±0.30	8.87^{e}±0.04	9.75^{e}±0.18	12.35^{fg}±1.70
Masr 1	14.40^{a}±1.2	14.66^{bc}±0.15	16.21^{b}±0.27	16.54^{cd}±1.20
Nubaria 1	11.96^{b}±0.92	15.59^{b}±0.08	15.87$^{b±}$0.23	20.54^{b}±0.38
Nuburia 3	4.80^{g}± 0.31	17.03^{a}±0.78	19.32^{a}±0.54	27.45^{a}±0.06
Nuburia 4	6.18f^{g}±0.05	9.70^{e}±0.037	11.22^{de}±1.3	10.50^{g}±0.31
Nuburia 5	7.98^{cd}±0.10	10.92^{d}±0.01	11.42^{d}±0.37	14.96^{def}±0.52
Sakha 1	9.14^{c}±0.68	11.59^{d}±0.46	13.16^{c}±0.12	14.89^{dfg}±0.05
Sakha 3	7.82^{cde}±0.46	11.84^{d}±0.70	13.10^{c}±0.05	13.10^{efg}±0.06
Sakha 4	5.20^{fg}±0.23	7.72^{f}±0.70	13.45^{c}±0.04	15.16^{de}±0.72
Wadi 1	6.60^{def}±0.41	12.00^{cd}±0.26	13.21^{c}±0.09	15.08^{de}±0.06

Table 2. Effect of salinity treatments on total phenolic compounds (µg gallic acid g^{-1} D Wt) content of faba bean leaves. Different letters in the column of each treatment indicate significant variations at p< 0.05.

	Treatment			
Varieties	0 (Control)	T1	T2	T3
Giza 716	121.0^{c}±1.20	130.9^{b}±0.4	154.4^{c}±1.7	183.6^{bcd}±4.50
Giza 843	74.6^{g}±2.80	76.3a^{b}±0.55	90.2^{g}±0.09	123.0^{de}±14.9
Masr 1	129.3^{b}±0.11	138.9^{b}±2.6	198.3^{a}±5.3	217.8^{b}±4.70
Nubaria 1	94.01^{ef}±2.30	103.8^{e}±4.1	113.7^{f}±10.9	118.5^{e}±5.10
Nuburia 3	141.6^{a}±1.90	150.0^{a}±0.43	167.9^{b}±4.5	396.8^{a}±57.0
Nuburia 4	95.9^{ef}±4.60	97.1^{f}±0.69	109.3^{f}±0.59	118.4^{e}±3.00
Nuburia 5	111.7^{e}±3.80	122.1^{d}±0.28	137.0^{d}±0.30	183.6^{bcd}±4.50
Sakha 1	101.4^{e}±4.80	106.6^{e}±0.96	113.7^{f}±3.0	120.5^{e}±1.00
Sakha 3	69.6g±1.70	83.90^{g}±2.30	109.6^{f}±1.04	140.3^{cde}±3.37
Sakha 4	91.6^{f}±3.60	102.6^{e}±0.77	118.6^{ef}±0.26	125.5^{de}±7.80
Wadi 1	125.6^{bc}±0.18	126.3^{cd}±0.49	128.8^{de}±0.83	128.6^{de}±3.80

Table 3. Effect of salinity treatments on POX enzyme activity and CAT enzyme activity of faba bean leaves. Different letters in the column of each treatment indicate significant variations at p< 0.05.

	Peroxidase enzyme activity (mg mg^{-1} protein min^{-1})			
Varieties	0 (Control)	T1	T2	T3
Giza 716	1.29^{cd}±0.06	2.70^{c}±0.220	3.40^{bc}±0.10	4.26^{c}±0.250
Giza 843	0.43^{de}±0.01	0.86^{f}±0.006	1.92^{cde}±0.03	2.24^{ef}±0.150
Masr 1	1.68^{c}±0.01	1.63^{cde}±0.030	1.87^{cde}±0.10	4.17^{b}±0.120
Nubaria 1	1.81^{c}±0.17	1.24^{ef}±0.120	2.08^{cde}±0.08	4.96^{b}±0.640
Nuburia 3	2.97^{b}±0.07	1.58^{de}±0.020	1.05^{de}±0.06	0.86^{g}±0.010
Nuburia 4	3.41^{a}±0.03	3.80^{b}±0.400	5.96^{a}±0.54	6.76^{b}±0.060
Nuburia 5	2.70^{b}±0.70	4.80^{a}±-0.200	4.83^{ab}±2.09	6.89^{a}±0.010
Sakha 1	1.34^{c}±0.09	2.04^{d}±0.040	2.42^{cde}±0.06	2.83^{de}±0.010
Sakha 3	0.12^{e}±0.02	1.86^{d}±0.030	2.35^{cde}±0.25	2.06^{f}±0.200
Sakha 4	3.41^{a}±0.03	1.29^{ef}±0.150	0.84^{e}±0.03	0.23^{g}±0.001
Wadi 1	2.07^{bc}±0.02	3.00^{c}±0.010	2.95^{ab}±0.04	3.11^{d}±0.100
	Catalase enzyme activity (µg mg^{-1} protein min^{-1})			
Giza 716	89.30^{d}±0.57	94.300^{c}±6.30	96.40^{e}±5.5	114.30^{hi}±10.9^{hi}
Giza 843	104.0^{d}±1.00	112.00^{a}±5.20	122.00^{d}±13.5	213.30^{gh}±6.10^{gh}
Masr 1	50.00^{d}±8.60	199.00^{c}±1.70	475.30^{c}±47.4	1253.3^{b}±68.8
Nubaria 1	831.3^{a}±41.5	827.60^{a}±39.5	848.60^{b}±9.80	874.30^{d}±5.1^{d}
Nuburia 3	814.6^{a}±16.1	502.30^{b}±68.7	1075.0^{a}±136.9	2194.3^{a}±12.5^{a}
Nuburia 4	99.10^{d}±0.17	99.400^{c}±0.47	117.30^{d}±6.60	317.60^{a}±90.7
Nuburia 5	31.60^{d}±2.80	47.000^{c}±1.70	49.300^{e}±1.10	71.30^{fg}±6.10
Sakha 1	40.30^{d}±6.40	81.000^{c}±5.20	113.60^{d}±5.00	403.30^{f}±6.88
Sakha 3	89.30^{d}±0.57	97.300^{c}±2.08	103.60^{d}±1.10	174.0^{hi}± 3.40
Sakha 4	728.3^{b}±50.33	770.00^{a}±12.7	911.00^{b}±18.1	1023.3^{c}±25.1
Wadi 1	304.6^{c}±56.0	502.30^{b}±68.7	562.00^{c}±3.40	681.00^{e}±32.9

It seems that the Nubaria genotypes are the most saline resistant genotypes in terms of photosynthetic performance under salinity stress. Strong relationships between the measured photosynthetic parameters indicate their homogeneous response under control and salinity treatments. *V. faba* genotypes with naturally high photosynthesis (Masr 1, Nubaria 1, and Nubaria 5) exhibited the greatest declines in photosynthetic activity under saline conditions. This was indicated by the noticeable decrease in the values of stomatal conductance of the three genotypes at T3 salinity treatment compared to the control. This phenomenon was reported for some olive cultivars with different sensitivity to salt stress in Greece (Loreto et al., 2003). On the other hand, Sakha genotype showed the highest number of leaves at T2 among all treatments, with a statistically similar transpiration rate and a statistically lower photosynthetic rate than the Nubaria genotypes. This may be one of the defense mechanisms that plants used to have more tolerance through more leaf tissue to accumulate ions and adjust osmotic potential. Plants suffer from ionic stress and leaf senescence due to long-term salt exposure, in addition to dryness (Chaves et al., 2009), which has an extra negative impact on plant growth. It is understood that suitable solute accumulations and activities, such as soluble sugars and sugar alcohols, play an essential role in osmotic adjustment (Ma and Celeste Dias, 2020).

Our results agree with Aboualhamed and Loutfy (2020) study on *V. faba*. They found that when *V. faba* was exposed to salt stress (150 mM NaCl), the quantity of soluble sugars and soluble proteins significantly increased. Salt stress causes an increase in soluble carbohydrates (Watanabe et al., 2000). Tolerant cultivars always have more soluble sugars in their leaves and developing tissue than sensitive ones (Watanabe et al., 2000). Nubaria 5 was the variety that accumulated the maximum content of soluble sugars, which probably explains its ability to tolerate the high salinity levels (T1, T2, and T3 salinity treatments). Soluble proteins are the most active components of plant cells, acting as a resource for enzymes and a modulator of metabolic processes (Zheng et al., 2018). Likewise, the current results, when *Pancratium maritimum* is exposed to high salt concentrations, its total soluble protein content rises (Khedr et al., 2003). According to Cristoffanini et al. (2021) the soluble proteins under salinity stress are essential for their protective role. They added that the protein-protein interaction networks may play important roles in salinity stress tolerance and need further investigation.

Plant cells produce various phenolic chemicals as low molecular weight non-enzymatic antioxidants that help remove reactive oxygen species (ROS) (Hodaei et al., 2018). Phenolic chemicals, including flavonoids, are plants' most abundant secondary metabolites. These chemicals perform various physiological and molecular activities in plants, including signaling pathways, plant defense, auxin transport mediating, antioxidant activity, and free radical scavenging (Tohidi et al., 2017). Because many of these phenolic compounds operate as reducing agents, they may also have antioxidant potential (Rice-Evans et al., 1997). This role may be attributed to the *p*-coumaric acid that expresses high radical scavenging activity due to its hydroxyl nature (Jamalian et al. 2013). At T3 salinity treatment, Nubaria 4 was the most sensitive variety for phenolics increase and greater tolerance than other types. High salinity causes major metabolic disruptions in plants by producing ROS, which disrupts the cellular redox system (Chutipaijit et al., 2009). So, antioxidant compounds (ascorbate, salicylate, glutathione, tocopherols, etc.) and antioxidant enzymes are essential for protection (Molassiotis et al., 2006, Fghire et al., 2013). H_2O_2 is a toxic reactive oxygen species (ROS) and is considered a source of other ROS. So, it is decomposed by both peroxidases (APX and GPOX, Glutathione peroxidase) and CAT. However, the affinity of APX for H_2O_2 breakdown is higher than that of CAT (Abogadallah, 2010), indicating that peroxidases may be related to scavenging ROS at lower concentrations of H_2O_2, while CAT quenches much higher concentrations of H_2O_2. The most increased activity for antioxidant enzymes CAT in Nubaria 3 indicates the salt tolerance of these varieties up to 100 mM NaCl.

CONCLUSION

All growth criteria were affected by salt stress especially at T3 treatment. Increasing salinity stress resulted in a considerable rise in total soluble proteins, soluble sugars, and phenolic compounds in the leaves of all genotypes. Except for Nubaria 3, increasing salt stress reduced photosynthetic rates in general under salinity stress. Antioxidant defence enzymes showed their highest activity at Nubaria genotypes. These current results indicated that Nubaria genotypes, especially 3 and Masr 1, can survive under high salt stress by accumulating osmolytes as soluble sugars or increasing their antioxidant protective role such as phenolic

compounds or enhancing antioxidant enzyme activity such as CAT.

ABBREVIATION

A: Photosynthetic rate
APX: Ascorbic Peroxidase
aCO_2: Ambient CO_2
CAT: Catalase
CAGR: Compound Annual Growth Rate
Ci: Internal CO_2
E: Transpiration rate
Gs: Stomatal conductance
iWUE: Intrinsic Water-use efficiency
POX: Peroxidase
ROS: Reactive Oxygen species
RUBISCO: Ribulose-1,5-bisphosphate carboxylase/oxygenase

AUTHOR'S CONTRIBUTIONS

E.M.Z. Conceptualization, methodology, data curation and writing the original draft.
Z.A.S. Conceptualization, methodology, data curation and writing the original draft.
E.A.F. Conceptualization, methodology, data curation, writing the original draft, reviewing and editing. All authors read the corrected and approved manuscript.

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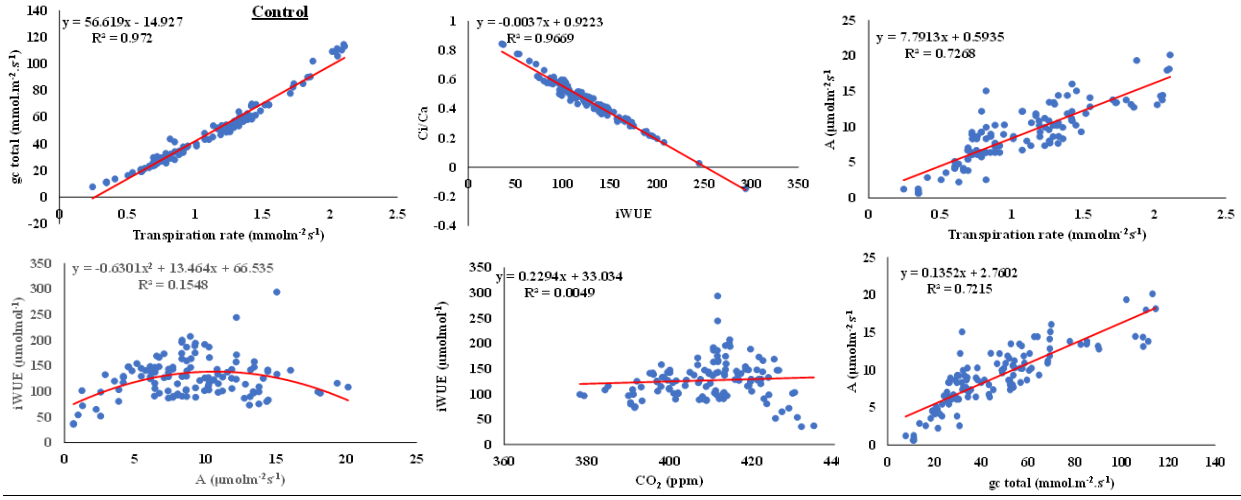
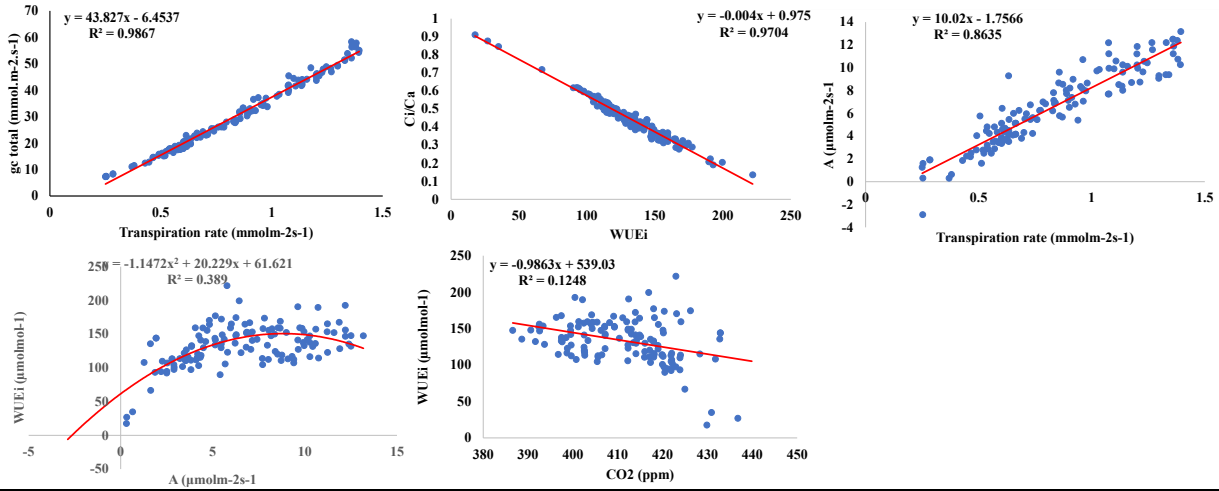
Supplementary Figures

S1: Relationships between the photosynthetic parameters of faba bean at control and salinity stress conditions (T1=50 mM NaCl, T2= 75 mM NaCl, and T3= 100 mM NaCl). gs= stomatal conductance total, A= photosynthetic capacity, Ci= intrinsic CO_2, Ca= ambient CO_2, iWUE= intrinsic water-use efficiency



S4: At 50 mM NaCl



S4:At 100 mM NaCl