Effect of salicylic acid in alleviates the adverse of salinity stress in fenugreek (Trigonella *foenum-graecum*)

.

ABSTRACT

Aims: Studying the effect of salicylic acid under different concentrations of NaCl on fenugreek plant.

Study design: The experimental design adopted a completely randomized block design (RCBD), with pots filled with a 1:2 mixture of sand and soil serving as the experimental units.
Place and Duration of Study: in a mini greenhouse covered with white polyethylene plastic sheet at Taiz University during the spring season 2021. The average temperature ranged from32 ℃ during the day to 29 ℃ at night.

Chemical analysis: All chemical analyses were performed at the pharmaceutical laboratory in Al-Saeed University.

Methodology: we study the resistance of Yemeni local fenugreek genotype to varying salinity levels (0, 50, 100, 150, and 200 mM) and the impact of salicylic acid (at concentrations of 0, 0.1, and 0.5 mg/l) in alleviating salt stress effects were examined at two time points: 15 and 30 days after 2 weeks of germination.

Results: showed that increasing salinity significantly reduced shoot and root length exhibited slight fluctuations influenced by salinity levels above 100 mM.
Salicylic acid (SA) significantly reduced the shoot length of the fenugreek plants. But had no effect on root length. fenugreek had no appreciable effect on shoot dry matter (SDM%). application of SA, which significantly decreased with SA application. Fenugreek exhibits a significant and sharp increase in RDM%. But there were no significant variations in RDM% seen in response to SA application in fenugreek plants. Salinity causes a significant and sharp decrease in the SDM% to RDM% ratio in treated plants, whereas SA treatment resulted in the lowest ratio when used 0.1 SA.

There were no significant modifications observed in the amounts of photosynthetic pigments in fenugreek leaves, including chl a, total chl, and chl a: chl b. Nevertheless, when Nacl levels rose, the amounts of carotenoids and chl b were significantly reduced.

The protein content of fenugreek plants treated with salt was much higher than that of control plants. Also, protein levels increased with SA treatment across all NaCl levels. Proline levels sharply increased with rising salinity, reaching a peak at approximately 22.28 ug/g DW. Application of 0.1 SA led to the highest proline level in comparison to other treatments. which

was the highest level of Nacl in the second measurement time. Proline levels in plants treated with 200 Nacl and 0.1 SA were the highest, averaging roughly 49.95 ug/g DW

APX significantly affected on fenugreek plants, the highest APX content was found in plants treated with 50 mM NaCl, the application of 0.1 SA produced the highest level of APX (0.373nm/g protein/mint) in fenugreek. Catalase (CA) levels significantly decreased, but SA application did not affect CA levels.

rigonella foenum-graecu
Keywords: (antioxidant, morphology, fenugreek, salinity, salicylic acid, photosynthetic pigments, Proline, protein *)*

1. INTRODUCTION

Salinity refers to the amount of dissolved substances known as aggregate salts. These salts encompass dissolved solids resulting from the weathering of rocks and soil by water. They freely move in soil and water and are taken up by the plants (Warrence *et al.*, 2002).Under natural conditions, abiotic stresses, particularly salt stress, has a triple effect on plant growth. First, it decreases water absorption due to the osmotic effect, caused by low water potential in saline soils, inhibiting water absorption into plant tissues. Second, it leads to ion imbalance or turbulence in ion homeostasis. Third, it results in plant toxicity (ionic effects); Ion toxicity occurs when salt accumulates to toxic concentration in leaves. Excessive levels of Na+ ion accumulation in plant tissues are one of the major factors causing salinity damage, reducing the plant's ability to absorb water from the soil (Al-Karaki 2000; Roussos *et al.*, 2007; Munns, 2005; Demiral and Türkan, 2005; Ashraf and Foolad, 2007; Yamaguchi and Blumwald, 2005; MILLER *et al.*, 2010; Roy *et al.*, 2014; Machado and Serralheiro, 2017; Dhiman *et al.*, 2021; Jiang *et al.*, 2017; Stavridou *et al.*, 2017). High osmotic potentials at the soil-root interface are a concern. According to FAO, more than 800 million hectares of world`s agricultural land are seriously affected by salinity (Munns, 2005). Salinity is the major abiotic stress and significant factor affecting crop production worldwide (Yamaguchi and Blumwald, 2005), especially in arid and semi-arid region. About 23% of the world's cultivated lands is saline, and 37% are sodic (Khajeh-Hosseini *et al.*, 2003). Environmental stresses, including salinity, effect on numerous characteristics of plant including morphological, physiological, biochemical, molecular and anatomical aspects (Alam *et al.*, 2019; Ahanger *et al.*, 2020; Kaya *et al.*, 2020b; Gupta *et al.*, 2021). as well as secondary metabolites (Misra and Dwivedi, 2004; Demiral and Türkan, 2006; Allakhverdiev *et al.*, 2000; Ashraf and Foolad, 2007; Rasool *et al.*, 2013). Increasing salt concentration inevitably affects the productivity in medicinal plants such as fennel, cumin, *Ammi majus*, *Trachyspermum ammi* and milk thistle. The fruit yield per plant, as well as the number of umbels, significantly decreases in stress-exposed plants (Ashraf and Orooj, 2006). Total carbohydrates affected throughout reduction of photosynthesis, hyperosmotic stress and nutritional imbalance as observed in fennel (Abd El-Wahab, 2006). Although salt stress affects all growth stages of a plant, seed germination and seedling growth stages are known to be more sensitive for most plant species(Cuartero *et al.*, 2006). Salicylic acid (SA) is a growth regulator that improves plant tolerance under salt stress (Abdelhameed *et al.*, 2021; JIA and JIANG, 2023), enhancing physiological traits and alleviate salt stress effects by influencing physiological processes such as increasing antioxidant activity enzymes and soluble sugars content in plants (Harati *et al.*, 2015). It regulates plant processes, including seed germination and plant growth and development (Koo *et al.*, 2020). Additionally, it may impact ion absorption and transport, stomatal conductance, the rate of photosynthetic activity, and transpiration (Hayat *et al.*, 2010a; Li *et al.*, 2019).

Nevertheless, the daily utilization of *Trigonella foenum-graecum* is prevalent in Yemeni households, the resilience of local genotypes of this plant to withstand salinity remains

uncertain. Additionally, previous studies specifically targeting these plants are lacking, underscoring the significance of this study.

2. material and METHODS.

Experimental Lay Out

Pots were filled with a medium containing a mixture of normal soil and sand in a ratio 2:1 (v: v). Seeds were planting in the post and irrigated with normal water until germination. After 15 days of germination, the pots were irrigated with salinity treatments, with or without Salicylic Acid. Some pots were harvested after 15 days from the beginning of the treatments, while others continued until they were harvested after 30 days of treatments.

Dry matter and Morphology using Tomato analyser

The method described by Al-Madhagi and Al-Sharagi,(2019) was employed., 3 Plants harvested from the plots and directly wished, and separated to the shoot and root then the fresh weight was recorded to the each part in digital balance. After weighting the fresh plants, they were spread on a labeled square black sheet. Plant photographs were taken with a mobile digital camera (Redmi not 10), and JPEG format photographs transferred to a laptop for processing. Images were enhanced using Photoshop software to blacken the background, saved at 200 resolutions, and corrected for size according to calibration ruler. Plants height (cm) was analyzed using the Tomato Analyser 3.0 software developed by Ohio State University (http://oardc.osu.edu/vanderknaap/tomato_analyzer.htm).

The dry matter was recorded after drying in normal room temperature using the following formula: (Al-doubibi *et al.*, 2021)

Estimation of photosynthetic pigments:

The extraction of chlorophyll followed the method outlined by Wasaya et al., (2021). Initially, 0.1g of fresh plant leaves was homogenized in a prechilled mortar using cold 85% (v/v) aqueous acetone. The resulting extract underwent centrifugation at 6000 rpm for 5 minutes. The supernatant was adjusted to specific volume using 85% acetone. The absorbance of the extract was spectrophotometry measured against a blank of pure 85% acetone at three different wavelengths of 470 nm, 644 nm, and 663 nm using a JENWAY 6305 Spectrophotometer. The concentration of Chlorophyll a, Chlorophyll b, and total Chlorophyll were determined using the following formulas:

- Chlorophyll a (mg/g FW) = [1.07 (OD 663) – 0.094 (OD 644) × V/1000 × W]
- Chlorophyll b (mg/g FW) = [1.77 (OD 644) – 0.28 (OD 663) × V/1000 × W]
- Total Chlorophyll (mg/g FW) = [0.79 (OD 644) + 1.076 (OD 663) × V/1000 × W]

Here V represents the volume of the leaf extract (mL), and W denotes the weight of fresh leaf tissue (g). Additionally, the total carotenoids was calculated using the formula : Carotenoids = 1000 (OD470) - 1.82Chl a - 85.02 Chl b /198 × V/1000 × W (Metzner *et al.*, 1965).

Biochemical Parameters:

Estimation of proteins (µg/g FW):

The method used to determine the shoot protein content was developed from Lowry et al.,(1951)The three steps were carried out as follows: . i- 8.33 ml of di potassium phosphate K_2HPO4 (8ml + 330mµ) and 1.67ml of mono potassium phosphate KH_2PO4 (1ml +670mµ) were combined to create the extraction solution and buffer, and the volume was then completed to 200 ml of distilled water. The dye was prepared by dissolving 0.1 g of Coomassie blue dye in a mixture of 50ml 95% ethanol and 100 ml phosphoric acid. Once the dye was completely dissolved, the mixture was poured into 1 litter, allowed to settle and then filtered. After

homogenized 0.1gm of frozen tissues with 1 ml of solution buffer that had been previously prepared, the sample was centrifuged for 10 minutes at 6000 rpm. The measuring tubes were filled with a mixture. exactly amount 1000 mμ of prepared (Coomassie blue dye) to 800 mμ of Buffer solution and added 200 mμ of supernatant, then the absorbance was read at 575 nm in A JENWAY 6305 spectrophotometer. The measuring tubes done by mixed exactly amount 1000 mμ of prepared (Coomassie blue dye) to 800 mμ of Buffer solution and added 200 mμ of supernatant, then the absorbance was read at 575 nm in A JENWAY 6305 spectrophotometer.

Estimation of Proline (μg/g DW)

The proline content in the shoot was measured using the protocol established by Bates et al., (1973).The reaction solution was prepared under dark condition by dissolving 1gm of ninhydrin with 60 ml of glacial acetic acid, followed by the addition of 20 ml ethanol of 70% and 20 ml of distilled water and kept cool until used. Proline of the shoot tissue extracted by homogenised 0.1 g of dry weight in mortar and pestle with 2 ml of 70% ethanol, which was then centrifuged at 6000 rpm for 10 mints. The supernatant was combined with reaction solution in a 1:2 v: v (supernatant: reaction solution) and incubated for 20 mints at 95 ^{o}C in water bath. The mixture was then cooled in an ice bath. Absorption was measured using a JENWAY 6305 Spectrophotometer, which has radiation at 520 -nanometre wavelength.

Estimation of antioxidants enzymes

The Velikova *et al*., (2000)method was employed to assess enzyme activation in the shoot. The preparation of solutions involved dissolving 34.836 grams of K2HPO4 in 200 millilitres of distilled water for the K2HPO4 solution, and 27.218 grams of KH2PO4 in 200 ml of distilled water for the KH2PO4 solution. For the enzyme extraction solution, 0.184 ml of mono potassium phosphate KH2PO4 (184 mμ) and 1.8 ml of dipotassium phosphate K2HPO4 (1 ml + 800 mμ) were combined and adjusted to a final volume of 20 ml with distilled water.

The extraction process involved homogenizing 0.1 g of frozen tissues with 1 ml of the previously prepared extraction solution, followed by centrifugation at 6000 rpm for 10 minutes. The resultant extract was kept on ice until further measurements. A buffer solution was prepared by mixing 1.230 ml of dipotassium phosphate K2HPO4 (1 ml + 230 mμ) with 0.77 ml of mono potassium phosphate KH2PO4 (770 mμ) and adding 40 ml of distilled water. Additionally, 0.834 ml of CAT H2O2 was prepared and adjusted to a final volume of 10 ml. For the APX H2O2 solution, 1.70 ml of H2O2 was brought up to a volume of 10 ml. The ascorbate solution was created by dissolving 0.0158 grams of ascorbate in 20 millilitres of purified water.

Setup of Measurement Tubes:

Catalase (CAT) activity was evaluated following Aebi, (1984) by mixing 2.7 ml of the prepared buffer solution with 0.05 ml of the supernatant. The absorbance was measured at 240 nm using a JENWAY 6305 Spectrophotometer at a rate of 0-30sens -1 min.

APX activation was determined according to Asada,(1994)by combining 1.8 ml of the prepared buffer solution with 0.1 ml of H2O2 solution, 1 ml of ascorbate solution, and 0.1 ml of the supernatant. Absorbance was measured at 290 nm using a JENWAY 6305 Spectrophotometer at a rate of 0-30 s within one minute.

The enzyme activity in nano-moles per gram of gram of protein was calculated using the specified formulas, with the extinction coefficient for peroxidases being 26.6 mM-1cm-1 and for catalase being 39.4 mM-1cm-1

Change in OD / (time mine) × 1

÷ extiniation cofficinttotal reaction volume

÷ volume of enzyme extract × total volume of enzyme

÷ fresh wt. × total protein (mg – 1ml) × 100

Change in OD / (time mine) × 1

÷ extiniation cofficinttotal reaction volume

÷ volume of enzyme extract

× total volume of enzyme

÷ fresh wt. × total protein (mg – 1ml) × 100)

Statistical analysis

The data were evaluated using Gene sat 12 software as a factorial experiment (RCBD), taking into account the concentration of salt, salicylic acid, and measuring period of time as factors. The significance of individual factor means was determined using the least significant difference (LSD) at a significant level of less than 0.05. Interactions means were evaluated using the Duncan's multiple range test (DMRT) method. Microsoft Excel was also used to estimate regression equations for linear effects.

3. results and discussion

Effect of Nacl and SA on morphological characteristic:

Table 1: displays the *F* probability for the effect of salicylic acid (SA) at different levels Nacl (N) and measurement Times (T) on fenugreek plant morphological characters

	Shoot length cm	Root length cm	Shoot DM%	Root DM%	Shoot: Root ratio
Nacl (N)	<.001	0.01	0.914	<.001	<.001
Salicylic acid (SA)	<.001	0.986	<.001	0.214	0.024
Time (T)	0.994	0.828	<.001	0.69	0.257
15	10.68	10.7	12.69b	13.69	1.24
30	10.68	10.57	17.4a	14.2	1.41
N ×SA	0.01	0.256	0.808	0.881	0.423
N×T	0.648	0.645	0.02	0.4	0.979
SA × T	0.551	0.834	0.709	0.001	0.125
N×.SA×T	0.594	0.084	0.478	0.451	0.116

F probability at the value less than 0.05 consider significant according to multiple ANOVA analysis

Effect of Nacl and SA on shoot length (cm).

The fenugreek plant's shoot length (in cm) exhibited a significant decrease dramatically with rising salinity levels. Comparing the control and 50 mM Nacl, there was a reduction of about 20% in shoot length, followed by an additional 10% decline for every 50 mM increment beyond 100 mM (Figuer1). The regression equation illustrated a reduction of approximately 1.463 cm for every 50 mM increase in NaCl (Figure 1).

Figure 1effect of salt on fenugreek shoot length (cm)

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$

Application of SA significantly diminished fenugreek shoot length. Plants treated with 0.1 SA and 0.5 SA showcased reductions in length by approximately 25% and 11%, respectively, compared to control plants (Figure 2). Remarkably, time did not significantly influence shoot length (Table 1, probability value ≈ 0.994).

Figure 2: effect of SA on fenugreek shoot length cm

Means with different litter indicate significant at less than *0.05*

Except for the interaction between salt and SA (N×SA), other studied factors' interactions had no significant effect on shoot length (Table 1). The N×SA impact is depicted in Figure 3.
Under most salinity levels, Plants treated with 0.1 or 0.5 SA did not display significantl differences, except at 100 and 150 mM, where a notable distinction emerged between the application of 0.1 and 0.5 of SA. Specifically, plants treated with 0.1 SA under 150 mM exhibited the shortest shoots (6.21 cm), while the control plants had the longest shoots (15.62cm)

Figure 3: Effect of interaction between Nacl and SA on fenugreek shoot length (cm)

Column with different alphabet indicate significant at the level less than *0.05*

Effect of Nacl and SA on root length (cm):

Fenugreek's root length exhibited slight fluctuations influenced by salinity levels above 100 mM. Control plants or thoes treated with 50 or 100 mM Nacl displayed the longest roots, while the 150 mM treated plants resulted in the shortest roots, measuring, 8.6 cm, representing a which declined of approximately 36% compared to the control plants (not treated plants) (Figrue 4).

Figure 4: effect of salt on fenugreek root length cm

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$.

Interestingly, fenugreek the root length remained largely unchanged unchange due to SA application of or variations in time factor, as indicated by *F* probability value of approximately 0.986 and 0.828, respectively (Table 1).

Figure 5: Effect of SA on fenugreek root length cm

Means with different litter indicate significant at less than *0.05*

The interaction between study factors showed no significant impact on fenugreek root length, with all interaction types the *F* probability greater than 0.05. (Table 1).

Effect of Nacl and SA on percentage of shoot dry matter (SDM%):

salinity overall, demonstrated no significant effect on the percentage of SDM% in fenugreek plant, with a probability value of about 0.914 (Table 1 and Figure 6).

Figure 6 : effect of salt on fenugreek shoot dry matter (SDM%)

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$.

However, the application of SA, particularly at the highest level of 0.5 mg/L, notably reduced the SDM% content in plants treated with 0.5 SA showing around a 28.4% decrease compared to the control. Conversely, there were no significant differences between plants treated with 0.1 SA and the and the control group (Figure 7).

Figure 7: Effect of SA on fenugreek shoot DM%

Means with different litter indicate significant at less than *0.05*

The time factor exhibited significance difference, with the second measurement recording a higher SDM% compared to the first (Table1).
Except for the interaction between Nacl and Time Factors (N×T), the other interactions (second and third) did not significantly impact SDM% (Table 1).

the interaction effect between NaCl and Time (N×T) is depicted in Figure 8, where plants treated with Nacl accumulated more dry matter over time, particularly at the second measurement time (30 days) than the first (15 day). Notably, there were no significant differences in dry matter content observed between different measurement times in the control plants.

The maximum accumulation of SDM% (19.30%) occurred in plants treated with 150 Nacl at 30 days, showing no significant differences from other salinity treatments (50 - 200 mM). during the first observation period, no significant difference were found between the control and salinity treatments (Figure 8).

Figure 8 : The effect of interaction between Nacl and Time on fenugreek shoot dry matter (SDM %).

Column with different alphabet indicate significant at the level less than *0.05*

Effect of Nacl and SA on root Dry matter percentage (RDM%):

Fenugreek RDM% demonstrates a significant increase dramatically with rising salinity levels. plants receiving 200 mM of irrigation exhibited the highest RDM% (20.91%), marking an approximately 180% increase compared to the control treatment. Another interpretation suggests that fenugreek RDM% increases by about 4.384 % for every addition 50 mM of Nacl above the control. Control plants had the lowest RDM%, averaging around 7.46% (Figure 9).

Figure 9 : The effect of salt on fenugreek root DM%

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$

there were no significant differences observed in RDM% related to the application of SA, with an *F* probability of about 0.214 (Table 1). Additionally the means of RDM% are presntented in figure 10.

Figure 10 : The effect of SA on fenugreek root DM%

Means with different litter indicate significant at less than *0.05*

The time factor also did not significantly affect fenugreek RDM%, with an *F* probability of about 0.96 (Table1).

Among the study factors, only the second interaction between SA and Time (S×T) demonstrated a a significant effect on root dry matter (RDM%). The results of S×T are depicted in Figure 11 During the first measurement period, plants treated with 0.1 SA showed the highest the greatest average RDM% value (18.64%), while those with 0.5 SA exhibited the lowest average value (10.51%). nosignificantly different was detected during the second measurement between the RDM% related to the application of SA and that of control plants.

Figure 11 : The effect of interaction between SA and Time (SA × T) on fenugreek Root dry matter (RDM %).

Column with different alphabet indicate significant at the level less than *0.05*

Effect of Nacl and SA on Root: Shoot ratio (SDM%: RDM%):

In general, excessive salinity significantly and sharply reduces the ratio of SDM% to RDM%, with the highest ratio (2.16) observed in control plants and the lowest ratio (0.68) in plants receiving 200mM Nacl of irrigation (Figure12). Alternatively, for every addition of 50 mM Nacl, the ratio decreases by about 0.3397.

Figure 12 : The effect of salt on shoot: root ratio

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$.

The ratio of shoot to root was significantly influenced by SA application. When using 0.1 SA in comparison to other treatments, the ratio between SDM% and RDM% was in the lower range. Application of 0.5 SA resulted in a control-like SDM%: RDM% ratio (Figure 13).

Figure 13 : The effect of SA on fenugreek shoot: root ratio

Means with different litter indicate significant at less than *0.05*

There was no significant difference in term of SDM%: RDM% ratio, related to the time factor with an F probability of about 0.257 (Table 1).

No significant effects were observed for the second and third interactions between study factors, where the *F* probability was greater than 0.05 in all interaction effects (Table 1)

Effect of Nacl and SA on photosynthesis pigments mg/g FW of fenugreek plant:

Overall, the amount of photosynthetic pigments in fenugreek leaves remained relatively stable, with no significantly changes observed in chl a, total chl, or chl a: chl b (Figuer 14 a,c and d). However, the levels of chl b and carotenoids significantly decreased with increasing Nacl levels (Table2). Specifically, chlorophyll b (chl b) peaked at at 50 mM Nacl (Figure 14 b). in carotene content became apparent only at higher NaCl levels, notably at 150 mM (Figure 14 e).

Table **2** : the *F* probability for the effect of salicylic acid (SA) at different levels Nacl (N) and measurement Times (T) on photosynthesis pigment of fenugreek plant

	chl_a	chl_b	Total_Chl	chl a: chl b	Carotein
	mg/g FW	mg/g FW	mg/g FW	mg/g FW	mg/g FW
Nacl (N)	0.221	0.038	0.062	0.397	0.006
Salicylic acid (SA)	0.071	0.017	0.013	0.346	0.038
Time (T)	<.001	<.001	<.001	0.106	0.322
15	0.91	1.27	2.18	0.773	0.23
30	1.48	2.35	3.81	0.651	0.22
N ×SA	0.037	<.001	0.001	0.332	0.345
N×T	0.028	0.004	0.002	0.752	0.001
SA × T	<.001	<.001	<.001	0.791	<.001
N×.SA×T	0.005	<.001	<.001	0.554	0.213

F probability at the value less than 0.05 consider significant according to multiple ANOVA analysis

Figure 14 : The effect of salt on photosynthesis pigments [a]: chlorophyll a (Chl a, [b]: is chlorophyll b, [c]: is total chlorophyll (Tchl), d: is the ratio between chlorophyll a to chlorophyll b (Chla / Chlb) and [e] is the total carotenoids.

Means with different alphabet in the same figure indicate significant different at the level of LSD less than *0.*

SA treatment did no effect chlorophyll a, chl a: chl b, or carotenoids in fenugreek leaves (Table 2). However, chl b and total chl, exhibited the significant impact, with F probability values of 0.017 and 0.013 respectively (Table 2). Notably, the application of 0.1 SA significantly increased Chl b and total Chl, whereas 0.5 SA did not significantly differ from the control (Figures 15 b and c).

Unlike chl a: chl b and carotenoids, all photosynthetic pigments including chl a, chl b total Chl, showed significant variation over time, with the second measurement period (which lasted 30 days) recording the highest values for these pigments (Table 2).

Figure 15 : The effect of SA on fenugreek photosynthesis pigments[a]: chlorophyll a (Chl a, [b] : is chlorophyll b , [c] : is total chlorophyll (Tchl) , d : is the ratio between chlorophyll a to chlorophyll b (Chla / Chlb) and [e] is the total carotenoids.

Means with different alphabet in the same figure indicate significant different at the level of LSD less than *0.05*

Interactions between study factors had a significant effect on chla, chlb, and total chl were notably in the second and third interactions (Table 2). No significant effect on chl a: chl b. For carotenoids, the Nacl × Time and SA× Time interaction were significant (Table 2).

Specifically, the third interaction (N× S× T) indicated that the plant receiving 0.1 SA under 100 mM Nacl or 0.5 SA under 50mM Nacl showedthe highest values of chl a, chl b and total chl in the second measurement time (Table 3, 4 and 5).

Table 3 Means of the interaction effect of N×.SA×T on the chl a of fenugreek plant.

SA		Time	Nacl				
			0	50	100	150	200
0		15	0.502hij	1.161^{bcdefgh}	0.424ij	0.29j	1.032^{cdefghi}
		30	1.442abcdef	1.451abcdef	1.652abcd	1.413^{abcdef}	1.571abcde
0.1		15	1.052cdefghi	1.067cdefghi	1.015defghi	0.412ij	1.072cdefghi
		30	1.716abc	1.531abcdef	1.968a	1.438abcdef	1.774ab
0.5		15	1.144bcdefgh	0.866fghij	1.249bcdefg	0.933efghij	1.394abcdef
		30	1.218bcdefg	1.689abcd	0.594ghij	1.825ab	0.847fghij

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test $p \leq 0.05$.

Table 4 : Means of the interaction effect of N×.SA×T on the chl b of fenugreek plant.

SA	Time	Nacl				
		0	50	100	150	200
0	15	0.627ijk	1.719defgh	0.607jk	0.358k	1.305hij
	30	2.17bcdefgh	2.303abcdefg	2.872ab	2.223abcdefgh	2.785abc
0.1	15	1.427ghij	1.53fghij	1.332hij	0.619ijk	1.91cdefgh
	30	2.472abcde	2.784abc	2.92ab	2.804abc	2.454abcdef
0.5	15	1.521fghij	1.311hij	1.756defgh	1.454ghij	1.56efghi
	30	2.534abcd	3.142a	0.624ijk	2.493abcde	0.697ijk

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test $p \leq 0.05$.

Table 5 : Means of the interaction effect of N×.SA×T on the total chl of fenugreek plant.

SA	Time	Nacl				
		0	50	100	150	200
0	15	1.129hij	2.88cde	1.031ij	0.649j	2.336defghi
	30	3.611abcde	3.754abcd	4.524a	3.636abcd	4.356ab
0.1	15	2.479defgh	2.597defg	2.348defghi	1.031ij	2.981bcde
	30	4.188abc	4.315abc	4.888a	4.243abc	4.228abc
0.5	15	2.665def	2.177efghi	3.005bcde	2.387defghi	2.955bcde
	30	3.752abcd	4.831a	1.218ghij	4.318abc	1.267fghij

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test $p \leq 0.05$.

carotenoid content, regarding carotenoid content, the NaCl × Time effect (Figure 16) showed that plants treated with 200 mM had significantly higher carotenoid levels during the first measurement time as compared with control. At the second measurement period, plants treated with 100mM had the lowest levels of carotenoid in comparison to other salinity treatments, but statically similar to the control (Figure 16).

Figure 16 ; The effect of interaction between Nacl and SA on fenugreek carotenoid

Column with different alphabet indicate significant at the level less than *0.05*

Figure 17: The effect of interaction between Nacl and SA on fenugreek carotenoid

Column with different alphabet indicate significant at the level less than *0.05*

The SA × time effect on the carotenoid content (figure 17) revealed distinct patterns. The control treatment showed the naturally occurring rise in carotenoid content of the leaves over time, but the SA treatment changed it. The highest carotenoid levels were found in plants treated with 0.5 SA mg/L at first measurement, while the lowest levels were found in the same plants at the second measurement. No significant differences were observed between time on plants treated with 0.1 mg/l SA, which gave the same value with control at the second measurement time.

Effect of Nacl and SA on biochemical content of fenugreek plant:

Table 6 : Shows the F probability for the effect of Salicylic acid (SA) under different levels of Nacl (N) at different measurement Time (T) on chemical analysis of fenugreek plant.

		Protein Mg/g/FW	Proline Mg/DW	A Peroxides Nanomole/min/ mg of protein	Catalase Nanomole/min/ mg of protein
Nacl (N)		<.001	<.001	0.05	0.317
Salicylic acid (SA)		<.001	<.001	0.002	0.903
Time (T)		0.329	0.153	0.214	0.124
15		26.6	12.63	0.25	0.02
30		25.15	11.49	0.4	0.01
N ×SA		<.001	<.001	0.057	0.322
N×T		<.001	<.001	0.07	0.183
SA × T		<.001	0.401	0.354	0.235
N×.SA×T		<.001	<.001	0.116	0.774

F probability at the value less than 0.05 consider significant according to multiple ANOVA analysis

Effect of Nacl and SA on protein mg/g FW:

Generally, salt treated plants exhibited considerably higher protein content compared to control fenugreek plants. when compared to control plants. As salt levels increased, protein concentration followed similar rise. Plants treated with 200 mM showed the highest protein concentration (31.63 mg/g/FW), while the control plants, (non-salinity plants) had the lowest concentration (17.62 mg/g/FW) (Figure 18).

Figure 18: effect of salt on fenugreek protein mg/g /FW

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$.

The protein content in Fenugreek was significantly affected by SA application. Plants treated with 0.1 SA exhibited the highest protein level (29.87 mg/FW) compared to controls or those treated with 0.5 SA, where no significant difference was observed between the control and 0.5 SA (Figure 19).

Notably, time measurements showed no discernible impact on protein content, (*F* probability about 0.329) (Table 6)

Figure 19: effect of SA on fenugreek protein mg/g /FW

Means with different litter indicate significant at less than *0.05*

However, all levels of interaction impact had significant impact on fenugreek protein content (Table 6). Specifically, the interaction across all study factors (N×SA×T) (Table7) indicated that plants treated with 0.1 SA under 100 Nacl (100 Nacl × 0.1 SA × 30 day) exhibited the highest average of protein content (around 51.06 mg/g /FW). Conversely, control plants under 0.1 SA × 15 days had the lowest protein value (7.62 mg/g /FW).

One notable finding was the decrease in protein content between the two measurement dates in control plants (without salinity and SA). Similarly, the protein levels in control plants with salinity treatments (without SA) followed this declining pattern, up to the treatment with 200 mM NaCl. However, the application of salicylic acid (except for treatments 0.5 and 0) reversed this declining trend in protein content.

Table 7: Means of the interaction effect of N×.SA×T on the protein of fenugreek plant.

SA	Time	Nacl				
		0	50	100	150	200
0	15	48.51 ^{a}	45.17 ^{abc}	35.34 ^{bcdef}	15.47 ^{hijk}	15.8 ^{hijk}
	30	7.02 ^{k}	9.48 ^{jk}	11.68 ^{ijk}	12.82 ^{hijk}	26.3 ^{fgh}
0.1	15	7.62 ^{k}	9.63 ^{jk}	25.08 ^{fghi}	31.74 ^{def}	47.56 ^{ab}
	30	17.08 ^{ghijk}	30.2 ^{efg}	51.6 ^{a}	33.6 ^{cdef}	44.55 ^{abcd}
0.5	15	10.4 ^{jk}	42.62 ^{abcde}	24.73 ^{fghi}	16.77 ^{hijk}	22.58 ^{fghij}
	30	15.11 ^{hijk}	18.12 ^{ghijk}	33.83 ^{cdef}	32.85 ^{cdef}	32.98 ^{cdef}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test $p \leq 0.05$.

Effect of Nacl and SA on proline mg /g DW:

Proline levels in fenugreek leaves displayed a a linearly increase corresponding to the salinity levels, peaking at 22.28 mg/DW in plants treated with 200 mM, while control plants recorded a lower level at 6.39 mg/DW. The increase in Proline was approximately 248.7% higher in plants treated with 200 mM- compared to the control. Alternatively, the proline level exhibited an increase of approximately 3.5 mg for every additional 50 mM NaCl above the control. Notably, plants treated with 50 and 100 mM Nacl or 100 and 150 mM Nacl did not show significant differences from each other (Figure 20).

Figure 20: effect of salt on fenugreek proline mg /DW

Different letters over bar indicates significant difference between treatment according to.
L.S.D test $p \leq 0.05$.

by the SA application significantly affected proline levels, with 0.1 SA leading the highest proline level compared to other treatments (0 and 0.5 SA). However, there was no statistically significant difference in proline levels between 0.1 SA and control plants (Figure 21).

Figure 21: effect of SA on fenugreek proline µg /g dw

Means with different litter indicate significant at less than *0.05*

The proline content of fenugreek leaves did not show significantly variation over time (Table 6). all levels of interaction factors showed significance (Table 6). The interaction effect between all study factors (Nacl× SA× Time) is presented in table 8. At the first measurement time (15 days),

plants treated with 200 Nacl and 0.1 SA exhibited the the highest proline levels, averaging about 49.95 mg/g DW. However, the proline levels dramatically dropped for the same treatment at the second measurement time (30 days). On the other hand, the lowest proline value approximately 2.73 mg/g DW was observed in a non-saline plant treated with 0.5 SA at 30 days.

Table 8: Means of the interaction effect of N×.SA×T on the proline of fenugreek plant.

SA	Time	Nacl mM				
		0	50	100	150	200
0	15	6.61^{hijk}	$9.92^{efghijk}$	8.34^{fghijk}	$9.74^{efghijk}$	15.47^{cdef}
	30	4.87^{ijk}	$9.75^{efghijk}$	8.26^{fghijk}	8.29^{fghijk}	20.65^{c}
0.1	15	$9.18^{efghijk}$	$10.59^{efghijk}$	14.73^{cdefg}	5.35^{ijk}	49.95^{a}
	30	6.72^{hijk}	14.52^{cdefg}	8.57^{fghijk}	30.05^{b}	19.34^{cd}
0.5	15	8.23^{fghijk}	5.05^{ijk}	11.16^{efghij}	12.83^{defgh}	12.29^{efghi}
	30	2.73^{k}	4.77^{jk}	10.17^{efghij}	7.72^{ghijk}	15.96^{cde}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test $p \leq 0.05$.

Effect of Nacl and SA on APX nnanomole/min/gm of portion:

Overall, APX concentration in fenugreek plants exhibited significant variation due to sodium chloride. The highest APX content was observed in plants treated with 50 mM Nacl, averaging 0.419 nanomole/min/mg of protein, with no significant difference between those treated with 200 mM Nacl. The control plant showed the lowest value at 0.238 nanomole/min/mg of protein (Figure 22).

Figure 22: effect of salt on fenugreek APX nanomole/min/mg of protein

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$.

The level of APX was significantly influenced by SA application, where, 0.1 SA led to the highest level of APX (0.373nanomole/min/mg of protein) compared to other treatments (0 and 0.5 SA). However, there was no significant difference in APX levels between 0.5 SA and control plants (Figure 23).

level of APX did no significant difference across both measurement times (Table 6). Among the examined factors, the second and third interactions did no display significant influence on APX (Table 6).

Figure 23: effect of SA on fenugreek APX nanomole/min/mg of protein

Means with different litter indicate significant at less than *0.05*

Effect of Nacl and SA on catalase nanomole/min/mg of protein:

Though the rise in salinity seems to lead to a linear reduction in catalase levels, this decline is not statistically significant at a level less than 0.05, indicated by an *F* probability of approximately 0.317 (Table 6). Figure 24 illustrates the average catalase values influenced by salinity.

Figure 24: effect of salt on fenugreek catalase nanomole/min/mg of protein

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$.

Figure 25: effect of SA on fenugreek catalase nanomole/min/mg of protein

Different letters over bar indicates significant difference between treatment according to L.S.D test $p \leq 0.05$.

Overall, applying SA at varying concentrations (0, 0.1, and 0.5) did not yield a significantly change in catalase levels, with an *F* probability of about 0.903 (Table 6). The averages are depicted in Figure 25. Similarly, the time factor showed no significant influence on catalase too, with an *F* probability around 0.124 (Table 6). Additionally, the interaction among all study factors (second and third) did not yield significant difference on catalase (Table 6).

DISCUSSIONS:

The impact of salinity on plant growth, particularly in in shoot length, was consistent across the studied plants compared to the control group. This aligns with previous findings (Singh and Singh, 1991; Mensah *et al.*, 2006; Sadat-noori *et al.*, 2008; Azooz *et al.*, 2011; Rasool *et al.*, 2013; Ahmad *et al.*, 2014b; Ahmad *et al.*, 2014a).

Among the plants examined, parsley, fennel, and fenugreek, salt content elicited distinct responses. While parsley roots were unaffected by salinity, fenugreek experienced reduced root length. This finding concurs with research(Beyk-Khormizi *et al.*, 2023) specifically on fennel.

Comparatively, the fenugreek appeared resilience. Studies by Banakar *et al.*, (2022) and Beyk-Khormizi *et al.*(2023) highlighted fenugreek's ability to tolerate environmental stresses by increasing endogenous trigonelline and triggering general physiological responses.

Salinity poses a significant challenge to plant growth, hindering water absorption due to osmotic stress from Na and Cl ions. This, in turn, reduces shoot length and development. Additionally, salt stress disrupts cell division, growth, and nutrient absorption, leading to ionic imbalances., Additionally, sodium accumulation can result in toxicity and competition with potassium ions, further aggravating ionic imbalances and hindering vital nutrient absorption like Ca and K (Hichem and Mounir, 2009; Ashraf *et al.*, 2010; Ahmad *et al.*, 2015; Ahmad *et al.*, 2016; Machado and Serralheiro, 2017).

The detrimental effects of salinity on plant growth and development have also been shown by additional studies (Tohma and Esitken, 2011; Per *et al.*, 2017b; Roshdy *et al.*, 2021). salinity-induced oxidative stress might also have a deleterious impact on root and vegetative growth (Alam *et al.*, 2019).

A reduction in plant development under salt stress conditions coincides with an elevated in lipid peroxidation and a decrease in MDA levels. This is a signifies heightened vulnerability of membrane (Siddiqui *et al.*, 2017; Akram *et al.*, 2017; Ahanger *et al.*, 2020; Kaya *et al.*, 2020a; Kaya *et al.*, 2020b).

Additionally,, ion toxicity, osmotic imbalance, and disruptions in nutrient and ion equilibrium have adverse effects on photosynthetic pigments, leading to a decline in photosynthetic rate , on rice (Jini and Joseph, 2017; Shahbaz *et al.*, 2017), and on soybean (Maswada *et al.*, 2018) .

Dry matter, a pivotal physiological parameter, represents the cumulative effect of photosynthesis by the leaf and ion absorption by the root. The alteration in the ratio between shoot dry matter (SDM%) and root dry matter (RDM%) compared to control plants signifies a plant's response to salinity. During this experiment, the response of experimental plants to mitigate NaCl toxicity was observed.

In fenugreek, SDM% in plants treated with NaCl remained similar to the control, whereas RDM% sharply increased with rising NaCl levels. This indicates root accumulation of Na ions

without their substantial transfer to the shoot, resulting in a significant decrease in the shoot-to-root ratio compared to the control.

Furthermore, the changes in root SDM% to RDM% compared to the control plants showed correlations. In fenugreek, this correlated with shoot height (r=0.38), These correlations highlight how plants redistribute biomass under salt stress, emphasizing either root or shoot growth, reflecting species-specific strategies. The decrease in dry matter accumulation is positively connected with the amount of photosynthetic pigment, and is a result of the chlorophyllase enzyme's suppression of Chl production and acceleration of pigment degradation. whilst fenugreek's distinct effect may be caused by the root's direct contact with salinity. Ion toxicity and oxidative stress may also be to blame for the decline in photosynthetic rate and dry matter (Smirnoff, 1996; Santos, 2004; Cabot *et al.*, 2014)

In the present investigation, the application of salicylic acid resulted in a reduction of the impact of salinity on the root and vegetative growth characteristics. This function could be attributed to the boost in dry matter in plants under salt stress after being subjected to SA, thereby elevating the plant's resistance and mitigating damage to the membrane (Misra and Saxena, 2009). Additionally, this could be due to the amelioration of the membrane, which reduces the deterioration in plants under salt stress, facilitating their maintenance and preserving the activity and functions of the membrane (Stevens *et al.*, 2006). Furthermore, it might also be due to an escalation in the soluble sugar content (Qados, 2015), and proline (Szepesi, 2006), or the curtailment of MDA content (Kováčik *et al.*, 2009; Lee *et al.*, 2010; Manaa *et al.*, 2014a).

The increase in the concentration of salt negatively affected the outputs of photosynthesis (Ahmad *et al.*, 2017). In this study, the increase in sodium chloride had a significant effect on reducing the content of photosynthesis pigments in all studied plants. It decreases in the content of all pigments (chla and chlb, total chlorophyll, and carotenoids). this finding took the same trend found by(Chondraki *et al.*, 2012; Desire and Arslan, 2021), and , as stated in the results on parsley of (Shafeiee and Ehsanzadeh, 2019) and (He *et al.*, 2014) on the fennel plant. While the fenugreek plant showed more resistance to increasing the concentration of NaCl in the irrigation water, the effect of increasing the salt concentration was not significant except with Chl b and carotenoids, in contradiction with the result of (Abdelhameed et al., 2021) regarding the content of carotenoids. This conflict may be mainly due to the genetic factor, as local cultivars were used in our experiments. Our results in fenugreek plant were consistent with (Chondraki *et al.*, 2012) on parsley and in chickpea (Ahmad *et al.*, 2016) .

The results from these experiments indicate that the chlorophyll a to chlorophyll b ratio no significant change was observed in fenugreek plants. Interestingly, there was no effect on the Chl a : Chl b ratio when application of SA in fenugreek plants.

Chlorophyll a play an essential role in photochemistry, while chlorophyll b is crucial for stabilizing major light-harvesting chlorophyll-binding proteins. It's synthesized from chlorophyll a and later catabolized after reconversion to chlorophyll a (Tanaka and Tanaka, 2011). A reduced Chl a/b ratio often signifies disruption in the photosynthetic apparatus, indicating the plant's sensitivity or stress response to saline conditions. This shift in ratio may suggest changes in pigment composition or physiological adaptations to cope with the saline environment.

The decline in the Chl a/b ratio occurs due to unequal destruction of chlorophylls, with relatively more Chl a than Chl b affected, resulting from their reaction with singlet oxygen produced under stress conditions. Therefore, monitoring alterations in the Chl a/b ratio offers valuable insights into these plants' sensitivity to salt stress (Shaw, 1995)

The effect of salinity on deterioration photosynthesis pigments may be due to damage and impaired biosynthesis (Neelam and Subramanyam, 2013), additionally the high salinity led to instability of chlorophyll associated with the protein complex and the accelerated decomposition of chlorine and filet due to the activities of chlorophyllase (Rasool *et al.*, 2013), an indication of the presence of photoprotection by reducing light absorption by reducing the chlorophyll content (Elsheery and Cao, 2008). Moreover, the lack of pigment is due to the low content of carbohydrates, which when reduced gives a positive effect on salt stress tolerance (Jahan *et al.*, 2018), where salinity reduces the activity of ribulose 1,5-diphosphate (Rubisco) carboxylase, which affects the process of carbohydrate synthesis and thus reduces its formation in leaves exposed to salt stress (El-Shihaby *et al.*, 2002). Also, the increase in salts affects the process of photosynthesis and the closure of the stoma, thus inhibiting biochemical reactions, and therefore the feedback prevents the process of carbon metabolism and is reflected on growth, as described by (Munns *et al.*, 2006).

The level of carotenoids in fenugreek increased in plant under 200 Nacl. This different response between those genius of plant is related to the genetic. In the case of carotenoids, the increase in its content is explained by its role in reducing oxidative damage, because this pigment acts as an antioxidant, destroys pigments and decreases their synthesis(Rasool *et al.*, 2013). It is also mechanism to protect chloroplasts from Reactive Oxygen Species (ROS) (Collins, 2001). Meanwhile, the low content of carotenoids is due to the inability of the plant to resist various stresses (Sehar *et al.*, 2019; Rasheed *et al.*, 2022) .

Application of SA enhanced the chlorophyll content in all plants used in this study. In fact the application of SA inhibit the harmful effects of salinity and maintained the optimal nutrition of minerals, improved the water content of cells, and led to an increase in the antioxidant response, as well as membrane protection (Nahar *et al.*, 2016; Fariduddin *et al.*, 2018; Ahanger *et al.*, 2019; Xu *et al.*, 2020; Bukhat *et al.*, 2020; Talaat, 2021; Es-sbihi *et al.*, 2021) and protecting pigment from ion toxicity as well as oxidation (Foyer and Shigeoka, 2011).

The application of SA increased the plant content of Chl as in the mustard plant(Nazar *et al.*, 2015), as the SA led to reduce the salinity stress in chlorophyll by enhancing the production of its enzymes and led to curb the defect in the photosynthetic system, thus reducing the breakdown of chlorophyll (Nahar *et al.*, 2016; Fariduddin *et al.*, 2018; Ahanger *et al.*, 2019; Bukhat *et al.*, 2020; Islam *et al.*, 2020; Kaya *et al.*, 2020 ; Es-sbihi *et al.*, 2021). which made the plant resist stresses under the influence of salt, such as osmotic stress, oxidative stress, ion imbalance, and protecting the plant from Reactive Oxygen Species (ROS), which have a clear effect on fats, proteins, and some molecules that may stop photosynthesis or cell death (Bhuyan *et al.*, 2020; Jahan *et al.*, 2020).

SA also reduced oxidative damage to plants under salt stress as reported by (Fariduddin *et al.*, 2003) in Brassica juncea; (Nazar *et al.*, 2011) in Mungbean; (Li *et al.*, 2013)

in Wheat, and this was the mitigation of toxic effects by increasing the activity of antioxidants (El-Tayeb, 2005; Syeed *et al.*, 2011; Ahanger *et al.*, 2020; Mehak *et al.*, 2021; Punia *et al.*, 2021; Kumar *et al.*, 2022b). Thus, how can explain why the experimental plants survived when using salicylic acid under higher concentrations of salinity and died at higher concentrations of salinity without using salicylic acid.

The proline content of stressed plants increased gradually with the increase of salt concentration, where the fenugreek plants accumulation of proline content, and salinity worked to reduce the process of creating proteins in the plants to varying degrees, the fenugreek plant showed more resistance to the negative impact on the synthesis of proteins.

Salinity causes a decrease in the amount of total proteins when compared to the control, as indicated by (Moussa and Hassan, 2016) in *Vicia faba* plant.

Also, the decrease in plant protein content when salt increases goes in the same direction as indicated by (El-Khallal *et al.*, 2009; Shahid *et al.*, 2011; Shakeel and Mansoo, 2012) , and this is in contrast to what was stated by (Özdemir *et al.*, 2004; Javed *et al.*, 2014) , who indicated that the protein content increased when the salt concentration increased.

While the activity of peroxidase was high with an increase in the concentration of Nacl in fenugreek plant. Salinity led to increasing the peroxidase activity of ROS antioxidant defines mechanisms (Harati *et al.*, 2015). Salinity did not show a significant effect the activity of catalase on fenugreek. That means the This finding took the same direction as what was stated by (Harati *et al.*, 2015) , as its results indicated that the activity of catalase did not have a significant effect under salt pressure, and this also in contrary to what was indicated by the plant of *Jatropha curcas* (Gao *et al.*, 2008), , in rice (Jini and Joseph, 2017),, and in maize (Tahjib-Ul-Arif *et al.*, 2018), where salt stress increased the activity of catalase, which is considered one of the defence mechanisms to confront stress, and this was in the same in Peanut (Manai *et al.*, 2014), in *Vignar adiatat* (Hayat *et al.*, 2010b), in Chickpea (Ahmad *et al.*, 2016)

The increase in proline content in response to stress, as stated by (Al-Khayri, 2002; İnal, 2002; Sakhabutdinova *et al.*, 2003), that the accumulation of proline was to alleviate the metabolic disturbances resulting from salt stress (Silveira *et al.*, 2009).The role of proline in the cell is: osmotic regulation, membrane stabilization, and detoxification of harmful ions in plants exposed to salt stress (Ashraf and Foolad, 2007), where proline originally acts as an enzyme protector (Delauney and Verma, 1993).This amino acid is believed to help protect enzymes and membrane integrity in plants under restrictive conditions (Ashraf and Foolad, 2007). The increase in proline in stressed plants is due to the role that proline plays against osmotic stress and ROS, as proline works to stabilize cell structures and enzymes and provide a balance of cellular oxidation. (Meena *et al.*, 2019) and its accumulation is more than amino acids, and its accumulation is an important indicator of the occurrence of plants under stress, as indicated by (Cherian and Reddy, 2003), while the increase in proteins had a role in modifying osmosis as mentioned by (Muhammad and Hussain, 2010; Harati *et al.*, 2015) and plant adaptation while the low content of proteins in stressed plants is due to the release of some proteins into the medium due to the osmotic shock resulting from the increase in salt, as indicated by (Maas *et al.*, 1979) , or a decrease in its synthesis as stated by (Hall and Flowers, 1973; Cherian and Reddy, 2003) . Thus, the increase in the proline content of the experimental plants can be explained by the fact that it is under the influence of salt stress.

Also, the increase in antioxidant enzymes APX, CAT(Ahmad *et al.*, 2018; Ahanger *et al.*, 2019). It is due to the increase in the resistance of harmful oxides that stress works to form. It was mentioned that the SOD accelerates the conversion of the superoxide anion to O2 + H2O2, while the latter hinders natural processes by causing damage to some molecules.

Experiments showed that the application of SA leads to the development of the stress response and the stimulation of the growth process after the stress response, as well as causing an increase in the plant's proline content (Manaa *et al.*, 2014b) .(Misra and Misra, 2012) indicated the role of SA in alleviating salinity stress through changes in turgor due to increased accumulation of proline, and further reported that the proline content and the activity of proline synthesis enzymes, P-5-CR and c-glutamyl kinase, significantly increased in the presence of salinity stress but a higher increase occurred when SA was applied to salt-stressed plants. Whereas, SA enhanced the activity of enzymes and the content of proline alone, and its interaction with sodium chloride had an additional effect on the activation process.

The application of SA had a positive effect on the protein content under salt stress. (El-Khallal *et al.*, 2009; Shakeel and Mansoo, 2012; Farheen *et al.*, 2018). reported an increase in protein content in salt-stressed plants when applying SA. (Aftab *et al.*, 2011) on Artemisia annua L *&* (Mishra and Choudhuri, 1999) stated that the application of SA has a role in enhancing the activities of antioxidant enzymes such as CAT *&* POX in salt-stressed plants, as stated by (Aftab *et al.*, 2011) in Artemisia annua L., (Harati *et al.*, 2015), and the increase in the activity of these enzymes is due to the formation of superoxide, hydrogen peroxide, which is harmful. on plant production and growth stages. Thus, it is possible to explain the reason for the resistance of the experimental plants when using salicylate and their survival compared to those that died due to salt stress.

References

Abd El-Wahab M. (2006). The efficiency of using saline and fresh water irrigation as alternating methods of irrigation on the productivity of Foeniculum vulgare Mill subsp. vulgare var. vulgare under North Sinai conditions. *Res J Agr Biol Sci 2*:571-577.

Abdelhameed R.E., Abdel Latef A.A.H., and Shehata R.S. (2021). Physiological Responses of Salinized Fenugreek (Trigonellafoenum-graecum L.) Plants to Foliar Application of Salicylic Acid. *Plants 10*:657.

Aebi H. (1984). [13] Catalase in vitro, Methods in Enzymology, Academic Press. pp. 121-126.

Aftab T., Khan M.M.A., da Silva J.A.T., Idrees M., Naeem M., and Moinuddin. (2011). Role of salicylic acid in promoting salt stress tolerance and enhanced artemisinin production in Artemisia annua L. *Journal of plant growth regulation 30*:425-435.

Ahanger M.A., Aziz U., Alsahli A.A., Alyemeni M.N., and Ahmad P. (2019). Influence of exogenous salicylic acid and nitric oxide on growth, photosynthesis, and ascorbate-glutathione cycle in salt stressed Vigna angularis. *Biomolecules 10*:42.

Ahanger M.A., Aziz U., Alsahli A.A., Alyemeni M.N., and Ahmad P. (2020). Influence of Exogenous Salicylic Acid and Nitric Oxide on Growth, Photosynthesis, and Ascorbate-Glutathione Cycle in Salt Stressed Vigna angularis. *Biomolecules 10*:42.

Ahmad F., Singh A., and Kamal A. (2017). Ameliorative effect of salicylic acid in salinity stressed Pisum sativum by improving growth parameters, activating photosynthesis and enhancing antioxidant defense system. *Biosci Biotechnol Res Commun 10*:481-489.

Ahmad P., Ozturk M., Sharma S., and Gucel S. (2014a). Effect of sodium carbonate-induced salinity–alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (Morus alba L.) cultivars. *Journal of Plant Interactions 9*:460 - 467.

Ahmad P., Ozturk M., Sharma S., and Gucel S. (2014b). Effect of sodium carbonate-induced salinity–alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (Morus alba L.) cultivars. *Journal of Plant Interactions 9*:460-467. DOI: 10.1080/17429145.2013.855271.

Ahmad P., Abdel Latef A.A., Hashem A., Abd_Allah E.F., Gucel S., and Tran L.-S.P. (2016). Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Frontiers in plant science 7*:347.

Ahmad P., Alyemeni M., Ahanger M., Egamberdieva D., Wijaya L., and Alam P. (2018). Salicylic Acid (SA) Induced Alterations in Growth, Biochemical Attributes and Antioxidant Enzyme Activity in Faba Bean (Vicia faba L.) Seedlings under NaCl Toxicity. *Russian Journal of Plant Physiology 65*:104-114. DOI: 10.1134/s1021443718010132.

Ahmad P., Hashem A., Abd-Allah E.F., Alqarawi A.A., John R., Egamberdieva D., and Gucel S. (2015). Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (Brassica juncea L) through antioxidative defense system. *Frontiers in plant science 6*. DOI: 10.3389/fpls.2015.00868.

Akram N.A., Shafiq F., and Ashraf M. (2017). Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Frontiers in plant science*:613.

Al-doubibi, M. H., Al-madhagi, I. A. H. andAl-munibary, M. (2021). The relationship and changing rate of strawberry crown carbohydrate as result of chilling hours and cold storage. *Big Data in Agriculture* 3 (1): 44-50.https://doi.org/10.26480/bda.01.2021.44.50

Al-Khayri J.M. (2002). Growth, proline accumulation and ion content in sodium chloride-stressed callus of date palm. *In Vitro Cellular & Developmental Biology-Plant 38*:79-82.

Al-Madhagi I., and Al-Sharagi H. (2019). *Schinus molle* leaves compost improves the growth, quality and productivity of strawberry (*Fragaria* × *Ananassa* Duch) in potting culture. *Journal of Horticulture and Plant Research 7*:26-39. DOI: https://doi.org/10.18052/www.scipress.com/jhpr.7.26.

Al-Karaki G.N. (2000). Growth, water use efficiency, and sodium and potassium acquisition by tomato cultivars grown under salt stress. *Journal of Plant Nutrition 23*:1-8. DOI: 10.1080/01904160009381992.

Alam P., Albalawi T.H., Altalayan F.H., Bakht M.A., Ahanger M.A., Raja V., Ashraf M., and Ahmad P. (2019). 24-Epibrassinolide (EBR) Confers Tolerance against NaCl Stress in Soybean Plants by Up-Regulating Antioxidant System, Ascorbate-Glutathione Cycle, and Glyoxalase System. *Biomolecules 9*:640.

Allakhverdiev S.I., Sakamoto A., Nishiyama Y., Inaba M., and Murata N. (2000). Ionic and Osmotic Effects of NaCl-Induced Inactivation of Photosystems I and II in Synechococcus sp.1. *Plant Physiology 123*:1047-1056. DOI: 10.1104/pp.123.3.1047.

Asada K. (1994). Production and action of active oxygen species in photosynthetic tissues. *Causes of photooxidative stress and amelioration of defense systems in plants*:77-104.

Ashraf M., and Orooj A. (2006). Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (Trachyspermum ammi [L.] Sprague). *Journal of Arid Environments 64*:209-220. DOI: https://doi.org/10.1016/j.jaridenv.2005.04.015.

Ashraf M., and Foolad M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany 59*:206-216.

Ashraf M.A., Ashraf M., and Ali Q. (2010). Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. *Pak J Bot 42*:559-565.

Azooz M.M., Youssef A.M., and Ahmad P. (2011). Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. *Int J Plant Physiol Biochem 3*:253-264.

Banakar M.H., Amiri H., Sarafraz Ardakani M.R., and Ranjbar G.H. (2022). Susceptibility and tolerance of fenugreek (Trigonella foenum-graceum L.) to salt stress: Physiological and biochemical inspections. *Environmental and Experimental Botany 194*:104748. DOI: https://doi.org/10.1016/j.envexpbot.2021.104748.

Bates L.S., Waldren R.a., and Teare I. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil 39*:205-207. DOI: https://doi.org/10.1007/BF00018060.

Beyk-Khormizi A., Sarafraz-Ardakani M.R., Hosseini Sarghein S., Moshtaghioun S.M., Mousavi-Kouhi S.M., and Taghavizadeh Yazdi M.E. (2023). Effect of Organic Fertilizer on the Growth and Physiological Parameters of a Traditional Medicinal Plant under Salinity Stress Conditions. *Horticulturae 9*:701.

Bhuyan M.B., Hasanuzzaman M., Parvin K., Mohsin S.M., Al Mahmud J., Nahar K., and Fujita M. (2020). Nitric oxide and hydrogen sulfide: two intimate collaborators regulating plant defense against abiotic stress. *Plant Growth Regulation 90*:409-424.

Bukhat S., Manzoor H., Athar H.-u.-R., Zafar Z.U., Azeem F., and Rasul S. (2020). Salicylic acid induced photosynthetic adaptability of Raphanus sativus to salt stress is associated with antioxidant capacity. *Journal of plant growth regulation 39*:809-822.

Cabot C., Sibole J.V., Barceló J., and Poschenrieder C. (2014). Lessons from crop plants struggling with salinity. *Plant Science 226*:2-13.

Cherian S., and Reddy M. (2003). Evaluation of NaCl tolerance in the callus cultures of Suaeda nudiflora Moq. *Biologia Plantarum 46*:193-198.

Chondraki S., Tzerakis C., and Tzortzakis N. (2012). INFLUENCE OF SODIUM CHLORIDE AND CALCIUM FOLIAR SPRAY ON HYDROPONICALLY GROWN PARSLEY IN NUTRIENT FILM TECHNIQUE SYSTEM. *Journal of Plant Nutrition 35*:1457-1467. DOI: 10.1080/01904167.2012.689906.

Collins A.R. (2001). Carotenoids and genomic stability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 475*:21-28.

Cuartero J., Bolarín M.C., Asíns M.J., and Moreno V. (2006). Increasing salt tolerance in the tomato. *Journal of Experimental Botany 57*:1045-1058. DOI: 10.1093/jxb/erj102.

Delauney A.J., and Verma D.P.S. (1993). Proline biosynthesis and osmoregulation in plants. *The Plant Journal 4*:215-223. DOI: https://doi.org/10.1046/j.1365-313X.1993.04020215.x.

Demiral T., and Türkan İ. (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environmental and Experimental Botany 53*:247-257. DOI: https://doi.org/10.1016/j.envexpbot.2004.03.017.

Demiral T., and Türkan I. (2006). Exogenous glycinebetaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. *Environmental and Experimental Botany 56*:72-79. DOI: https://doi.org/10.1016/j.envexpbot.2005.01.005.

Desire M., and Arslan H. (2021). The Effect of Salicylic Acid On Photosynthetic Characteristics, Growth Attributes, and Some Antioxidant Enzymes On Parsley (Petroselinum crispum L.) Under Salinity Stress. *Gesunde Pflanzen 73*:435-444. DOI: 10.1007/s10343-021-00565-3.

Dhiman P., Rajora N., Bhardwaj S., Sudhakaran S.S., Kumar A., Raturi G., Chakraborty K., Gupta O.P., Devanna B.N., Tripathi D.K., and Deshmukh R. (2021). Fascinating role of silicon to combat salinity stress in plants: An updated overview. *Plant Physiology and Biochemistry 162*:110-123. DOI: https://doi.org/10.1016/j.plaphy.2021.02.023.

El-Khallal S.M., Hathout T.A., Ahsour A., and Kerrit A.-A.A. (2009). Brassinolide and salicylic acid induced antioxidant enzymes, hormonal balance and protein profile of maize plants grown under salt stress. *Research Journal of Agriculture and Biological Sciences 5*:391-402.

El-Shihaby O.A., Younis M.E., El-Bastawisy Z.M., and Nemat Alla M.M. (2002). Effect of kinetin on photosynthetic activity and carbohydrate content in waterlogged or seawater-treated Vigna sinensis and Zea mays plants. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 136*:277-290. DOI: 10.1080/11263500212331351189.

El-Tayeb M. (2005). Response of barley grains to the interactive e. ect of salinity and salicylic acid. *Plant Growth Regulation 45*:215-224.

Elsheery N.I., and Cao K.-F. (2008). Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. *Acta Physiologiae Plantarum 30*:769-777.

Es-sbihi F.Z., Hazzoumi Z., Aasfar A., and Amrani Joutei K. (2021). Improving salinity tolerance in Salvia officinalis L. by foliar application of salicylic acid. *Chemical and Biological Technologies in Agriculture 8*:1-12.

Farheen J., Mansoor S., and Abideen Z. (2018). Exogenously applied salicylic acid improved growth, photosynthetic pigments and oxidative stability in mungbean seedlings (Vigna radiata) at salt stress. *Pak J Bot 50*:901-912.

Fariduddin Q., Hayat S., and Ahmad A. (2003). Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity, and seed yield in Brassica juncea. *Photosynthetica 41*:281-284.

Fariduddin Q., Khan T., Yusuf M., Aafaqee S., and Khalil R. (2018). Ameliorative role of salicylic acid and spermidine in the presence of excess salt in Lycopersicon esculentum. *Photosynthetica 56*:750-762.

Foyer C.H., and Shigeoka S. (2011). Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant physiology 155*:93-100.

Gao S., Ouyang C., Wang S., Xu Y., Tang L., and Chen F. (2008). Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in Jatropha curcas L. seedlings. *Plant Soil Environ 54*:374-381.

Gupta S., Schillaci M., Walker R., Smith P.M.C., Watt M., and Roessner U. (2021). Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: Current knowledge, perspectives and future directions. *Plant and Soil 461*:219-244. DOI: 10.1007/s11104-020-04618-w.

Hall J., and Flowers T. (1973). The effect of salt on protein synthesis in the halophyte Suaeda maritima. *Planta 110*:361-368.

Harati E., Kashefi B., and Matinizadeh M. (2015). Investigation Reducing Detrimental Effects of Salt Stress on Morphological and Physiological Traits of (Thymus vulgaris) by Application of Salicylic Acid. *Journal of plant physiology 5*:1383-1391.

Hayat Q., Hayat S., Irfan M., and Ahmad A. (2010a). Effect of exogenous salicylic acid under changing environment: a review. *Environmental and experimental botany 68*:14-25.

Hayat S., Hasan S.A., Yusuf M., Hayat Q., and Ahmad A. (2010b). Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in Vigna radiata. *Environmental and experimental botany 69*:105-112.

He Y., Yu C., Zhou L., Chen Y., Liu A., Jin J., Hong J., Qi Y., and Jiang D. (2014). Rubisco decrease is involved in chloroplast protrusion and Rubisco-containing body formation in soybean (Glycine max.) under salt stress. *Plant Physiology and Biochemistry 74*:118-124.

Hichem H., and Mounir D. (2009). Differential responses of two maize (Zea mays L.) varieties to salt stress: changes on polyphenols composition of foliage and oxidative damages. *Industrial Crops and Products 30*:144-151.

İnal A. (2002). Growth, Proline Accumulation and Ionic Relations of Tomato (Lycopersicon esculentum L.) as Influenced by NaCl and Na_2SO_4 Salinity. . *Turkish Journal of Botany 26*:285-290.

Islam M.A., PANG J.-h., MENG F.-w., LI Y.-w., Ning X., Chao Y., and Jun L. (2020). Putrescine, spermidine, and spermine play distinct roles in rice salt tolerance. *Journal of Integrative Agriculture 19*:643-655.

Jahan, B., AlAjmi, M. F., Rehman, M. T. andKhan, N. A. (2020). Treatment of nitric oxide supplemented with nitrogen and sulfur regulates photosynthetic performance and stomatal behavior in mustard under salt stress. *Physiologia Plantarum* 168(2): 490-510.https://doi.org/10.1111/ppl.13056

Jahan I., Parvin S., Miah M.G., and Ahmed J.U. (2018). Effect of salinity on the physiological and biochemical responses of neem. *International Journal of Environmental and Agriculture Research 4*.

Javed S., Bukhari S.A., Mahmood S., and Iftikhar T. (2014). Effect of salinity on growth, biochemical parameters and fatty acid composition in safflower (carthamus tinctorius l.). *Pakistan Journal of Botany 46*:1153-158.

JIA X., and JIANG Z. (2023). SALICYLIC ACID ALLEVIATES SALT STRESS IN SOYBEAN BY IMPROVING ANTI-OXIDANT CAPACITY AND GROWTH PERFORMANCE. *Pak. J. Bot 55*:37-44.

Jiang C., Zu C., Lu D., Zheng Q., Shen J., Wang H., and Li D. (2017). Effect of exogenous selenium supply on photosynthesis, Na+ accumulation and antioxidative capacity of maize (Zea mays L.) under salinity stress. *Scientific Reports 7*:42039. DOI: 10.1038/srep42039.

Jini D., and Joseph B. (2017). Physiological Mechanism of Salicylic Acid for Alleviation of Salt Stress in Rice. *Rice Science 24*:97-108. DOI: https://doi.org/10.1016/j.rsci.2016.07.007.

Kaya C., Ashraf M., Alyemeni M.N., and Ahmad P. (2020a). The role of endogenous nitric oxide in salicylic acid-induced up-regulation of ascorbate-glutathione cycle involved in salinity tolerance of pepper (Capsicum annuum L.) plants. *Plant Physiology and Biochemistry 147*:10-20. DOI: https://doi.org/10.1016/j.plaphy.2019.11.040.

Kaya C., Higgs D., Ashraf M., Alyemeni M.N., and Ahmad P. (2020b). Integrative roles of nitric oxide and hydrogen sulfide in melatonin-induced tolerance of pepper (Capsicum annuum L.) plants to iron deficiency and salt stress alone or in combination. *Physiologia Plantarum 168*:256-277. DOI: https://doi.org/10.1111/ppl.12976.

Kaya C., Ashraf M., Alyemeni M., Corpas F., and Ahmad P. (2020). Salicylic acid-induced nitric oxide enhances arsenic toxicity tolerance in maize. *Journal of hazardous materials 399*. DOI: doi: 10.1016/j.jhazmat.2020.123020. Epub.

Khajeh-Hosseini M., Powell A.A., and Bingham I.J. (2003). The interaction between salinity stress and seed vigour during germination of soyabean seeds. *Seed Science and Technology 31*:715-725.

Koo Y.M., Heo A.Y., and Choi H.W. (2020). Salicylic acid as a safe plant protector and growth regulator. *The plant pathology journal 36*:1.

Kováčik J., Klejdus B., Hedbavny J., and Bačkor M. (2009). Salicylic acid alleviates NaCl-induced changes in the metabolism of Matricaria chamomilla plants. *Ecotoxicology 18*:544-554. DOI: 10.1007/s10646-009-0312-7.

Kumar S., Abass Ahanger M., Alshaya H., Latief Jan B., and Yerramilli V. (2022b). Salicylic acid mitigates salt induced toxicity through the modifications of biochemical attributes and some key antioxidants in capsicum annuum. *Saudi Journal of Biological Sciences 29*:1337-1347. DOI: https://doi.org/10.1016/j.sjbs.2022.01.028.

Lee S.K., Sohn E.Y., Hamayun M., Yoon J.Y., and Lee I.J. (2010). Effect of silicon on growth and salinity stress of soybean plant grown under hydroponic system. *Agroforestry Systems 80*:333-340. DOI: 10.1007/s10457-010-9299-6.

Li G., Peng X., Wei L., and Kang G. (2013). Salicylic acid increases the contents of glutathione and ascorbate and temporally regulates the related gene expression in salt-stressed wheat seedlings. *Gene 529*:321-325. DOI: https://doi.org/10.1016/j.gene.2013.07.093.

Li X., Zhang L., Ahammed G.J., Li Y.-T., Wei J.-P., Yan P., Zhang L.-P., Han X., and Han W.-Y. (2019). Salicylic acid acts upstream of nitric oxide in elevated carbon dioxide-induced flavonoid biosynthesis in tea plant (Camellia sinensis L.). *Environmental and experimental botany 161*:367-374.

Lowry O., Rosebrough N., Farr A.L., and Randall R. (1951). Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry 193*:265-275. DOI: https://doi.org/10.1016/S0021-9258(19)52451-6.

Maas E.V., Ogata G., and Finkel M.H. (1979). Salt-induced Inhibition of Phosphate Transport and Release of Membrane Proteins from Barley Roots. *Plant physiology 64*:139-143. DOI: 10.1104/pp.64.1.139.

Machado R.M.A., and Serralheiro R.P. (2017). Soil Salinity: Effect on Vegetable Crop Growth. Management Practices to Prevent and Mitigate Soil Salinization. *Horticulturae 3*:30.

Manaa A., Gharbi E., Mimouni H., Wasti S., Aschi-Smiti S., Lutts S., and Ben Ahmed H. (2014a). Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (Solanum lycopersicum) cultivars. *South African Journal of Botany 95*:32-39. DOI: https://doi.org/10.1016/j.sajb.2014.07.015.

Manaa A., Mimouni H., Terras A., Chebil F., Wasti S., Gharbi E., and Ben Ahmed H. (2014b). Superoxide dismutase isozyme activity and antioxidant responses of hydroponically cultured Lepidium sativum L. to NaCl stress. *Journal of Plant Interactions 9*:440-449. DOI: 10.1080/17429145.2013.850596.

Manai J., Gouia H., and Corpas F.J. (2014). Redox and nitric oxide homeostasis are affected in tomato (Solanum lycopersicum) roots under salinity-induced oxidative stress. *Journal of plant physiology 171*:1028-1035. DOI: https://doi.org/10.1016/j.jplph.2014.03.012.

Maswada H.F., Djanaguiraman M., and Prasad P.V.V. (2018). Response of photosynthetic performance, water relations and osmotic adjustment to salinity acclimation in two wheat cultivars. *Acta Physiologiae Plantarum 40*:105. DOI: 10.1007/s11738-018-2684-x.

Meena M., Divyanshu K., Kumar S., Swapnil P., Zehra A., Shukla V., Yadav M., and Upadhyay R.S. (2019). Regulation of L-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon 5*:e02952. DOI: https://doi.org/10.1016/j.heliyon.2019.e02952.

Mehak G., Akram N.A., Ashraf M., Kaushik P., El-Sheikh M.A., and Ahmad P. (2021). Methionine-induced regulation of growth, secondary metabolites and oxidative defense system in sunflower (Helianthus annuus L.) plants subjected to water deficit stress. *PLOS one 16*:e0259585.

Mensah J.K., Akomeah P., Ikhajiagbe B., and Ekpekurede E. (2006). Effects of salinity on germination, growth and yield of five groundnut genotypes. *African journal of biotechnology 5*: 1973-1979. DOI: https://doi.org/10.5897/AJB2006.000-5098.

Metzner H., Rau H., and Senger H. (1965). Untersuchungen zur Synchronisierbarkeit einzelner Pigmentmangel-Mutanten von Chlorella. *Planta 65*:186-194. DOI: 10.1007/bf00384998.

MILLER G., SUZUKI N., CIFTCI-YILMAZ S., and MITTLER R. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell & Environment 33*:453-467. DOI: https://doi.org/10.1111/j.1365-3040.2009.02041.x.

Mishra A., and Choudhuri M.A. (1999). Effects of Salicylic Acid on Heavy Metal-Induced Membrane Deterioration Mediated by Lipoxygenase in Rice. *Biologia Plantarum 42*:409-415. DOI: 10.1023/a:1002469303670.

Misra N., and Dwivedi U.N. (2004). Genotypic difference in salinity tolerance of green gram cultivars. *Plant Science 166*:1135-1142. DOI: https://doi.org/10.1016/j.plantsci.2003.11.028.

Misra N., and Saxena P. (2009). Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Science 177*:181-189. DOI: https://doi.org/10.1016/j.plantsci.2009.05.007.

Misra N., and Misra R.P. (2012). Salicylic acid changes plant growth parameters and proline metabolism in Rauwolfia serpentina leaves grown under salinity stress. *American-Eurasian Journal of Agricultural and Environmental Science 12*:1601-1609.

Moussa H.R., and Hassan M.A.E.-F. (2016). Growth Enhancers to Mitigate Salinity Stress in Vicia faba. *International Journal of Vegetable Science 22*:243-250. DOI: 10.1080/19315260.2015.1020585.

Muhammad Z., and Hussain F. (2010). Vegetative growth performance of five medicinal plants under NaCl salt stress. *Pak. J. Bot 42*:303-316.

Munns R. (2005). Genes and salt tolerance: bringing them together. *New phytologist 167*:645-663.

Munns R., James R.A., and Läuchli A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany 57*:1025-1043. DOI: 10.1093/jxb/erj100.

Nahar, K., Hasanuzzaman, M., Rahman, A., Alam, M. M., Mahmud, J.-A., Suzuki, T. andFujita, M. (2016). Polyamines Confer Salt Tolerance in Mung Bean (Vigna radiata L.) by Reducing Sodium Uptake, Improving Nutrient Homeostasis, Antioxidant Defense, and Methylglyoxal Detoxification Systems. *Frontiers in plant science* 7.10.3389/fpls.2016.01104

Nazar R., Iqbal N., Syeed S., and Khan N.A. (2011). Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. *Journal of plant physiology 168*:807-815. DOI: https://doi.org/10.1016/j.jplph.2010.11.001.

Nazar R., Umar S., Khan N.A., and Sareer O. (2015). Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. *South African Journal of Botany 98*:84-94. DOI: https://doi.org/10.1016/j.sajb.2015.02.005.

Neelam S., and Subramanyam R. (2013). Alteration of photochemistry and protein degradation of photosystem II from Chlamydomonas reinhardtii under high salt grown cells. *Journal of Photochemistry and Photobiology B: Biology 124*:63-70. DOI: https://doi.org/10.1016/j.jphotobiol.2013.04.007.

Özdemir F., Bor M., Demiral T., and Türkan İ. (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (Oryza sativa L.) under salinity stress. *Plant Growth Regulation 42*:203-211. DOI: 10.1023/b:grow.0000026509.25995.13.

Per T.S., Khan N.A., Reddy P.S., Masood A., Hasanuzzaman M., Khan M.I.R., and Anjum N.A. (2017b). Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. *Plant Physiology and Biochemistry 115*:126-140. DOI: https://doi.org/10.1016/j.plaphy.2017.03.018.

Punia H., Tokas J., Mor V.S., Bhuker A., Malik A., Singh N., Satpal, Alsahli A.A., and Hefft D.I. (2021). Deciphering Reserve Mobilization, Antioxidant Potential, and Expression Analysis of Starch Synthesis in Sorghum Seedlings under Salt Stress. *Plants 10*:2463.

Qados A.M.S.A. (2015) Effects of salicylic acid on growth, yield and chemical contents of pepper (Capsicum annuum L) plants grown under salt stress conditions.

Rasheed F., Anjum N.A., Masood A., Sofo A., and Khan N.A. (2022). The key roles of salicylic acid and sulfur in plant salinity stress tolerance. *Journal of plant growth regulation 41*:1891-1904. DOI: 10.1007/s00344-020-10257-3.

Rasool S., Ahmad A., Siddiqi T.O., and Ahmad P. (2013). Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiologiae Plantarum 35*:1039-1050. DOI: 10.1007/s11738-012-1142-4.

Roshdy A.E.-D., Alebidi A., Almutairi K., Al-Obeed R., and Elsabagh A. (2021). The Effect of Salicylic Acid on the Performances of Salt Stressed Strawberry Plants, Enzymes Activity, and Salt Tolerance Index. *Agronomy 11*:775.

Roussos P.A., Gasparatos D., Tsantili E., and Pontikis C.A. (2007). Mineral nutrition of jojoba explants in vitro under sodium chloride salinity. *Scientia Horticulturae 114*:59-66. DOI: https://doi.org/10.1016/j.scienta.2007.05.001.

Roy S.J., Negrão S., and Tester M. (2014). Salt resistant crop plants. *Current Opinion in Biotechnology 26*:115-124. DOI: https://doi.org/10.1016/j.copbio.2013.12.004.

Sadat-noori S.A., Mottaghi S., and Lotfifar O. (2008). Salinity Tolerance of Maize in Embryo and Adult Stage. *American-Eurasian J. Agric. & Environ. Sci 3* 717-725.

Sakhabutdinova A.R.D., Fatkhutdinova D., Bezrukova M., and Shakirova F.M. (2003). Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol. 29*:314-319.

Santos C.V. (2004). Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Scientia Horticulturae 103*:93-99. DOI: https://doi.org/10.1016/j.scienta.2004.04.009.

Sehar Z., Masood A., and Khan N.A. (2019). Nitric oxide reverses glucose-mediated photosynthetic repression in wheat (Triticum aestivum L.) under salt stress. *Environmental and experimental botany 161*:277-289. DOI: https://doi.org/10.1016/j.envexpbot.2019.01.010.

Shafeiee M., and Ehsanzadeh P. (2019). Physiological and biochemical mechanisms of salinity tolerance in several fennel genotypes: Existence of clearly-expressed genotypic variations. *Industrial Crops and Products 132*:311-318. DOI: https://doi.org/10.1016/j.indcrop.2019.02.042.

Shahbaz M., Abid A., Masood A., and Waraich E.A. (2017). Foliar-applied trehalose modulates growth, mineral nutrition, photosynthetic ability, and oxidative defense system of rice (Oryza sativa L.) under saline stress. *Journal of Plant Nutrition 40*:584-599. DOI: 10.1080/01904167.2016.1263319.

Shahid M.A., Pervez M.A., Balal R.M., Mattson N.S., Rashid A., Ahmad R., Ayyub C.M., and Abbas T. (2011). Brassinosteroid (24-Epibrassinolide) Enhances Growth and Alleviates the Deleterious Effects Induced by Salt Stress in Pea ('Pisum sativum' L.). *Australian Journal of Crop Science 5*:500-510.

Shakeel S., and Mansoo S. (2012). Pretreatment Effect of Salicylic Acid on Protein and Hydrolytic Enzymes in Salt Stressed Mung Bean Seedlings. *asian journal of agricultural sciences 4*:122-125.

Shaw B. (1995). Changes in the levels of photosynthetic pigments in Phaseolus aureus Roxb. exposed to Hg and Cd at two stages of development: a comparative study. *Bulletin of environmental contamination and toxicology 55*.

Siddiqui M.N., Mostofa M.G., Akter M.M., Srivastava A.K., Sayed M.A., Hasan M.S., and Tran L.-S.P. (2017). Impact of salt-induced toxicity on growth and yield-potential of local wheat cultivars: oxidative stress and ion toxicity are among the major determinants of salt-tolerant capacity. *Chemosphere 187*:385-394. DOI: https://doi.org/10.1016/j.chemosphere.2017.08.078.

Silveira J.A.G., Araújo S.A.M., Lima J.P.M.S., and Viégas R.A. (2009). Roots and leaves display contrasting osmotic adjustment mechanisms in response to NaCl-salinity in Atriplex nummularia. *Environmental and experimental botany 66*:1-8. DOI: https://doi.org/10.1016/j.envexpbot.2008.12.015.

Singh A.K., and Singh B.B. (1991). Genotypic Variability in Biomass Protein and Nucleic Acids in Four Linum Species to Sodicity Stress. *Journal of Agronomy and Crop Science 167*:1-7. DOI: https://doi.org/10.1111/j.1439-037X.1991.tb00927.x.

Smirnoff N. (1996). BOTANICAL BRIEFING: The Function and Metabolism of Ascorbic Acid in Plants. *Annals of Botany 78*:661-669. DOI: https://doi.org/10.1006/anbo.1996.0175.

Stavridou E., Hastings A., Webster R.J., and Robson P.R. (2017). The impact of soil salinity on the yield, composition and physiology of the bioenergy grass Miscanthus× giganteus. *Gcb Bioenergy 9*:92-104.

Stevens J., Senaratna T., and Sivasithamparam K. (2006). Salicylic Acid Induces Salinity Tolerance in Tomato (Lycopersicon esculentum cv. Roma): Associated Changes in Gas

Exchange, Water Relations and Membrane Stabilisation. *Plant Growth Regulation 49*:77-83. DOI: 10.1007/s10725-006-0019-1.

Syeed S., Anjum N.A., Nazar R., Iqbal N., Masood A., and Khan N.A. (2011). Salicylic acid-mediated changes in photosynthesis, nutrients content and antioxidant metabolism in two mustard (Brassica juncea L.) cultivars differing in salt tolerance. *Acta Physiologiae Plantarum 33*:877-886. DOI: 10.1007/s11738-010-0614-7.

Szepesi Á. (2006). Salicylic acid improves the acclimation of Lycopersicon esculentum Mill. L. to high salinity by approximating its salt stress response to that of the wild species L. pennellii. *Acta Biologica Szegediensis 50*:177.

Tahjib-Ul-Arif M., Siddiqui M.N., Sohag A.A.M., Sakil M.A., Rahman M.M., Polash M.A.S., Mostofa M.G., and Tran L.-S.P. (2018). Salicylic Acid-Mediated Enhancement of Photosynthesis Attributes and Antioxidant Capacity Contributes to Yield Improvement of Maize Plants Under Salt Stress. *Journal of plant growth regulation 37*:1318-1330. DOI: 10.1007/s00344-018-9867-y.

Talaat N.B. (2021). Polyamine and nitrogen metabolism regulation by melatonin and salicylic acid combined treatment as a repressor for salt toxicity in wheat (Triticum aestivum L.) plants. *Plant Growth Regulation 95*:315-329. DOI: 10.1007/s10725-021-00740-6.

Tanaka R., and Tanaka A. (2011). Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes. *Biochimica et Biophysica Acta (BBA)-Bioenergetics 1807*:968-976.

Tohma O., and Esitken A. (2011). RESPONSE OF SALT STRESSED STRAWBERRY PLANTS TO FOLIAR SALICYLIC ACID PRE-TREATMENTS. *Journal of Plant Nutrition 34*:590-599. DOI: 10.1080/01904167.2011.538285.

Velikova V., Yordanov I., and Edreva A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science 151*:59-66. DOI: https://doi.org/10.1016/S0168-9452(99)00197-1.

Warrence N.J., Bauder J.W., and Pearson K.E. (2002). Basics of salinity and sodicity effects on soil physical properties. *Departement of Land Resources and Environmental Sciences, Montana State University-Bozeman, MT 129*:1-29.

Wasaya A., Affan M., Ahmad Yasir T., Atique-ur-Rehman, Mubeen K., Rehman H.u., Ali M., Nawaz F., Galal A., Iqbal M.A., Islam M.S., El-Sharnouby M., Rahman M.H.u., and EL Sabagh A. (2021). Foliar Potassium Sulfate Application Improved Photosynthetic Characteristics, Water Relations and Seedling Growth of Drought-Stressed Maize. *Atmosphere 12*:663.

Xu J., Yang J., Xu Z., Zhao D., and Hu X. (2020). Exogenous spermine-induced expression of SlSPMS gene improves salinity–alkalinity stress tolerance by regulating the antioxidant enzyme system and ion homeostasis in tomato. *Plant Physiology and Biochemistry 157*:79-92. DOI: https://doi.org/10.1016/j.plaphy.2020.09.033.

Yamaguchi T., and Blumwald E. (2005). Developing salt-tolerant crop plants: challenges and opportunities. *Trends in Plant Science 10*:615-620. DOI: https://doi.org/10.1016/j.tplants.2005.10.002.