



The effect of salinity stress on the *Phaseolus vulgaris* L. plant

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Abstract

Soil salinity is one of the world's most important problems. Many studies have tried to find a solution to this problem. Therefore, this report aimed to determine the tolerance of salt concentrations for pole bean (*Phaseolus vulgaris* L.) plant growth naturally. The current experiment examined the effect of irrigation with saline water on seed germination and seedling growth. Seeds were grown in media containing 0.0, 50, 100, 150, and 200 mM NaCl. Therefore, the number of germinated seeds decreased according to the media concentration. 15-day-old pole bean seedlings were irrigated with saline water at different concentrations (0.0, 50, 100, 150, and 200 mM NaCl). Later, seedlings are watered with tap water for 15 days. After that, the soil and plant shoot samples were collected for analysis purposes. Our results showed that soil pH, EC, and moisture enhanced as salinity levels increased. The responses of pole bean plants to salt stress were documented. Pole bean shoots have gradually increased both soluble carbohydrates and protein content, with the increase of salt levels and free amino acids compared to control. However, photosynthetic pigments and carotenoids of the pole bean leaves were higher in control than under salt concentrations.

Keywords: *Phaseolus vulgaris* L., Salinity stress, Germination

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1. Introduction

Salinity is one type of abiotic stress that is defined as excessive levels of soluble salts in the soil solution (Tang *et al.*, 2015 and Yadav *et al.*, 2020). Salinity spreads in arid and semi-arid regions around the world, where degraded land and a scarcity of water exist (Geissler *et al.*, 2010; and Velmurugan *et al.*, 2020). In the soil, salts occur in the form of ions (Shrivastava and Kumar, 2015; and Velmurugan *et al.*, 2020). Rengasamy (2006) and Cuevas *et al.* (2019) mentioned in the reports that the salinization of soil is normally classified as "primary" resulting from natural and "secondary" salinity caused by human activities. Soil is complex and evolves continuously with other environmental components that are sensitive to human activities and changes in climate (Smith *et al.*, 2012; and Arora *et al.*, 2017). The continuous experience in the saline environment would delay plant growth, cause senescence, and end with death (Zhu, 2007; and Miryeganeh, 2021). Increased levels of sodium chloride result in hyperosmotic (Munns, 2005; and Ma *et al.*, 2020) and hypertonic

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(Paranychianakis and Chartzoulakis, 2005; and Almeida *et al.*, 2017) stresses before declining plant growth, plant metabolism, and physiological processes cause a decrease in plant development and then productivity (Rahnama *et al.*, 2010; and Otlewska *et al.*, 2020).

The pole bean plant is considered the most edible vegetable crop (Kumar *et al.*, 2021). According to Gentry (1969), a plant would be described as either an annual or a short-time-life cycle crop. The plant has a high nutrient value for human consumption. It provides carbohydrates, proteins, vitamins, and minerals such as phosphorus, potassium, magnesium, and calcium to our body (Başdinç and Çirka, 2021).

2. Materials and methods

2.1. Germination test

Pole bean plant seeds were purchased from the Alyaseen Agri Company, Qassim district, Saudi Arabia. Ten similar-sized seeds were put in a petri dish (9 cm in diameter) with two layers of filter paper (Whatman No.1). These papers were wetted with 10 ml of different saline solutions (0.0, 50, 100, 150, and 200 mM of NaCl) by the method of Mena *et al.* (2015). Each concentration had three replicates (10 seeds per replicate) and was kept in the dark at 25°C. In order to avoid salt accumulation, the filter papers must be changed every two days (Rahman *et al.*, 2008). The germination of seeds test lasted for five days, with a daily check starting to count the germinated seeds on the next day (Abiri *et al.*, 2016). The germinated seeds were counted when the emergence of radicle was 2 mm (Mena *et al.*, 2015) or any appearance of "radicle protrusion" (Bahrami and Razmjoo, 2012). Then the final-germination percentage (FG%) was calculated based on the following equation created by Nasri *et al.* (2011):

$$FG \% = 100 \times (\text{total germinated seeds} / \text{total number of seeds})$$

2.2. Experiment design

Pole bean plant seeds have been sown on May 17, 2021 under normal conditions in plastic pots which (26 × 24 cm) have holes in the bottoms, allowing the extra water to be excluded. These pots contained homogeneously mixed sand and meat moss (2:1 v/v). Plants were irrigated with tap water three times a week until the emergence of the second real leaf (after 15 days), El-Fouly, and El-Nour (2021), when two healthy selected seedlings had been chosen to complete the experiment. Then five different concentrations of salt (0.0, 50, 100, 150, 200 mM of NaCl) were applied as described by Mena *et al.* (2015). After approximately 15 days of saline treatment, soil and plant samples were collected for analysis purposes (Azimychetabi and Sabokdast, 2020).

2.3. Chemical analyses of soil

2.3.1. Soil pH and soil electrical conductivity

1:5 weight-to-volume (EC1:5 w/v) method: (1:5) soil to water suspensions were prepared by adding 250 ml of distilled water to 50 g of oven-dried soil sample. The containers, including suspensions, were shaken overnight and then filtered using filter paper to obtain the extracts.

2.3.2. Soil pH

A pH meter was used to gauge the level of pH of the solution according to Conklin (2005).

2.3.3. Soil Electrical Conductivity (EC)

An EC meter was utilized to evaluate the electrical conductivity ($\mu\text{dS/m}$) for soil extracts following the method of Page *et al.* (1982).

2.3.4. Soil moisture content (SMC)

Soil water content for each sample was measured according to the method provided by Conklin (2005) and Yousef (1999). 50 g of soil sample was weighted as wet weight. After that, the sample was placed in an air-drying oven at 105°C for 24 h to record its dry weight. The following formula was used to calculate the amount of water in the soil sample:

$$SMC \% = [(Wet\ soil\ (g) - Dry\ soil\ (g)) / (Wet\ soil\ (g))] \times 100$$

2.4. Biochemical analysis

2.4.1. Determination of chlorophylls and carotenoid contents

Fresh leaves were collected from all tested pots to determine the content of chlorophylls (*a* and *b*) and carotenoids after 12 days of treatment and then washed with deionized water. Following the method of Metzner *et al.* (1965), 0.5 g of a clean, green, and fresh leaf was ground with 10 ml of 85% acetone for 5 min at room temperature. This solute was filtered and collected in a flask (70 ml volume) to complete the volume until 50 ml with acetone (85%). Later, three replicates for each sample were taken to measure the absorbance of the concentrations of chlorophyll *a*, chlorophyll *b*, and carotenoids at wavelengths (663, 644, and 440 nm) against a blank (containing pure 85% of acetone) by a spectrophotometer machine. The concentrations of chlorophyll *a* (mg·g⁻¹ fresh weight), chlorophyll *b* (mg·g⁻¹ fresh weight), and carotenoids (mg·g⁻¹ fresh weight) were calculated by using the following (Metzner *et al.*, 1965) equations:

$$\text{Chlorophyll } a = 10.3 E_{663} - 0.918 E_{644}$$

$$\text{Chlorophyll } b = 19.7 E_{644} - 3.87 E_{663}$$

$$\text{Carotenoids} = 4.2 E_{440} - [(0.0264 \text{ Chl } a + 0.426 \text{ Chl } b)]$$

2.4.2. Determination of soluble and total carbohydrates

The colorimetric anthrone method (Fales, 1951; and Schlegel, 1956) was used for the determination of carbohydrates in shoot samples. In a test tube, 0.2 ml of the extracted sample was placed, and 4.5 ml of anthrone reagent was added. This tube was mixed gently and boiled in a water bath for 7 min at 100°C, then left to cool for 5 min. The absorbance was determined at 620 nm by a spectrophotometer against a blank containing only water and anthrone reagent (Lowry *et al.*, 1951).

2.4.3. Determination of soluble and total proteins

The soluble and total protein content in the shoot extraction was estimated according to Lowry *et al.* (1951). 5 ml of the alkaline reagent solution was added to 1 ml of the extracted sample in a test tube. After mixing, the tube was left for 10 min at room temperature to stand. After that, 0.5 ml of Folin-Ciocalteu's reagent was added, mixed, and placed in the dark for 30 min. Then the absorbance was measured by a spectrophotometer at 750 nm against a blank.

2.4.4. Determination of free amino acid

The free amino acid content in the shoots' extraction was estimated according to the method of Moore and Stein (1948). 0.5 ml of the water extract plant samples were mixed in a test tube with 1 ml of stannous chloride reagent. The test tube was placed in a water bath for 20 min to boil and then cooled. Later, 4 ml of diluent solvent was added and mixed. Then the absorbance was read by a spectrophotometer at 570 nm against a blank.

2.4.5. Statistical analyses

Data were analyzed using a sigma stat (3.5) with Tukey test ($p < 0.05$). Correlation analysis using Spearman Rank Order Correlation in sigma stat (3.5) was conducted to determine the relationship between the measurement parameters.

3. Results

3.1. Seed germination percentage

In this study, the results showed that there was a significant decrease in seeds germination under different treatments of NaCl. All seeds germinated under control (100%), whereas, the germination percentage was decreased significantly by 97, 90, 87, 83 %, respectively at 50, 100, 150, and 200 mM NaCl, (Table 1).

Table 1: Impact of different concentrations of NaCl on the percentage of seeds germination in *Phaseolus vulgaris* L.

NaCl (mM)	Seed Germination (%)
0.0	100 ± 11.01 ^a
50	97 ± 11.64 ^b
100	90 ± 10.05 ^c
150	87 ± 10.44 ^d
200	83 ± 9.13 ^e

Note: Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant ($p > 0.05$), whereas others with different superscript letters are significant ($p < 0.05$).

3.2. Soil analysis

Soil pH values have directly affected plant growth under various NaCl concentrations. Data in Table (2) showed that soil pH values were not significantly influenced by different concentrations of NaCl. The soil pH values were 7.63, 7.78, 7.77, and 7.91 at (50, 100, 150, and 200 mM NaCl) respectively, as compared to the control (7.45). Moreover, this study showed that soil electrical conductivity has significantly increased under high concentrations of NaCl. The soil electrical conductivity was 662.33, 713.33, 951.66, 1181.66, and 1357.0 $\mu\text{S}/\text{m}$, respectively, at 0.0, 50, 100, 150, and 200 mM NaCl (Table 2).

Table 2: Impact of different concentrations of NaCl on soil pH and soil electrical conductivity in *Phaseolus vulgaris* L.

NaCl (mM)	Soil pH	Soil EC ($\mu\text{S}/\text{m}$)
0.0	7.45 ± 0.44 ^d	662.33 ± 78.02 ^e
50	7.63 ± 0.41 ^c	713.33 ± 102.25 ^d
100	7.78 ± 0.53 ^b	951.66 ± 65.14 ^c
150	7.77 ± 0.51 ^b	1181.66 ± 26.54 ^b
200	7.91 ± 0.60 ^a	1357.05 ± 235.75 ^a

Note: Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant ($p > 0.05$), whereas others with different superscript letters are significant ($p < 0.05$).

3.3. Soil moisture

The soil moisture has been affected by the addition of high concentrations of NaCl as compared with the control (19.35%), whereas, these values are 23.35, 24.84, 24.86, and 26.21% at 50, 100, 150 and 200 mM NaCl respectively as shown in Table 3.

Table 3: Impact of different concentrations of NaCl on soil moisture in *Phaseolus vulgaris* L.

NaCl (mM)	Soil moisture (%)
0.0	19.35 ± 1.33 ^d
50	23.35 ± 2.18 ^c
100	24.84 ± 2.16 ^b
150	24.86 ± 2.10 ^b

Table 3 (cont.)	
NaCl (mM)	Soil moisture (%)
200	26.21 ± 2.08 ^a

Note: Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant ($p > 0.05$), whereas others with different superscript letters are significant ($p < 0.05$).

3.4. Photosynthetic pigments (chlorophyll a and b) and carotenoid contents

In our study, we found that all different concentrations of NaCl significantly decreased the chlorophyll (a and b) and carotenoids content as compared to the control plants, as shown in Table 4.

Table 4: Impact of different concentrations of NaCl on chlorophylls (a and b) and carotenoids in <i>Phaseolus vulgaris</i> L.			
NaCl(mM)	Chlorophyll (a)(mg·g ⁻¹ fresh weight)	Chlorophyll (b)(mg·g ⁻¹ fresh weight)	Carotenoids(mg·g ⁻¹ fresh weight)
0.0	9.78 ± 1.24 ^a	3.28 ± 0.36 ^a	6.32 ± 0.75 ^a
50	6.92 ± 0.22 ^b	2.24 ± .004 ^b	4.59 ± 0.11 ^b
100	5.37 ± 0.63 ^c	1.66 ± 0.23 ^d	3.69 ± 0.43 ^c
150	5.26 ± 0.46 ^c	1.63 ± 0.17 ^d	3.66 ± 0.29 ^c
200	5.26 ± 0.60 ^c	1.81 ± 0.24 ^c	3.86 ± 0.39 ^c

Note: Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant ($p > 0.05$), whereas others with different superscript letters are significant ($p < 0.05$).

3.5. Soluble and total carbohydrate contents

The results in Table (5) showed that soluble carbohydrates increased under NaCl stress. The maximum soluble carbohydrate content was observed at 50 mM NaCl at 280.65 mg/g DW. Although the soluble carbohydrates decreased with the increase of NaCl levels, which were (237.06, 210.59, and 206.19 mg/g DW) at 100, 150, and 200 mM NaCl, respectively, which is significantly higher as compared with the control plants (195.47 mg/g DW). Regarding total carbohydrate, our data represented a reduction in the total carbohydrate level under saline conditions (331.30, 288.40, and 262.65 mg/g DW) at 50, 100, and 150 mM NaCl, respectively, which is significantly lower as compared with the control plants (351.86 mg/g DW). The minimum reduction in the total carbohydrate level was recorded at 200 mM NaCl (224.68 mg/g DW) as shown in Table (5).

Table 5: Impact of different concentrations of NaCl on total and soluble carbohydrate contents in <i>Phaseolus vulgaris</i> L.		
NaCl(mM)	Total carbohydrates(mg·g ⁻¹ Dry weight)	Soluble carbohydrates(mg·g ⁻¹ Dry weight)
0.0	351.86 ± 35.2 ^a	195.47 ± 17.5 ^d
50	331.30 ± 33.8 ^b	280.65 ± 19.8 ^a
100	288.40 ± 26.1 ^c	237.06 ± 18.1 ^b
150	262.65 ± 21.7 ^d	210.59 ± 20.2 ^c
200	224.68 ± 20.2 ^e	206.19 ± 18.6 ^c

Note: Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant ($p > 0.05$), whereas others with different superscript letters are significant ($p < 0.05$).

3.6. Soluble and total protein contents

In the present investigation, the content of soluble protein affected obviously related to NaCl treatments. The soluble protein values were higher under 50 and 100 mM NaCl by 98.43 and 98.91 mg/g DW, respectively, as compared with the control plants (90.11 mg/g DW). However, the soluble protein dropped remarkably more at 150 mM NaCl by 79.76 mg/g DW and at 200 mM NaCl by 88.65 mg/g DW. Related to total protein, the highest value was noted at 50 mM NaCl (145.92 mg/g DW). Whereas, the lowest values were recorded at both 200 and 100 mM NaCl by 118.38 and 117.03 mg/g DW, respectively. The total protein level under treatments with 150 mM NaCl has no significant value as compared to the control plants (126.88 and 127.83 mg/g DW) as shown in Table (6).

NaCl (mM)	Total protein (mg.g ⁻¹ Dry weight)	Soluble protein (mg.g ⁻¹ Dry weight)
0.0	127.83 ± 6.35 ^b	90.11 ± 8.10 ^b
50	145.92 ± 8.71 ^a	98.43 ± 7.87 ^a
100	117.03 ± 9.05 ^c	98.91 ± 7.78 ^a
150	126.88 ± 7.56 ^b	79.76 ± 6.85 ^c
200	118.38 ± 8.93 ^c	88.65 ± 6.21 ^b

Note: Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant ($p > 0.05$), whereas others with different superscript letters are significant ($p < 0.05$).

3.7. Free amino acid content

The results are shown in Table (7) revealed that there is a significant variation in the accumulation of free amino acid in various treatments with NaCl. Free amino acid content increased obviously at 50 and 100 mM NaCl by 47.86 and 39.95 mg/g DW as compared to control samples (33.86 mg/g DW). However, the free amino acid amount decreased by 22.76 mg/g DW when plants were treated with 150 mM NaCl and then decreased sharply when plants were treated with 200 mM NaCl by 17.87 mg/g DW when compared to the control plants (33.86 mg/g DW).

NaCl (mM)	Free amino acid(mg.g ⁻¹ Dry weight)
0.0	33.86 ± 1.69 ^c
50	47.86 ± 4.11 ^a
100	39.95 ± 2.78 ^b
150	22.76 ± 1.35 ^d
200	17.87 ± 1.45 ^e

Note: Values are represented as mean ± SD of 3 replicates. Means marked with the same superscript letters are not-significant ($p > 0.05$), whereas others with different superscript letters are significant ($p < 0.05$).

4. Discussion

Salinity stress is the most important abiotic stress that has adverse effects on soil properties and plant growth. This current study was on pole beans, which is one of the most important plants in Saudi Arabia (Purushothaman *et al.*, 2018). According to the recent results (Table 1), the final germination percentage gradually decreased at enhanced salinity. Similar findings have been supported our results in many crops (Siddiqui *et al.*, 2013; Nyagah and Musyimi, 2009; Almodares *et al.*, 2007; and Tobe *et al.*, 2004). Also, this results in agreement with

the data of Khodarahmpour *et al.* (2012) who reported that the growth percentage of eight hybrids of maize reduced under different salt concentrations. Our data showed a significant reduction in seeds germinated under 200 mM NaCl by 83% as compared to all saline treatments. Supporting our result, Al Hassan *et al.* (2017) reported that, the germination of *L. narbonense* seeds obviously reduced at 300 mM NaCl. In high concentrations of salt, the germination was affected by enhancing ABA amounts and decreasing the amounts of seed germination stimulants such as GAs, and influence membrane permeability, which is consistent with Uçarl (2020) study in crops plants who reported that high concentrations of salt showed a significant decrease in seed germination. Moreover, Kaveh *et al.* (2011) mentioned that the reason behind delaying the seed germination percentage under saline conditions could be the ions toxicity uptake affecting embryo protoplasm (Datta *et al.*, 2009). The toxicity of the saline environment causes hinders in the amount of water available to seed germination (Jamil *et al.*, 2005) by an osmotic barrier (Neamatollahi *et al.*, 2009).

Our data indicated (Table 2) that there was a correlation between the soil pH and soil EC under different salinity levels compared to control soil. Soil pH, which is a soil chemical property, is an important measurement to indicate whether the soil is alkalinity or acidity type. Soil pH acts as an indicator to determine the essential nutrients available for plants' development and growth. Soil pH in this study was slightly alkaline at 7.65–7.91, similar to what Kaur *et al.* (2021) pronounce. Soil pH was a little higher compared to control as salinity increased, which is in agreement with Ghazali *et al.* (2020). Moreover, Mori *et al.* (2011) reported the same result by noticing a slight enhancement in the soil pH of the snap bean after applying different concentrations of salinity. Also, our data agreed with Selvakumar *et al.* (2018) study, who noticed a slight increase in the soil pH under different salt levels on snap bean (*Phaseolus vulgaris*). Garcia *et al.* (2019) explained that the cause behind this enhancement of soil pH under salt was the existence of sodium ions in the soil. Soil electrical conductivity is a measure of the number of salts in soil (Kaur *et al.*, 2021). Regarding soil tests, soil EC might be used as an important indicator of plant nutrient availability (Sahu *et al.*, 2016). Moreover, Abbas *et al.* (2018) and Yang *et al.* (2020) studies agreed with our findings of the increase in the values of soil pH and soil EC under saline stress. The gradual increment of the soil EC amount may be related to the gradual high applied salt concentrations. However, a few studies have shown the adverse influence of salinity on pH and EC under different concentrations of salts (Cristaldi *et al.*, 2020).

Soil moisture measurement is important to identify the soil water content. Soil moisture is considered as a transporter of materials to plant (Li *et al.*, 2018). Our data indicated (Table 3) that soil moisture reached high levels under saline treatments compared to the control. Our results agreed with many reports. Yuan *et al.* (2019) pronounced that the rate of soil water content (soil moisture) increased with increasing salinity levels. This phenomenon, as a result of the presence of salt ions derived from saline irrigation water, could prevent plants from absorbing water. An increase in the soil water content under salinity may be as a result of the high levels of Na⁺ and Cl⁻ which prevent plant water uptake from the soil. Taghizadehghasab *et al.* (2021) mentioned that soil water content is raised when the soil depth increases under saline conditions.

Salinity alters plant growth, physiology, and metabolism in crop plants, as reported in a study by Isayenkov and Maathuis (2019). In the present study, the photosynthetic pigments and carotenoids of the pole bean plant were significantly reduced under all salt levels (Table 4). Mazumdar *et al.* (2019), Sharma *et al.* (2013), and Alharby *et al.* (2019a) reported the same results. Also, Ali *et al.* (2008) pronounced that high saline media has negative effects on the chlorophyll content in Brassica juncea. Furthermore, Sadak (2016a) noticed that the saline treatment had reduced the chlorophyll levels but enhanced the synthesis of chlorophyll a, leading to increased levels of chlorophyll and less chlorophyll b. This may be as a result of the degeneration and transformation of chlorophyll b into chlorophyll a (Loggini *et al.*, 1999). Some studies showed that there were no changes in total chlorophyll as well as carotenoid content (Cocetta *et al.*, 2018; and Barbieri *et al.*, 2011). Rahneshan *et al.* (2018) stated that saline conditions affected the level of carotenoids slightly compared to photosynthetic pigments. Whereas, according to Karimi *et al.* (2015), safflower plants elevated the carotenoids content, which could be a part of the plant's defence system during salinity stress. Rady *et al.* (2015) mentioned that the reduction of chlorophyll a, b, and carotenoids is probably due to the oxidation process, which degrades these photosynthetic pigments and damages the biosynthesis of plant pigments. Sadak and Ahmed (2016) explain that plants under salinity stress can highly synthesise the chlorophyllase enzyme, resulting in chlorophyll destruction. Growth parameters decreased at different concentrations of salt. In addition, salt stress perturbed the energy migration of chlorophyll complexes into the reaction centers and maybe the cause of the decrease in photosynthetic pigments. Paunov *et al.* (2018) recorded similar results in wheat growth.

Carbohydrates are considered osmolytes, having the ability to regulate physiological functions and maintain the balance of internal cells under salt stress (Kaur *et al.*, 2015). In the present work (Table 5), the data presents the incensement of soluble carbohydrates when the plant experiences salinity compared to the non-tested group. Many reports are in agreement with our results. The highest value of soluble carbohydrate was at 50 mM (280.65 mg/gDW) and then started to decrease until the 200 mM NaCl concentration (206.19 mg/gDW). This data contradicted the findings of Karimi *et al.* (2005), who reported an increase in soluble carbohydrates under 200 mM of salt. However, our result is in agreement with Abid *et al.* (2020), who found a decrease in soluble sugars in some kiwifruit germplasm under high salt. According to our study, high salt concentration causes all plant growth to be negatively affected, including all metabolism. This may be due to the ionic harmful imbalance and reduction in the internal osmotic cellular volume. Plants can tolerate salinity stress at certain concentrations by the accumulation of soluble and/or total carbohydrates depending on the plant's species. Ahanger *et al.* (2019) and El-Badri *et al.* (2021) reported that the solubility of carbohydrates increased when the salinity level increased. Furthermore, Zheng *et al.* (2008) and Kerepesi and Galiba (2000) found a significant increase in soluble carbohydrates in salt-tolerant and salt-sensitive wheat (*Triticum aestivum* L.). During saline stress, plants are able to increase the soluble sugar content (nonstructural carbohydrates) as an adaptive and protective property (Bartels and Sunkar, 2005). In this current study, the total carbohydrate decreased as salinity increased compared to control. It is clear that the total carbohydrate content was higher at 50 mM NaCl (331.309 mg/gDW). Whereas total carbohydrate content dropped at 150 and 200 mM NaCl by 262.65 and 224.68 mg/gDW, respectively, which is agreed with Gowayed and Abd El-Moneim (2021) results, who stated that total carbohydrate content has not changed at 150 mM NaCl. Moreover, Ali *et al.* (2021) recorded a reduction in the total carbohydrate in the saline condition, which agrees with our results. As the total carbohydrate is composed of soluble and insoluble carbohydrates, their content would be different under abiotic stresses. So, Parvaiz and Satyawati (2008) mentioned the role of non-soluble sugar deterioration in the synthesis of soluble sugars aiming to reduce the dehydration of cells. Plants species under various environmental stresses could tolerate them differently by either increasing or decreasing the soluble and insoluble carbohydrates.

According to our results (Table 6), the content of soluble protein raised and then dropped when the salinity level increased compared to control. Our findings showed that a significant decrease in protein solubility usually increases in the presence of high salt content at 150 and 200 mM NaCl by 79.76 and 88.65 mg/gDW, respectively. In agreement with our results, the solubility of the proteins decreased; however, proline increased (Naliwajski and Skłodowska, 2021). Moreover, under abiotic stress such as salinity, Ramezani *et al.* (2011) agreed with our data and interpreted that the decline of soluble protein content was accompanied by an accumulation of nitrogenous amino acids and free proline in the cytoplasm, thus playing an important role in the osmotic balance of plants. Also, Cherian and Reddy (2003) explained that high levels of soluble protein under abiotic stresses may promote the synthesis of other proteins. Gadallah (1999) explains that the reason behind the increment of soluble proteins is the degradation of some structural proteins. Also, Bano *et al.* (2021) reported that soluble protein levels declined under saline conditions, especially at 800 mM, which is similar to our result.

The total protein was higher at 50 and 150 mM NaCl and decreased at 200 mM NaCl (145.92, 126.88, and 118.38 mg/g DW) respectively. This is in contrast to Bano *et al.* (2021), who noticed an increase in the total protein under high saline conditions. However, the same results were documented in Kumar *et al.* (2010) study, who noticed the rise of total protein content increased under saline stress, but this content decreased under high levels of salt, which agreed with our study. Total protein consists of many types of protein, including soluble types. The plant might tolerate saline stress at a specific level by transforming more soluble protein from total protein.

The data in Table (7) shows that the free amino acid content of pole bean shoots was obviously increased by irrigation of plants with different concentrations of salt. In our results, we showed an obvious increase in the free amino acid content under both 50 and 100 mM NaCl by 47.56 and 39.95 mg/g DW, over control, with many studies. Sadak (2019) documented an increase in the level of free amino acids in wheat leaf plants under salinity control. Mansour (2000) reported similar findings. Sreelakshmy *et al.* (2021) mentioned that the reason behind the increase in free amino acid content in tomatoes (*Solanum lycopersicum*) treated with saline water was protein hydrolysis. Some reporters explained that the main reason for increasing free amino acid under saline conditions is that plants utilise free amino acid as a substitutional substance in mitochondrial respiration

as carbohydrate and chlorophyll decrease (Hildebrandt, 2018; Hildebrandt *et al.*, 2015; and Araújo *et al.*, 2011). Under high salinity levels, at 150 and 200 mM NaCl, the amount of free amino acid dropped significantly by 22.76 and 17.87 mg/g DW compared to control plants. Our results agreed with the discussion of Roychoudhury *et al.* (2021) and Shaddad *et al.* (2010) who indicated that the free amino acid content decreased under saline conditions in order to produce new proteins.

El-Badri *et al.* (2021) explained that the reduction of the content of free amino acids in (*Brassica* species) grown in saline media might be due to the accumulation of amino acids derivatives (compatible osmolytes) such as proline (Szabados *et al.*, 2011). Proline might contribute to the protection of plant cells by reducing the harmful effects of reactive oxygen species and maintaining the structure of cell membranes and functions of proteins and enzymes (Hayat *et al.*, 2012). Some articles mentioned that proline could act as protein under some stress conditions (Strizhov *et al.*, 1997).

5. Conclusion

Pole beans (*Phaseolus vulgaris* L.) are considered one of the most consumed global crops and have economic importance due to their health benefits. Pole beans were exposed to different concentrations of NaCl during this study.

As pole beans had been irrigated once only, the results showed different responses to all NaCl treatments. Although the salinity inhibited the physiological processes of pole beans, pole beans proved their ability to tolerate and grow under 200 mM of NaCl.

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