



# Physiological and biochemical responses of two faba bean (*Vicia faba* L.) varieties grown in vitro to salt stress

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## Abstract

Faba bean (*Vicia faba* L.) is one of the most important legume crops worldwide. High salinity is a major constraint for faba bean productivity in many countries, including Egypt. Here, we examined the effects of salinity-induced toxicity on the growth of two local faba bean genotypes by analyzing physiological and biochemical responses to identify the salt-tolerant attributes between the genotypes. In vitro experiments were carried out to characterize the response of two faba bean genotypes (Sakha 3 and Nubaria 2) to salinity imposed by different sea-salt concentrations (1000, 3000, 5000 and 7000 ppm). For both genotypes, salinity induced a marked reduction in dry matter gain along with a reduction in shoots height, roots length, leaves number and branches number. In addition, the photosynthetic pigments (chlorophyll a, b) were significantly decreased with the increase in salinity. Changes in tissue ion levels, peroxidase (POD) and polyphenol oxidase (PPO) activities depended on genotype, tissue and salinity level. The deteriorating effect of salt stress on the growth performance of genotype Nubaria 2 was lower than that of Sakha 3. This is maybe ascribed to its better antioxidant enzymes activities. Moreover, Nubaria 2 accumulated low quantities of Na<sup>+</sup> in the shoots with a higher accumulation of ions in the roots compared to Sakha 3. The obtained results suggested Nubaria 2 seedlings have a strong ability to sustain sea-salt stress by the regulation of transport and distribution of ions and this genotype may be characterized as a salt excluder.

**Keywords** Antioxidants · Chlorophyll · Ion accumulation · Salt stress · *Vicia faba* L.

## Introduction

Environmental abiotic stress such as salinity and drought imposed undesirable effects on plant growth and productivity in many parts of the world specifically in arid and semi-arid regions. With climate change on the way, the problems will dramatically increase and salt stress becoming more severe as agriculture is intensified. Consequently, salinity may lead to increased pressure on food availability worldwide. The adverse effect of salinity on plants is a combination of ion

toxicity, osmotic stress and production of reactive oxygen species (ROS) in plant cells which lead to damage lipids, proteins and DNA (Yasar et al. 2006). The negative effect of salinity on plant growth has been also attributed to physiological disorder, such as severe ion imbalance and inhibition of enzyme activities; particularly those involved in the defense against oxidative stress (Türkan and Demiral 2009). High extracellular NaCl concentration is also well known to inhibit the expression of genes encoding carboxylation enzymes such as rubisco (Koch 1996) that could be the reason for reduced plant development. Studying the response of crops to salinity and developing crops with elevated salt stress tolerance are long-held objectives of many research groups worldwide to expand the use of the saline area for cultivation. Therefore, understanding the mechanisms of salt-tolerance is imperative for crop improvement (Mahl-ooggi et al. 2018).

Plants react to changes in their environment by an array of biochemical and physiological alterations to cope with these challenges. The mechanisms of stress tolerance may vary from one plant species to another and even at different

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developmental stages (Foolad and Lin 2001). For example, the plants developed an antioxidant system such as catalase (CAT), ascorbic acid (AsA), glutathione reductase (GR) and superoxide dismutase (SOD) to repair the oxidative damage to macromolecules (Ahmad et al. 2010).

Faba bean susceptibility to biotic and abiotic stresses is a major constraint to yield (Fouad et al. 2013). Genetic variation in salinity tolerance has been reported in many crops such as rice (Yeo and Flowers 1983), barley (Epstein et al. 1980), wheat (Munns et al. 2000; Munns and James 2003) and alfalfa (Noble et al. 1984). However, little information is available regarding the genetic variability of salt tolerance in faba bean. The available data indicate that the source of salinity tolerance in this species may be more limited than in other crops (Cordovilla et al. 1999). In literature, there are many reports dealing with the evaluation of the salt tolerance of faba bean. The response of four Egyptian faba bean genotypes (Misr 1, Giza 843, Giza 429 and Giza 3) was investigated (Abdelhamid et al. 2010). They reported relative high salt tolerance of Giza 429, Misr 1 and Giza 843 genotypes and could be watered with 100 mM NaCl solution. They ascribed their results to the high degree of physiological tolerance characterizing these genotypes through creating osmotic adjustment by accumulation more quantities of N, P, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and lower quantities of Na<sup>+</sup> and Cl<sup>-</sup>, as well as higher K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratios.

In this connection, previous reports showed that salt stress activated the antioxidant enzymes such as superoxide dismutase (SOD) and polyphenol oxidase (PPO) in leaves, shoots and roots of faba bean (Adss and Eldakrory 2015; Anaya et al. 2017). Salt tolerant peas showed also a close correlation with antioxidant capacity (Hernandez et al. 2000). Salt stressed green bean (*Phaseolus vulgaris* L.) decreased the accumulation of the toxic ions in plant shoot to tolerate the adverse effect of salt stress (Assimakopoulou et al. 2015). Consequently, the purpose of the present work is to study the effect of different sea-salt concentrations on several plant growth parameters, ion contents, chlorophyll contents, peroxidase and polyphenol oxidase activities in shoots and roots of two commercial faba bean genotypes, widely distributed in Egypt to depict the response of these genotypes to salt stress.

## Materials and methods

### Plant material

Two genotypes, Sakha 3 and Nubaria 2 commonly cultivated in Egypt, were selected for this study based on our previous study that showed their low content of phenolic compounds with high in vitro regeneration ability (Hanafy Ahmed et al.

2020). The seeds were obtained from Agriculture Research Center, Egypt.

### Plant growth and treatments

Uniform seeds were surface sterilized and then sown on MS—basal medium (Murashige and Skoog 1962) supplemented with different concentrations (1000, 3000, 5000 and 7000 ppm) of sea-salt (Sigma-Aldrich, S9883) for six weeks. The response of the two genotypes to salt stress was investigated under in vitro culture condition. The vegetative growth of faba bean plants was characterized by measurement of shoot height, root length and counting the number of leaves and branches after six weeks of seed sowing. For the determination of total fresh and dry weights, five plants of each genotype from each treatment were harvested and weighted to determine the total fresh weight. These plants were then oven-dried at 65 °C for 48 h to record total plant dry weight. The fresh shoots and root samples were kept at -20 °C for further analyses. To determine the relative-salt stress tolerance of the plants, the tolerance indices were calculated by dividing the individual plant dry weights by the mean value of their corresponding controls. Tolerance indices (Ti) of faba plants to sea-salt stress were determined according to Shetty et al. (1995) as follows:

$$\text{Tolerance index} = \frac{\text{Total dry weight of salt-treated plants}}{\text{total dry weight of control plants}} \times 100.$$

### Chlorophyll determination

The contents of chlorophyll (Chl *a*, Chl *b*), and total Chl (*a* + *b*) were determined spectrophotometry as described earlier (Arnon 1949). The extraction of chlorophyll from the leaves was done according to Horborne (1973). Briefly, one gram of finely cut fresh shoots was homogenized in 20 ml of 80% acetone. Then, the homogenate was centrifuged at 5000 rpm for 5 min. The supernatant was transferred to a new tube and the procedure was repeated till the supernatant became clear. The estimation of chlorophyll was done by measuring the OD of the solution at 645 nm and 663 nm in a UV–VIS spectrophotometer and calculated using the formula given by Arnon (1949).

### Tissue ion accumulation

Oven (80 °C) dried samples (100 mg) were extracted in 50 ml deionized water with continuous shaking and used for the determination of tissue ion percentage on a microprocessor-based Ion Analyzer (Elico, India) using ion specific electrode to sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), Magnesium (Mg<sup>2+</sup>) and chloride (Cl<sup>-</sup>) according to Faithfull (2002).

## Activity of antioxidant enzymes

Enzymes were extracted from fresh leaves (0.5 g) using a mortar and pestle with 10 ml of ice-cold extraction buffer containing 50 mM phosphate buffer (pH 7.8), 1 mM Na<sub>2</sub>-EDTA and 2% polyvinylpyrrolidone (PVP). The homogenate was filtered through 4 layers of cheese cloth and centrifuged at 5000 xg for 10 min at 4°C (Zhang et al. 2007). The supernatant was collected to assay the activity of peroxidase (POD) and polyphenoloxidase (PPO) spectrophotometrically. POD activity was assayed in a reaction mixture containing 100 mM sodium phosphate buffer (pH 5.8), 7.2 mM guaiacol, 11.8 mM H<sub>2</sub>O<sub>2</sub> and 100 µl enzyme extract. The activity of POD was assayed according to Kato and Shimizu (1987). Samples of 3 ml of the reaction mixture containing 100 mM sodium phosphate buffer (pH 5.8), 7.2 mM guaiacol, 11.8 mM H<sub>2</sub>O<sub>2</sub> and 100 µl enzyme extract was used for the assay. The absorbance was measured at 470 nm and the change in O.D. was recorded at 30 s intervals up to 3 min and the averages were calculated. POD activity was expressed as  $\Delta_{470} \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$ . PPO activity was determined according to Mayer et al. (1966). The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 100 mM sodium phosphate buffer (pH 6.5). The reaction was started by the addition of 200 µl of 100 mM catechol. The change in the absorbance was measured at 490 nm and the change in O.D. was recorded at 30 s interval up to 3 min. The average of reading was calculated. PPO activity was expressed as  $\Delta_{490} \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$ .

## Statistical analysis

All of the experiments were conducted in at least triplicate and the results were tabulated as mean  $\pm$  standard error (SE). Data were analyzed by analysis of variance (ANOVA). The means were compared using the least significant difference (LSD) test at the 0.05 probability level.

## Results and discussions

### Plant growth

Data recorded in Fig. 1, Table S1 and Table S2 show the effect of different levels of sea-salt stress (1000, 3000, 5000 and 7000 ppm) on the growth of two faba bean genotypes (Sakha 3 and Nubaria 2). Shoot height (SH), root length (RL), leaves number (LN), branches number (BN), total fresh weight (FW), total dry weight (DW) and salt-tolerance indexes (Ti) of faba plants were recorded after sowing faba bean seeds in vitro for 6 weeks. In general, the obtained data reveal that the growth performance of faba bean genotypes were significantly affected, depending on the level of sea-salt

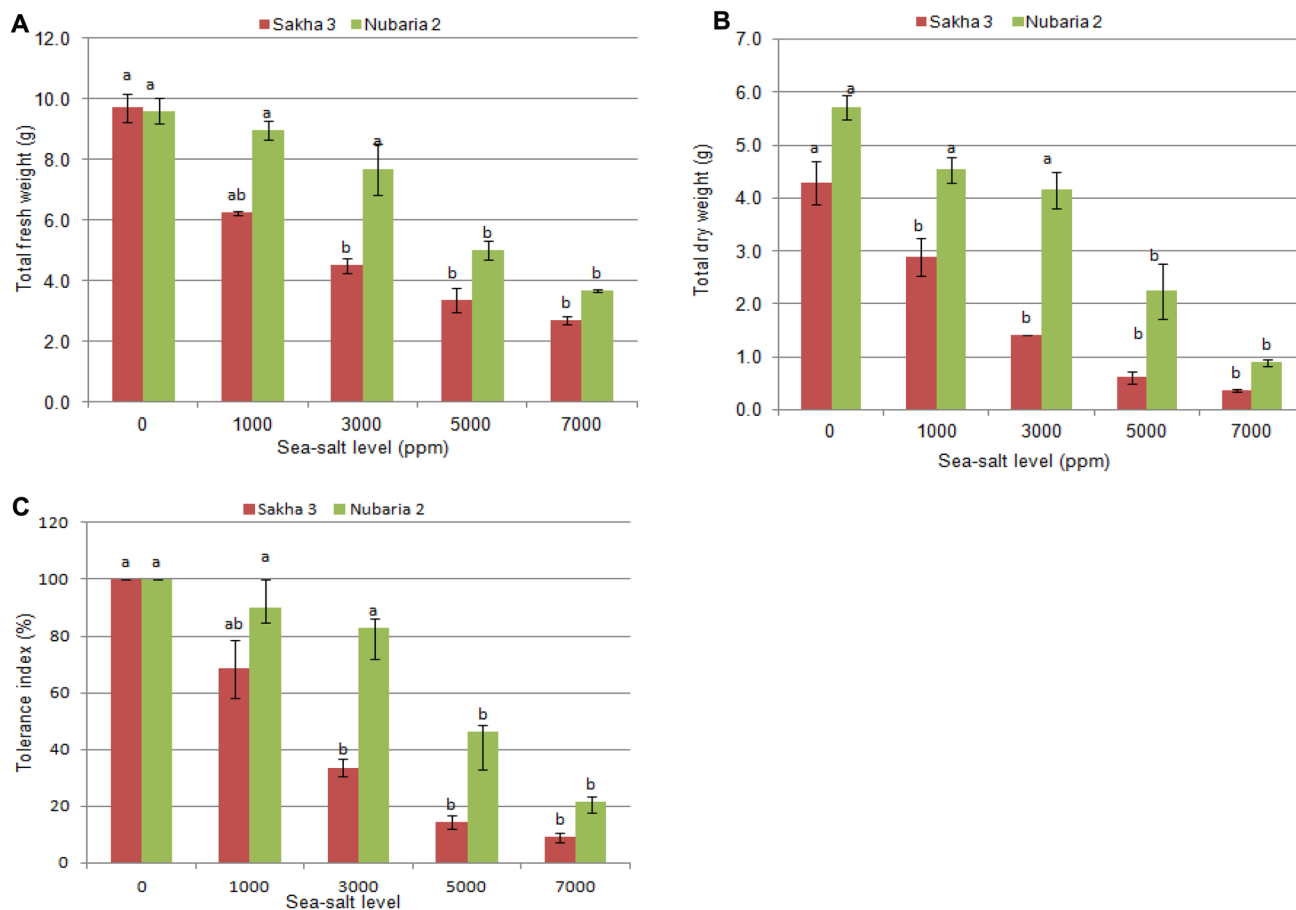
stress (Table S1 and Table S2). This was more pronounced with a severe sea-salt stress level of 7000 ppm with both genotypes. Nubaria 2 exhibited the highest values of FW, DW and Ti under sea-salt level of 3000 ppm as compared to Sakha 3 (Fig. 1A, B, C and Table S2). The reductions in Ti were 10.2, 17.1, 53.88 and 78.55% at 1000, 3000, 5000 and 7000 ppm of sea-salt stress in Nubaria 2, respectively. However, the reduction in salt tolerance index in Sakha 3 were 31.5, 66.44, 85.62 and 91.02% at 1000, 3000, 5000 and 7000 ppm of sea-salt stress, respectively (Fig. 1C).

The decrease in plant growth and dry-matter accumulation under salt stress has been reported in several crops, including legumes (Jamil et al. 2007; Kh et al. 2012; El-Rodeny et al. 2014; Adss and Eldakrory 2015; Kaur et al. 2022). The retardation of plant growth could be attributed to the inhibition of specific growth metabolism, impairment of cell division and expansion due to the loss of cell turgor (Farooq et al. 2012; Ludwiczak et al. 2021). It has been reported that salt stress had a negative effect on growth performance and the physiological attributes of faba beans (Abdelhamid et al. 2010; Assimakopoulou et al. 2015; Eldardiry et al. 2017; Rady et al. 2017; Alzahrani et al. 2019; Desouky et al. 2021). The obtained results are in accordance with El-Rodeny et al. (2014) and (El-Bastawisy et al. 2018) who mentioned that faba bean cultivar Nubaria 2 is relatively tolerant to salt stress.

### Chlorophyll contents

High salt stress (5000 and 7000 ppm) caused a significant decrease in the concentration of Chl *a* in both genotypes (Fig. 2A), while the concentration of Chl *b* was unaffected by salinity stress of 5000 ppm of sea-salt in Nubaria 2 as compared with the results obtained with the same sea-salt level in Sakha 3 (Fig. 2B). There were no significant differences between the two genotypes in total chlorophyll under the salinity levels and it was reduced significantly under severe sea-salt stress of 5000 and 7000 ppm (Fig. 2C). The obtained data revealed that the relative reduction in the Chl *b* was lesser in Nubaria 2 compared to Sakha 3 under high salt stress of 5000 ppm (Fig. 2B). The ANOVA table for chlorophyll content is summarized in table S3.

The obtained results are in agreement with Radi et al. (2013); Hussein et al. (2017) and Mogazy and Hanafy (2022) who reported the gradual reduction of photosynthetic pigments with an increasing salt concentration in faba bean. Moreover, this phenomenon has been reported in many *Fabaceae* species such as *Medicago ciliaris* (Salah et al. 2011; Mbarki et al. 2020), *Phaseolus vulgaris* L (Turan et al. 2007; Azizi et al. 2022) and *Vigna subterranea* L (Taf-fouo et al. 2010; Ambede et al. 2012). The reduction in chlorophyll levels in salt-stressed plants has been identified as the main symptom of oxidative stress due to the excessive



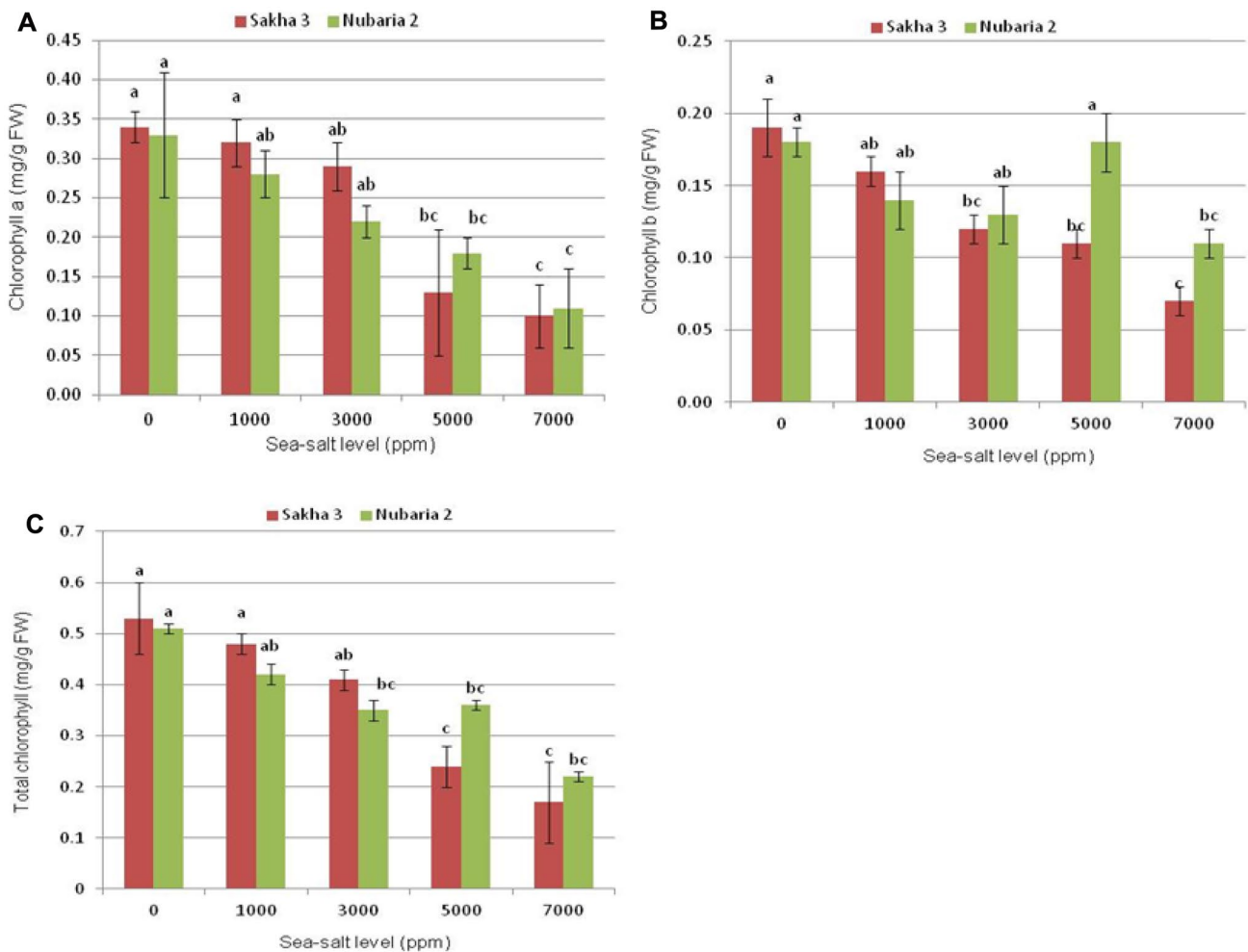
**Fig. 1** Interactive effects of sea-salt stress on **A** plant fresh weight (g), **B** plant dry weight plant (g), and tolerance index of faba bean plants at six weeks after sowing. The vertical bars with different letters are significantly different from each other at  $P < 0.05$  level of probability

accumulation of sodium ions in leaf tissues that leads to lesser biosynthesis, alteration of chlorophyll pigments and/or lesser photosynthetic efficiency (Najar et al. 2019). Also, the activity of a chlorophyll degrading enzyme, chlorophyllase maybe a vital reason for decreasing the *Chl* content under salt stress (Santos 2004; Hundare et al. 2022).

### Ion accumulation

The response of the plants to cope with the toxicity of  $\text{Na}^+$  varies from one plant species to another. Some plants accumulate sodium ions in the vacuole of the leaves, while others extrude the  $\text{Na}^+$  at the level of the roots (Tejera et al. 2006; Souana et al. 2020; Taïbi et al. 2021). However, enhancing the potassium uptake is also a well-known strategy to prevent the entry of sodium to plant cells (Serrano et al. 1999; Souana et al. 2020). The increase of shoot  $\text{K}^+$  and  $\text{Na}^+$  in Sakha 3 under high sea-salt level (7000 ppm) indicated that sodium is accumulated in the shoots not being extruded (Fig. 3A, B). Moreover, the significant reduction in the  $\text{K}^+$  and  $\text{Na}^+$  in the roots of this genotype suggested that the

mechanism to block  $\text{Na}^+$  transfer to growing tissues was not effective at high sea-salt concentration in Sakha 3 (Fig. 4A, B). However, the accumulation of  $\text{K}^+$  and  $\text{Na}^+$  in the shoots of Nubaria 2 under salinity stress was on the same level as in the control (Fig. 4A, B). On the other side, the significant decrease of  $\text{K}^+$  and the increase of  $\text{Na}^+$  in the roots of Nubaria 2 under high salinity indicated that the mechanism to block  $\text{Na}^+$  transfer to growing tissues was effective at high sea-salt concentrations in Nubaria 2 (Fig. 4A, B). Notable, Nubaria 2 has a relatively effective mechanism to control the transport of  $\text{Na}^+$  from roots to shoots. These observations indicate that Nubaria 2 is more tolerant to salinity than Sakha 3. These results are in agreement with those of (Assimakopoulou et al. 2015) in *Phaseolus vulgaris* L. who reported that the salt-tolerant green bean cultivar "Corallo" tolerated  $\text{NaCl}$  stress better due to its ability to retain the  $\text{Na}^+$  in the roots, maintaining an appropriate  $\text{K}^+/\text{Na}^+$  ratio and limiting the accumulation of  $\text{Na}^+$  into shoots. Also, they mentioned that the salt-sensitive green bean (cv. Romano) accumulated a higher concentration of  $\text{Na}^+$  in the leaves and a lower concentration in the roots in response to salt stress.



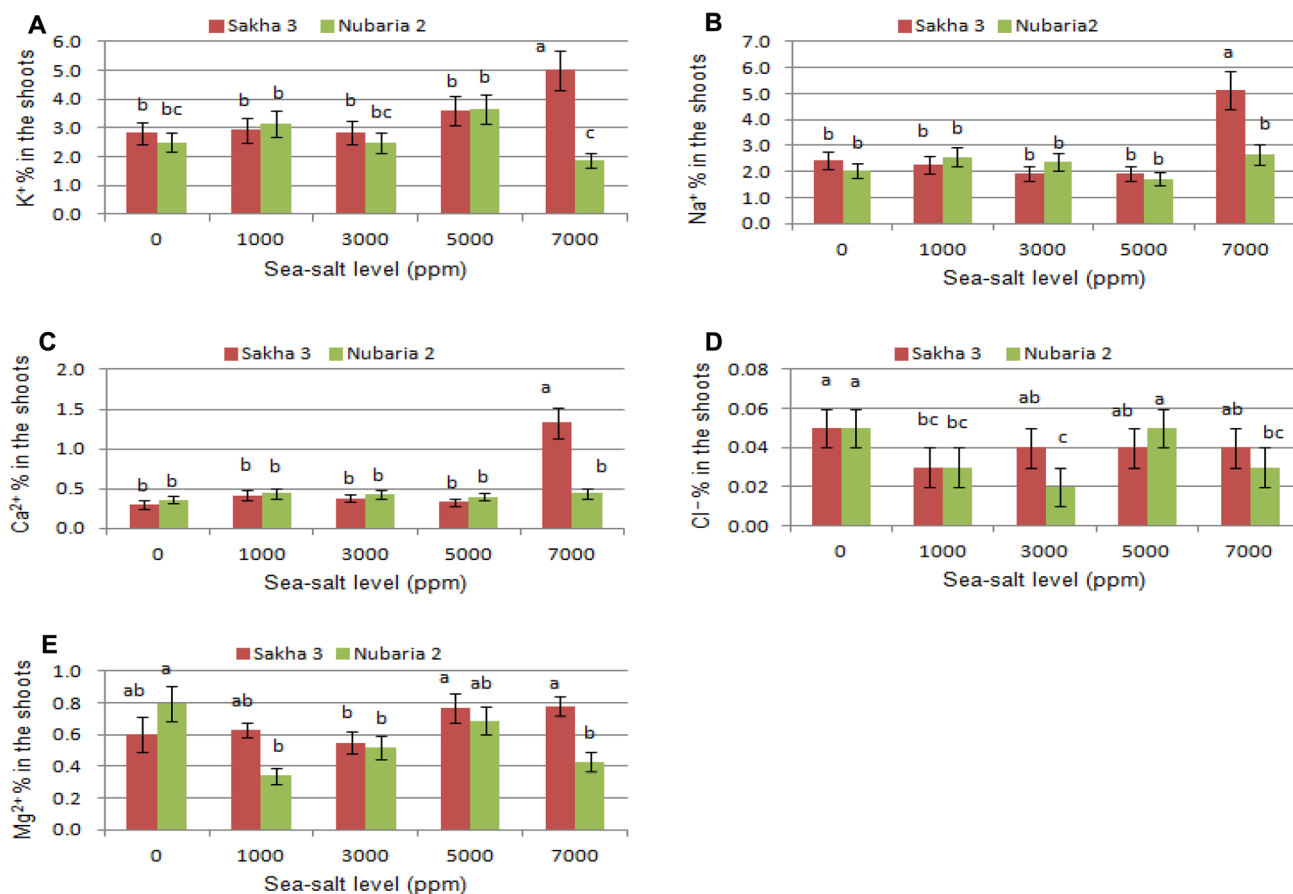
**Fig. 2** Effect of different sea-salt stress levels on Chl *a* (A), Chl *b* (B) and total chlorophyll (C) content in faba bean genotypes Sakha 3 and Nubaria 2. The vertical bars with different letters are significantly different from each other at  $P < 0.05$  level of probability

Calcium is crucial for the preservation of the membrane integrity under stress, influencing  $K^+/Na^+$  selectivity and plays important role in osmoregulation (Rengel 1992). In the present study, shoots and roots  $Ca^{2+}$  percentage were unaffected under sea-salt stress treatments except under sea-salt level of 7000 ppm where the  $Ca^{2+}$  percentage increased significantly in the shoots of Sakha 3 and in the roots of Nubaria 2 (Figs. 3C and 4C). Previous studies reported that  $Ca^{2+}$  uptake from the soil may be decreased due to ion interactions, increase in ionic strength that resulted in the reduction of the  $Ca^{2+}$  activity and precipitation (Janzen and Chang 1987; Ciriello et al. 2022). In accordance with the obtained results, the accumulation of  $Ca^{2+}$  showed differences with the increase of salt stress in alfalfa cultivars (Khorshidi et al. 2009).

In the same line, sea-salt stress had a negligible effect on the shoot  $Cl^-$  and  $Mg^{2+}$  contents measured in Sakha 3, but sea-salt level 1000 ppm significantly reduced the shoot  $Cl^-$  (Fig. 3D) and sea-salt level 7000 decreased the root

$Mg^{2+}$  significantly in this genotype (Fig. 4E). However, salt stress-induced decreases in shoot  $Cl^-$  and  $Mg^{2+}$  contents in Nubaria 2. Except at sea-salt level of 5000 ppm,  $Cl^-$  and  $Mg^{2+}$  percentage were almost in the same percentage as control (Fig. 3D, E). While the root  $Cl^-$  and  $Mg^{2+}$  contents of Sakha 3 were significantly decreased under severe sea-salt stress of 7000 ppm, the roots  $Cl^-$  and  $Mg^{2+}$  of Nubaria 2 were increased significantly under sea-salt stress of 7000 ppm as compared with the control plants (Fig. 4D, E).

In addition to the role of Magnesium ( $Mg^{2+}$ ) in chlorophyll synthesis and as an enzyme cofactor, it plays an important role in the export of photosynthesis in plants (Marschner and Cakmak 1989). In the present study, the accumulation of  $Mg^{2+}$  was either unaffected or reduced in the aerial parts of both genotypes (Fig. 3E). In contrary to the aerial parts, the root  $Mg^{2+}$  was significantly decreased in Sakha 3 and markedly increased in Nubaria 2 under severe sea-salt stress of 7000 ppm (Fig. 4E). Similar results were obtained in sunflower (Ashraf and O'leary 1997).



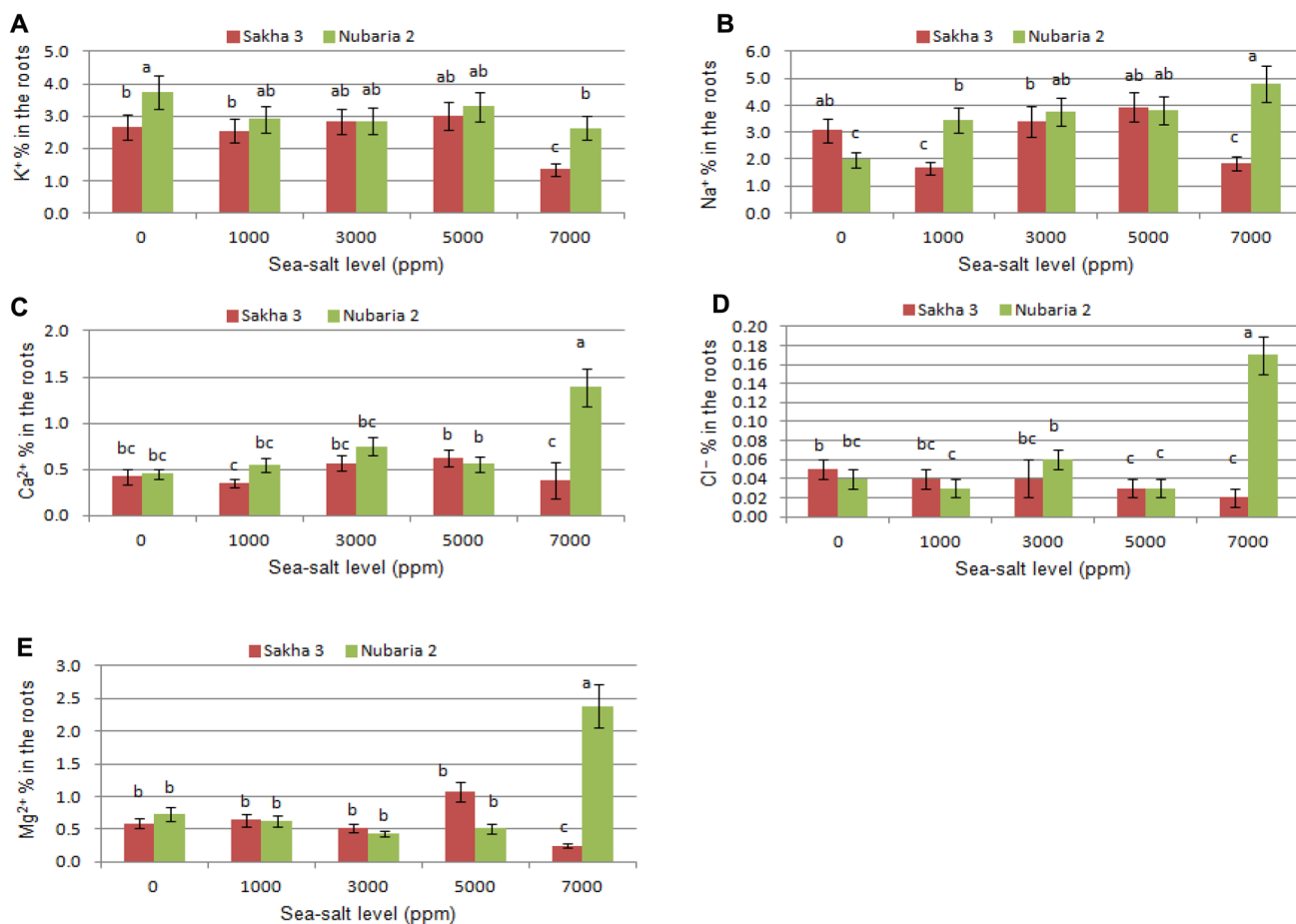
**Fig. 3** Effect of different sea-salt stress levels on **A** potassium, **B** sodium, **C** calcium, **D** chloride and **E** magnesium ions in shoots of faba bean genotypes Sakha 3 and Nubaria 2 at 6 weeks after sowing.

The vertical bars with different letters are significantly different from each other at  $P < 0.05$  level of probability

Also, NaCl application decreased  $Mg^{2+}$  content in the roots of olive (Loupassaki et al. 2002). The obtained results of the current study in the line with those obtained by Khorshidi et al. (2009) and Ashrafi et al. (2018) who reported that  $Mg^{2+}$  concentration in alfalfa depended on the cultivar and the salinity level. Our results indicated that Nubaria 2 accumulated significantly low  $Cl^{-}$  percentage in the shoots under severe sea-salt stress (7000 ppm) with high root  $Cl^{-}$  relative to the control and the other genotype (Fig. 3D and Fig. 4D). The obtained results are in agreement with those reported by Lacerda et al. (2001) and de Oliveira et al. (2020) who found that the transfer of  $Na^{+}$  and  $Cl^{-}$  to the shoots was higher in salt-sensitive sorghum genotypes. In general, the obtained data suggest that Nubaria 2 genotype is relatively tolerant to salinity and this is may be due to the plant ability to prevent the accumulation of toxic ions such as  $Na^{+}$  and  $Cl^{-}$  or maintaining the level of the essential ions at adequate concentrations in the plant aerial part. The ANOVA tables for ion accumulation in shoots and roots are summarized in table S4 and table S5.

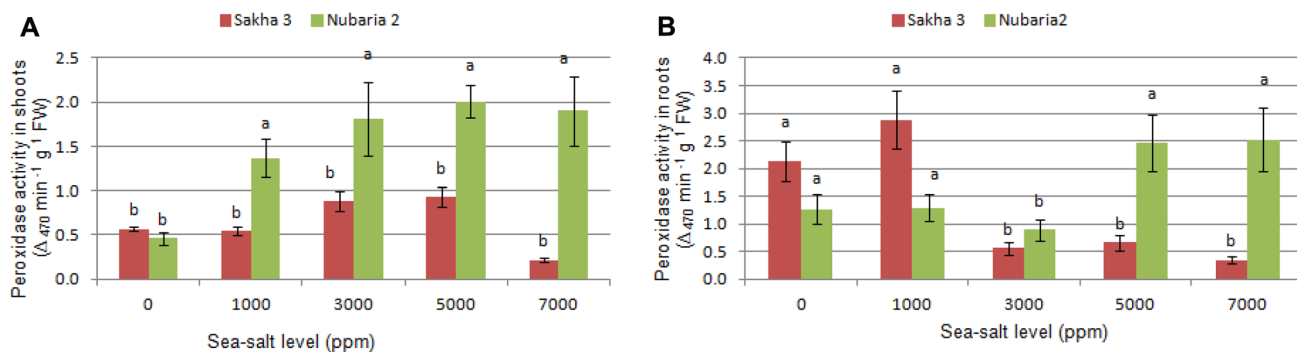
### Antioxidant enzyme activities

To overcome oxidative damage caused by abiotic stress, plants developed antioxidative systems to scavenge reactive oxygen species (ROS) which caused severe oxidative damage to plant cell (Alscher et al. 2002). In this context, the activities of two important antioxidative enzymes were assayed under control and sea-salt stress conditions. Our results showed that salt stress had a negligible effect on the activity of POD in the shoots of Sakha 3. However, the activity of this enzyme was significantly increased with increasing levels of sea-salt stress in shoots of Nubaria 2 (Fig. 5A). The activity of POD in the roots of Sakha 3 was significantly decreased under sea-salt stress levels of 3000, 5000 and 7000 ppm (Fig. 5B). However, sea-salt stress had an insignificant effect on the activity of POD in the roots of Nubaria 2, except with a sea-salt level of 3000 where its activity was decreased significantly compared with that in the control plant (Fig. 5B). It was also observed that the activity of PPO was unchangeable in the shoots of Sakha 3 up to sea-salt levels of 3000 ppm relative to control, after which its



**Fig. 4** Effect of different sea-salt stress levels on **A** potassium, **B** sodium, **C** calcium, **D** chloride and **E** magnesium ions in roots of faba bean genotypes Sakha 3 and Nubaria 2 at 6 weeks after sowing.

The vertical bars with different letters are significantly different from each other at  $P < 0.05$  level of probability

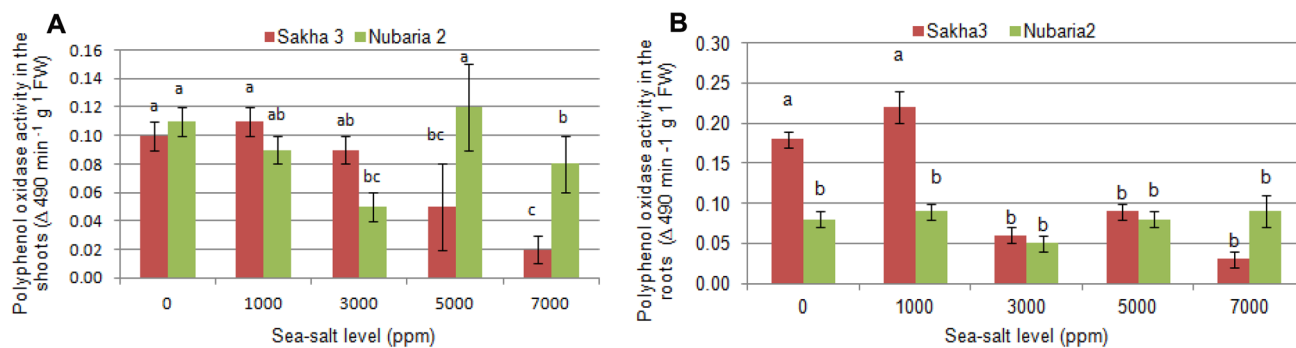


**Fig. 5** Effect of different sea-salt stress levels on the activity of peroxidase (POD) activity in **A** shoots and **B** roots of faba bean genotypes (Sakha 3, Nubaria 2) at 6 weeks after sowing. The vertical bars with

different letters are significantly different from each other at  $P < 0.05$  level of probability

activity was significantly declined (Fig. 6A). The activity of this enzyme was adversely affected in the roots of Sakha 3 under sea-salt stress of 3000, 5000 and 7000 ppm. Sea-salt stress reduced the PPO activity in the shoots of Nubaria

2 under sea-salt levels of 3000 and 7000 ppm (Fig. 6A). Interestingly, PPO activity in the shoots of Nubaria 2 under sea-salt level of 5000 ppm was reached its level in control plants. However, sea-salt stress had a negligible effect on



**Fig. 6** Effect of different sea-salt stress levels on the activity of PPO activity in **A** shoots and **B** roots of faba bean genotypes (Sakha 3, Nubaria 2) at 6 weeks after sowing. The vertical bars with different letters are significantly different from each other at  $P < 0.05$  level of probability

PPO activity in the roots of this genotype (Fig. 6B). The results obtained are in agreement with Ashraf (2009) and Hassanein et al. (2009) who demonstrated that salt stress induced the activities of antioxidant enzymes in the leaves of *Vicia faba*. These findings confirm the fact that the activities of the antioxidant enzymes acting as a tolerant mechanism against salt stress in different plants. Thus, it could be possible to suggest that Nubaria 2 was more tolerant than the other genotype, because the maximum values for these enzymes activity in the shoots were recorded compared to Sakha 3 genotype (Figs. 5A and 6A). In addition, the values of POD in the roots of this genotype were higher under severe salt stress than that in the roots of Sakha 3 (Fig. 5B). The ANOVA table for antioxidant enzyme activities in shoots and roots are summarized in table S6. The determination of reducing, non-reducing, total sugars, soluble phenols, conjugated phenols and total phenols contents in shoots and roots of faba bean genotypes (Sakha 3, Nubaria 2) at 6 weeks after sowing content in shoots and roots revealed no important differential differences between both genotypes (tables S7-S10).

## Conclusion

The results of the morphological, biochemical and physiological traits assessed in this study showed significant genotypic variation, confirming that traits which show significant genotypic variation may be used as screening criteria for salinity tolerance in faba bean. Generally, the studied genotypes showed a significant difference in their tolerance to salinity. However, the studied traits of both genotypes were severely affected by salinity at varying degrees, indicating that salinity tolerance differs between both genotypes which are widely used in Egypt. Nubaria 2 was found to be more tolerant compared to Sakha 3 based on the majority of growth parameters assessed, being the most differential factors are the biomass, ions translocation and antioxidant

activities. The obtained results elucidate that several processes are involved in salinity tolerance.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s12892-022-00168-y>.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.

## References

- Abdelhamid MT, Shokr MM, Bekheta M (2010) Growth, root characteristics, and leaf nutrients accumulation of four faba bean (*Vicia faba* L.) cultivars differing in their broomrape tolerance and the soil properties in relation to salinity. *Commun Soil Sci Plant Anal* 41(22):2713–2728
- Adss IA, Eldakrory M (2015) Effects of salt stress on gene expression and activities of POD and PPO in of broad bean (*Vicia faba* L.) cultivars. *Alex J Agric Sci* 60(2):55
- Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S (2010) Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit Rev Biotechnol* 30(3):161–175
- Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot* 53(372):1331–1341
- Alzahrani SM, Alaraidh IA, Migdadi H, Alghamdi S, Khan MA, Ahmad P (2019) Physiological, biochemical, and antioxidant properties of two genotypes of *Vicia faba* grown under salinity stress. *Pak J Bot* 51(3):786–798
- Ambede JG, Netondo GW, Mwai GN, Musyimi DM (2012) NaCl salinity affects germination, growth, physiology, and biochemistry of bambara groundnut. *Braz J Plant Physiol* 24:151–160



- Anaya F, Fghire R, Wahbi S, Loutfi K (2017) Antioxidant enzymes and physiological traits of *Vicia faba* L. as affected by salicylic acid under salt stress. *J Mater Environ Sci*. 8(7):2549–2563
- Arnon DI (1949) Copper enzymes in isolated chloroplasts polyphenoloxidase in beta vulgaris. *Plant Physiol* 24(1):1
- Ashraf M (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol Adv* 27(1):84–93
- Ashraf M, O'leary J (1997) Responses of a salt-tolerant and a salt-sensitive line of sunflower to varying sodium/calcium ratios in saline sand culture. *J Plant Nutr* 20(2–3):361–377
- Ashrafi E, Razmjoo J, Zahedi M (2018) Effect of salt stress on growth and ion accumulation of alfalfa (*Medicago sativa* L.) cultivars. *J Plant Nutr* 41(7):818–831
- Assimakopoulou A, Salmas I, Nifakos K, Kalogeropoulos P (2015) Effect of salt stress on three green bean (*Phaseolus vulgaris* L.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 43(1):113–118
- Azizi F, Amiri H, Ismaili A (2022) Melatonin improves salinity stress tolerance of *Phaseolus vulgaris* L. cv. Pak by changing antioxidant enzymes and photosynthetic parameters. *Acta Physiol Plant* 44(4):1–12
- Ciriello M, Formisano L, Soteriou GA, Kyratzis A, De Pascale S, Kyriacou MC, Roupheal Y (2022) Differential response to NaCl osmotic stress in sequentially harvested hydroponic red and green basil and the role of calcium. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2022.799213>
- Cordovilla MDP, Ligerio F, Lluch C (1999) Effect of salinity on growth, nodulation and nitrogen assimilation in nodules of faba bean (*Vicia faba* L.). *Appl Soil Ecol* 11(1):1–7
- de Oliveira DF, Lopes LdS, Gomes-Filho E (2020) Metabolic changes associated with differential salt tolerance in sorghum genotypes. *Planta* 252(3):1–18
- Desouky AF, Ahmed AHH, Stützel H, Jacobsen H-J, Pao Y-C, Hanafy MS (2021) Enhanced abiotic stress tolerance of *Vicia faba* L. plants heterologously expressing the PR10a gene from potato. *Plants*. 10(1):173
- El-Bastawisy ZM, El-Katony TM, Abd El-Fatah SN (2018) Genotypic variability in salt tolerance of *Vicia faba* during germination and early seedling growth. *J King Saud Univ Sci* 30(2):270–277
- El-Rodeny W, Kimura M, Hirakawa H, Sabah A, Shirasawa K, Sato S, Tabata S, Sasamoto S, Watanabe A, Kawashima K (2014) Development of EST-SSR markers and construction of a linkage map in faba bean (*Vicia faba*). *Breed Sci* 64(3):252–263
- Eldardiry EI, Abd El-Hady M, Ageeb G (2017) Maximize faba bean production under water salinity and water deficit conditions. *Middle East J Appl Sci* 7(4):819–826
- Epstein E, Norlyn JD, Rush DW, Kingsbury RW, Kelley DB, Cunningham GA, Wrona AF (1980) Saline culture of crops: a genetic approach. *Science* 210(4468):399–404
- Faithfull NT (2002) Methods in agricultural chemical analysis: a practical handbook. Cabi
- Farooq M, Hussain M, Wahid A, Siddique K (2012) Drought stress in plants: an overview. *Plant Responses Drought Stress*. [https://doi.org/10.1007/978-3-642-32653-0\\_1](https://doi.org/10.1007/978-3-642-32653-0_1)
- Foolad MR, Lin G (2001) Relationship between cold tolerance during seed germination and vegetative growth in tomato: analysis of response and correlated response to selection. *J Am Soc Hortic Sci* 126(2):216–220
- Fouad M, Mohammed N, Aladdin H, Ahmed A, Xuxiao Z, Shiyang B, Tao Y (2013) Genetic and genomic resources of grain legume improvement. Faba bean. Elsevier Inc.
- Hanafy Ahmed A, Desouky A, Reda A, Stützel H, Hanafy MS (2020) Impact of chitosan on shoot regeneration from faba bean embryo axes through its effect on phenolic compounds and endogenous hormones. *Plant Arch* 20(1):2269–2279
- Hassanein RA, Hassanein AA, Haider AS, Hashem HA (2009) Improving salt tolerance of *Zea mays* L. plants by presoaking their grains in glycine betaine. *Aust J Basic Appl Sci*. 3(2):928–942
- Hernandez JA, Jiménez A, Mullineaux P, Sevilla F (2000) Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. *Plant Cell Env* 23(8):853–862
- Horborne J (1973) Phytochemical methods. Chapman and Hall Ltd, London, pp 49–188
- Hundare A, Joshi V, Joshi N (2022) Salicylic acid attenuates salinity-induced growth inhibition in in vitro raised ginger (*Zingiber officinale* Roscoe) plantlets by regulating ionic balance and antioxidative system. *Plant Stress* 4:100070
- Hussein M, Embiale A, Husen A, Aref IM, Iqbal M (2017) Salinity-induced modulation of plant growth and photosynthetic parameters in faba bean (*Vicia faba*) cultivars. *Pak J Bot* 49(3):867–877
- Jamil M, Rehman S, Rha E (2007) Salinity effect on plant growth, PSII photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea capitata* L.). *Pak J Bot*. 39(3):753–760
- Janzen H, Chang C (1987) Cation nutrition of barley as influenced by soil solution composition in a saline soil. *Can J Soil Sci* 67(3):619–629
- Kato M, Shimizu S (1987) Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidative degradation. *Can J Bot*. 65(4):729–735
- Kaur H, Hussain SJ, Kaur G, Poor P, Alamri S, Siddiqui MH, Khan MIR (2022) Salicylic acid improves nitrogen fixation, growth, yield and antioxidant defence mechanisms in chickpea genotypes under salt stress. *J Plant Growth Regul* 10:1–14
- Kh T, Taïbi F, Belkhdja M (2012) Effects of external calcium supply on the physiological response of salt stressed bean (*Phaseolus vulgaris* L.). *Gen Plant Physiol* 2(24):177–186
- Khorshidi M, Yarnia M, Hassanpanah D (2009) Salinity effect on nutrients accumulation in alfalfa shoots in hydroponic condition. *J Food Agric Environ* 7:787–790
- Koch K (1996) Carbohydrate-modulated gene expression in plants. *Annu Rev Plant Biol* 47(1):509–540
- Lacerda CFd, Cambraia J, Cano MAO, Ruiz HA (2001) Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. *Rev Bras Fisiol Veg* 13:270–284
- Loupassaki M, Chartzoulakis K, Dgalaki N, Androulakis I (2002) Effects of salt stress on concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium in leaves, shoots, and roots of six olive cultivars. *J Plant Nutr* 25(11):2457–2482
- Ludwiczak A, Osiak M, Cárdenas-Pérez S, Lubińska-Mielińska S, Piernik A (2021) Osmotic stress or ionic composition: which affects the early growth of crop species more? *Agronomy* 11(3):435
- Mahlooji M, Sharifi RS, Razmjoo J, Sabzalain M, Sedghi M (2018) Effect of salt stress on photosynthesis and physiological parameters of three contrasting barley genotypes. *Photosynthetica* 56(2):549–556
- Marschner H, Cakmak I (1989) High light intensity enhances chlorosis and necrosis in leaves of zinc, potassium, and magnesium deficient bean (*Phaseolus vulgaris*) plants. *J Plant Physiol* 134(3):308–315
- Mayer A, Harel E, Ben-Shaul R (1966) Assay of catechol oxidase—a critical comparison of methods. *Phytochemistry* 5(4):783–789
- Mbarki S, Skalicky M, Vachova P, Hajihashemi S, Jouini L, Zivcak M, Tlustos P, Brestic M, Hejnak V, Zoghiami Khelil A (2020) Comparing salt tolerance at seedling and germination stages in local populations of *Medicago ciliaris* L. to *Medicago intertexta* L. and *Medicago scutellata* L. *Plants* 9(4):526

- Mogazy AM, Hanafy RS (2022) Foliar spray of biosynthesized zinc oxide nanoparticles alleviate salinity stress effect on *Vicia faba* plants. *J Soil Sci Plant Nutr* 22:1–16
- Munns R, Hare R, James R, Rebetzke G (2000) Genetic variation for improving the salt tolerance of durum wheat. *Aust J Agric Res* 51(1):69–74
- Munns R, James RA (2003) Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil* 253(1):201–218
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assay with tobacco tissue cultures. *Physiol Plant* 15:473–497
- Najar R, Aydi S, Sassi-Aydi S, Zarai A, Abdely C (2019) Effect of salt stress on photosynthesis and chlorophyll fluorescence in *Medicago truncatula*. *Plant Biosyst Int J Dealing Aspects Plant Biol* 153(1):88–97
- Noble C, Halloran G, West D (1984) Identification and selection for salt tolerance in lucerne (*Medicago sativa* L.). *Aust J Agric Res* 35(2):239–252
- Radi AA, Farghaly FA, Hamada AM (2013) Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. *J Biol Earth Sci* 3(1):72–88
- Rady MM, Taha RS, Semida WM, Alharby HF (2017) Modulation of salt stress effects on *Vicia faba* L. plants grown on a reclaimed-saline soil by salicylic acid application. *Rom Agric Res* 34:175–185
- Rengel Z (1992) The role of calcium in salt toxicity. *Plant, Cell Environ* 15(6):625–632
- Salah IB, Slatni T, Gruber M, Messedi D, Gandour M, Benzarti M, Haouala R, Zribi K, Hamed KB, Perez-Alfocea F (2011) Relationship between symbiotic nitrogen fixation, sucrose synthesis and anti-oxidant activities in source leaves of two *Medicago ciliaris* lines cultivated under salt stress. *Environ Exp Bot* 70(2–3):166–173
- Santos CV (2004) Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Sci Hortic* 103(1):93–99
- Serrano R, Mulet JM, Rios G, Marquez JA, De Larrinoa IF, Leube MP, Mendizabal I, Pascual-Ahuir A, Proft M, Ros R (1999) A glimpse of the mechanisms of ion homeostasis during salt stress. *J Exp Bot* 50:1023–1036
- Shetty K, Hetrick B, Schwab A (1995) Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. *Environ Pollut* 88(3):307–314
- Souana K, Taïbi K, Abderrahim LA, Amirat M, Achir M, Boussaid M, Mulet JM (2020) Salt-tolerance in *Vicia faba* L. is mitigated by the capacity of salicylic acid to improve photosynthesis and antioxidant response. *Sci Hortic* 273:109641
- Taffouo V, Wamba O, Youmbi E, Nono G, Akoa A (2010) Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranea* (L.) Verdc.) landraces grown under saline conditions. *Int J Bot* 6(1):53–58
- Taïbi K, Abderrahim LA, Boussaid M, Bissoli G, Taïbi F, Achir M, Souana K, Mulet JM (2021) Salt-tolerance of *Phaseolus vulgaris* L. is a function of the potentiation extent of antioxidant enzymes and the expression profiles of polyamine encoding genes. *S Afr J Bot* 140:114–122
- Tejera N, Soussi M, Lluch C (2006) Physiological and nutritional indicators of tolerance to salinity in chickpea plants growing under symbiotic conditions. *Environ Exp Bot* 58(1–3):17–24
- Turan MA, Turkmen N, Taban N (2007) Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. *J Agron*. <https://doi.org/10.3923/ja.2007.378.381>
- Türkan I, Demiral T (2009) Recent developments in understanding salinity tolerance. *Environ Exp Bot* 67(1):2–9
- Yasar F, Kusvuran S, Ellialtioglu S (2006) Determination of anti-oxidant activities in some melon (*Cucumis melo* L.) varieties and cultivars under salt stress. *J Hortic Sci Biotechnol*. 81(4):627–630
- Yeo A, Flowers T (1983) Varietal differences in the toxicity of sodium ions in rice leaves. *Physiol Plant* 59(2):189–195
- Zhang F-Q, Wang Y-S, Lou Z-P, Dong J-D (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere* 67(1):44–50

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