



Alleviation of salt injuries on green bean's morpho-physiological properties by application of calcium with other amendments

Sayed F. El-Sayed¹, Omailma S. Darwish¹, Ahmed M. Ibrahim² and Rasha R. Eid³

1. Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt

2. Potato and Vegetatively Propagated Vegetables Dep., Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

3. M.SC student at Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza Egypt

ABSTRACT

Salinity stress is considered a great challenge affecting agriculture sector worldwide. Green bean is very sensitive to salinity stress and faces losses in production and quality of pods with any increase in salt concentration in irrigation water. This challenge requires implementing some amendments to alleviate salt injuries on green bean. This experiment was carried out in the experimental farm of Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt, in the winter season of 2020/2021 and 2021/2022. The effect of anti-stressors under different salinity levels (0, 1.5, and 2.4 dS m⁻¹ of sea salt) was studied on plant growth parameters, yield, proline, peroxides, abscisic acid, and nutrient content of green bean. The anti-stressors were varied in their content of Calcium, Potassium, humic, and fulvic acid, while the control (untreated with any anti-stressor). Our findings revealed that all tested plant growth parameters of treated plants with anti-stressors were significantly higher than the control without significant differences among each other. All anti-stressors obtained total yield and marketable yield higher than control at each salinity level. The treated plants with anti-stressors recorded significantly higher proline content than the control. At both highest levels of salinity (1.5 and 2.4 dS/m), all anti-stressor recorded higher peroxidase and abscisic acid than the control without significance among them. It is very obvious that the anti-stressor with calcium with other amendments limited the negative impacts of salinity on green beans, while there was no superiority to one of these anti-stressors on the others.

Keywords: Green bean, *Phaseolus vulgaris* L, salinity, salt injuries, calcium, humic acid.

1-Introduction

Salinity stress poses a serious threat to crop productivity and food security worldwide (**Wani and Gosal 2011; Yu et al. 2013; Wani et al. 2013, and Rizwan et al., 2015**). Worldwide, one of the major challenges to agriculture sector is the production of 70% more food crops for an additional 2.3 billion people by 2050. (**Al-Naggar et al., 2015**). Salt stress is considered the main factor determining the

increase in food demand. In addition, more than 20% of irrigated land around the world is affected by salinity, and the amount is greatly increasing (Peleg *et al.*, 2011). Arid and semi-arid regions of the world are mainly greatly affected by salinity and drought, which are responsible for the decline in crop yield (Singh *et al.*, 2015). So, food production enhancement in salt-prone areas is the key to facing future food demands (Rizwan *et al.*, 2015). Typically, salinity stress is associated with a lack of cell wall extension and expansion, resulting in the cessation of plant growth. The ionic effect inhibits nutrient imbalance and reduces net photosynthetic rates in afflicted plants (Arif *et al.*, 2020). Increasing NaCl reduces carbohydrates essential for cell proliferation, causing a nutritional imbalance in plant water availability and absorption (Tuteja *et al.*, 2012). The plants are affected by a factor that reduces their growth rate and alters their metabolic processes (Arif *et al.*, 2020). The green bean (*Phaseolus vulgaris* L.) is one of the world's most essential vegetable commodities. It is a significant source of protein, carbohydrates, vitamins, and minerals for millions of individuals around the world (Montoya *et al.*, 2010; Celmeli *et al.*, 2018). Green bean is considered salt-sensitive plant throughout their entire growth cycle. When salinity increased from 1 to 3 dS m⁻¹, its growth was reduced by approximately 10% (Garcia *et al.*, 2019). In addition, salinity levels of 2 and 4 dS m⁻¹ caused a considerable decrease in the root and shoot dry matter production of bean seedlings (Asfaw, 2020). At a salinity of 6.3 dS m⁻¹, germination percentage, and stem length were reduced by an average of 31% and 76.9%, while at a salinity of 9 dS m⁻¹, they were reduced by an average of 49% and 86.9% (Can-Chulim *et al.*, 2017). In addition, bean seedling root and stalk dry matter production decreased simultaneously with increasing salinity levels above 8 dS m⁻¹ (Asfaw, 2021). In addition, Mori *et al.* (2011) reported that irrigation with salinity levels of 0.7, 3.0, and 6.0 dS m⁻¹ decreased the growth and yield of green beans. Bean yield was reduced to about 43.5% (Mori *et al.*, 2011) or 50% (Garcia *et al.*, 2019) when the salinity level raised from 1 to 3 dS m⁻¹. The decrease in green bean yield reached 79.9% at 6 dS m⁻¹, but with increasing salt concentration up to 9.0, 12.0 dS m⁻¹ (Mori *et al.*, 2011) and 16 dS m⁻¹ (Asfaw, 2021), the plants did not reach ripening, and consequently, no yield was obtained. By reabsorbing Na⁺ from the xylem, the green bean is known to exclude Na⁺ from the shoot and translocate Cl to the leaves. High concentrations of Cl in the leaf inhibit plant growth by modifying the plant's nutritional balance, CO₂ uptake, and water relations (Chen *et al.*, 2018).

Green bean is one of Egypt's most widely cultivated legumes for local consumption and export. In 2020, the acreage of green beans in Egypt was 26028 ha, while the production was 2,64,959 tons. Exports increased from 13901 tons in 2017 to 25573 tons in 2020 (FAO statistics division, 2023). 20–30% of bean-producing regions in the Middle East, including Egypt, are affected by soil salinity (Mahdy *et al.*, 2022). Numerous efforts have been made to mitigate the negative impact of salinity on vegetable crops (Zhang *et al.*, 2010; Akladios and Mohamed, 2018; Saidimoradi *et al.*, 2019; Hamaiel *et al.*, 2020; Jan *et al.*, 2020; Abbas *et al.*, 2022). Calcium is one of the primary elements that play a significant role in mitigating the detrimental effects of salinity. It plays a significant role in higher plants, serving as an essential inorganic nutrient with structural, metabolic, and detoxification functions (Soetan *et al.*, 2010). Inhibition of Na⁺ uptake depends on Ca level, which reduces the Na⁺ effect in plants and promotes plant growth (Zhang *et al.*, 2010) and could prevent the entry of Na⁺ ions via HKT2 transporters (Kronzucker and Britto, 2011). Tobe *et al.* (2002, 2004) reported that the deleterious effects of chloride, sulfate, and Na could be mitigated by calcium at low concentrations during germination or seedling development. Humic compounds consist of numerous compounds, including humic acid (HA), one of the organic soil constituents. According to Suddarth *et al.* (2019), humic substances applied to saline soils can increase Na⁺ leaching and decrease both exchangeable sodium percentage and soil salinity. In addition, humic

acid treatment increased plant growth, photosynthetic pigments, nutrient content, and non-enzymatic antioxidants in plants grown under normal or saline salinity (Akladious and Mohamed, 2018). Similarly, administration of HA under salinity stress substantially enhanced the vegetative growth and yield of strawberry (Saidimoradia et al., 2019) and potato (Hamaiel et al., 2020). Potassium (K) is regarded as one of the primary elements that influence the majority of biochemical and physiological processes that impact plant growth and metabolism. It is crucial to the survival of plants under biotic and abiotic stresses such as parasites, drought, salinity, high or low temperature, and waterlogging (Wang et al., 2013). In addition, Jan et al. (2020) reported that the administration of K enhanced plant growth and acted as a formidable barrier against the negative effects of salinity. By promoting Na⁺ exclusion, K⁺ accumulation, and osmotic adjustment, potassium application improved salinity tolerance, as measured by plant water status, biomass produced under stress, osmotic adjustment, and ionic balance, according to Chakraborty et al., 2016. Recently, Abbas et al. (2022) demonstrated that potassium and humic acid application synergistically increased salt tolerance and nutrient assimilation in diverse wheat via ionic homeostasis and activation of antioxidant enzymes.

Consequently, the purpose of this study was to examine the effect of commercial preparations containing primarily calcium in addition to a few other compounds such as HA and potassium on the growth, yield, and phytochemicals of green beans under varying levels of salinity stress.

2-Materials and methods

2.1. Plant material and experimental design:

This experiment was carried out the experimental farm of Vegetable Crops Department, Faculty of Agriculture, Cairo university, Giza, Egypt (30° N, 31°: 28'E with an altitude of 19 m) in two successive winter seasons (2020/2021 and 2021/2022). It was laid out in a split plot design. The main plot was salt stress level and sub plot was anti-stressors at 3 replicates in each season. The levels of salt stress were 0, 1.5 and 2.4 dS m⁻¹ of sea salt, while 0 dS m⁻¹ was the irrigation water at the farm (its salinity was 0.76 dS m⁻¹). The anti-stressors were; 1-Salix Plus Ca (Ca 14 % + K 15 %), 2-Salcali Cod (Calcium acetate 18 % + poly carboxylic acid 35 %), 3-Equilibrium (Complex Ca 5.7+ Humic acid 5.9% + Fulvic acid 5.9 % + organic substances 11 %), all anti-stressors were added as a foliar application (1cm/ L of each) and 4-control (foliar application with distilled water). A plot area was 5 m². The number of plants was 40 plants/ m². Seeds of green bean cv. Pulista were sown on 23th and 26th of September in the first and second season, respectively. The soil of experimental site was clay loam soil (Physicochemical properties of experimental site soil are shown in table 1). The plants were treated with sea salt at the different levels or anti-stressors after 3,4,5 and 6 weeks of sowing date at both seasons.

Table 1. Physicochemical properties of experimental site soil.

Particle Size Distribution (%)	
Coarse sand	6.0
Fine sand	37.0
Silt	22.0
Clay	35.0
Textural class	Clay loam
Chemical properties	

pH (1:2.5)	8.1
EC (mmohs/cm)	0.63
CaCO ₃ (%)	4.8
Anions (meq/L)	
HCO ₃ ⁻	2.8
SO ₄ ²⁻	2.5
Cl ⁻	1.0
Cations (meq/L)	
K ⁺	0.58
Na ⁺	2.6
Ca ²⁺	1.0
Mg ²⁺	1.8
Macro elements (ppm)	
N	121
P	116
K	590
Micro elements (ppm)	
Mn	11.5
Cu	2.06
Fe	5.6
Zn	2.34

2.2. Measurements:

2.2.1. Plant growth parameters:

Plant growth parameters were recorded 60 days after sowing date at full blooming stage. Ten plants were taken from each plot for measuring plant length, number of branches per plant and plant fresh weight.

2.2.2. Yield and its components:

Weight of green pods of three harvest picks was recorded as a total yield (kg/ha). In addition, marketable yield was calculated (%) after sorting of total yield. Pod fresh weight was recorded at the second picking on 10 pods harvested from each plot randomly.

2.2.3. Phytochemical analysis:

2.2.3.1. Proline:

Proline ($\mu\text{g/g}$ fresh leaves) was quantified spectrophotometry at 520 nm by ninhydrin method (**Bates (1973)**). Plant sample was extracted (0.1 g of fresh leaves) with 5 mL of 3 % sulfosalicylic acid solution and then centrifuged. The extract was mixed with 2 mL of glacial acetic acid and 2 mL of ninhydrin (1.25 g ninhydrin warmed in 30 mL of glacial acetic acid and 20 ml of 6 M phosphoric acid until dissolved) for 1 h at 100 C, after that the mixture was put into an ice bath. The reaction mixture was extracted with 1 ml of toluene and the absorbance was determined at 520 nm.

2.2.3.2. Peroxides (POD):

POD was quantified according to method described by **Aebi (1984)**. POD was isolated by freezing 0.5 g of leaves sample in liquid nitrogen. The centrifuged at 3930 rpm for 20 mins was used to crushed the samples with 10 mL of extraction buffer (50 mM phosphate buffer, pH 7, including 0.5 mM EDTA and 2 percent PVPP (w/v)). The peroxidase action was determined spectrophotometry depending on the structure of guaiacol in a 1 mL effect mixture (450 μ l of 25 mM guaiacol, 450 μ l of 225 mM H₂O₂) and 100 μ l of crude enzymes.

2.2.3.3. Absciscic Acid (μ g/g fresh weight)

Absciscic acid (ABA) content in green bean leaves was analyzed using the method described by **Fales *et al.* (1973)**. Freeze-dried green bean leaves was homogenized with 15 mL of methanol/butylated hydroxytoluene (80% v/v) solution at 4 °C in the dark, the extractions and quantification of ABA was performed as described at **AOAC (1990)** guidelines.

2.2.4. Nutrients composition

The sample of green bean plants was dried in an oven at 70 °C for 24 h (**Helrich (1990)**). The dried samples were crushed for determination of the elements. The wet digestion of 0.2 g of samples with sulfuric and perchloric acids was carried out on samples by adding concentrated sulfuric acid (5 mL). The mixture was heated for 10 min, followed by the addition of 0.5 mL perchloric acid, with heating continued until a clear solution was obtained (**Helrich 1990 and Jackson, 1973**). Total N content was quantified by using the modified micro-Kjeldahl method as described by **Helrich (1990)**. Total P was determined calorimetrically by using the chloro-stannous molybdophosphoric blue color method in sulfuric acid according to **Jackson (1973)**. Total K, Na, and Ca concentrations were determined using a flame photometer apparatus.

2.2.5. Statistical analysis:

Regular analysis of variance of split plot design. With 3 replicates was performed for the obtained data of each season, combined analysis over seasons and differences between means were compared to the estimated value of L.S.D at 5% level of probability (**Snedecor and Cochran, 1982**).

3- Results

Data in Fig. 1 show the combined analysis over both seasons of different anti-stressors (Saliks Plus Ca (SPCa), Salcalci cod (SCod), Equilibrium (Equi) and control) on A: plant height, B: number of branches/plant and C: plant fresh weight of green bean plants grown under different concentrations of salt stress (0, 1.5 and 2.4 dS m⁻¹). At the first level (0 dS m⁻¹), no significant differences were obtained among all values of plant height obtained from all different anti-stressors (Fig.1A). On the other hand, at the second and third levels of salinity, the plant height of all anti-stressors was significantly higher than the control (untreated with any anti-stressor). The same trend was observed in the number of branches/plant (Fig. 1B). At the first and second levels of salinity (0 and 1.5 dS m⁻¹), all different anti-stressors produced higher values of the number of branches than the control (without anti-stressor), but without significant differences among each other. However, at the third level of salinity, no significant differences were recorded between all anti-stressors and control. The number of branches of different treatments and control under 2.4 dS m⁻¹ was significantly lower than the number of branches at the lowest level of salinity. Plant fresh weight values of plants at different anti-stressors were higher than control under each salinity level (Fig.1C). Firstly, under first level (0 dS m⁻¹), the plant fresh weight of Equi. was

significantly higher than control and SPCa. At the second level of salinity, no significant differences were obtained among treatments; however, the control was lower than all anti-stressors. At the highest level of salinity, Equi caused higher values of plant fresh weight as compared to control. Meanwhile, no significances were recorded among all anti-stressors.

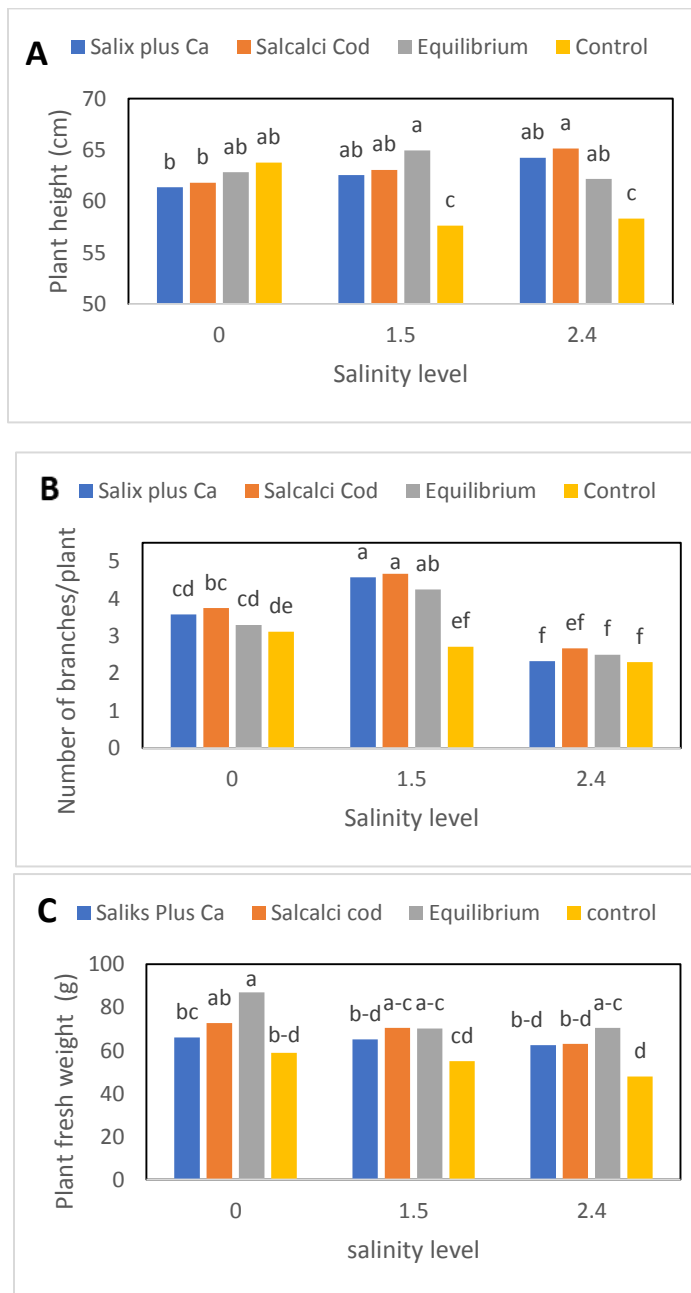
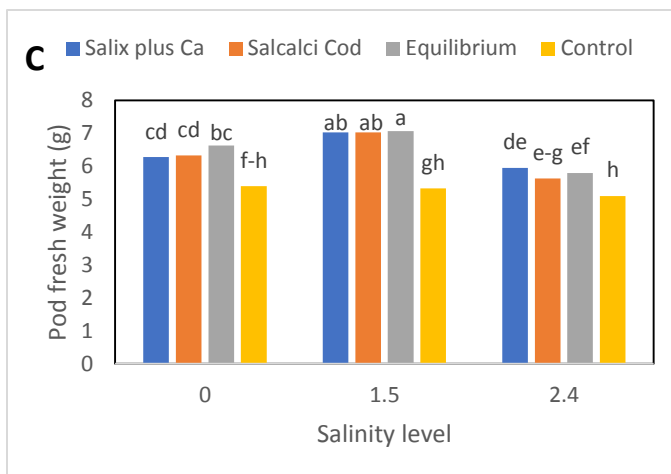
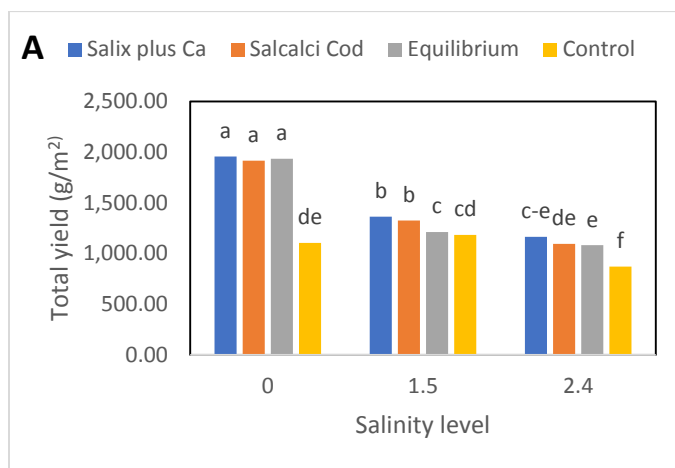


Fig.1. The combined over both seasons (2019-2020 and 2020-2021) of different anti-stressors (Saliks Plus Ca, Salcalci cod, Equilibrium and control) on (A) plant height (cm), (B) number of branches/plant and (C) plant fresh weight of green bean plants grown under different concentrations of salt stress (0, 1.5 and 2.4 dS m⁻¹), 7 weeks after seed sowing. (n=3). letters in common are not indicate a significant difference between treatments (LSD test at 5%).

Data in Fig.2 show the combined effects over both seasons of different anti-stressors (SPCa, SCoD, Equi and control) on (A) total yield, (B) marketable yield (%) and (C) pod fresh weight of green bean plants grown under different concentrations of salt stress (0, 1.5 and 2.4 dS m⁻¹). Total yield was decreased by increasing salinity level (Fig.2A). All anti-stressors produced a higher total yield than the control at each salinity level. At the first level of salinity, all the treated plants with all anti-stressors recorded significantly total yield higher than control, while no significances were obtained among them. At the second level of salinity, SPCa and SCoD gave significantly the highest total yield. At the highest level of salinity, no significant differences were obtained among all anti-stressors; however, all of them recorded values of total yield higher than the control. The percentage of marketable yield of green beans was decreased by increasing salinity level (Fig.2B). At the first level of salinity, SCoD obtained the highest marketable yield compared to control and Equi. At the second level, no significant differences were recorded among all anti-stressors and the control. At the third level, the values of marketable yield at all anti-stressors were significantly higher than the control except SCoD. However, no significant differences were recorded among anti-stressors. Pod fresh weight of all anti-stressors was significantly higher than the control at all salinity levels without significant differences among anti-stressors (Fig. 2C).



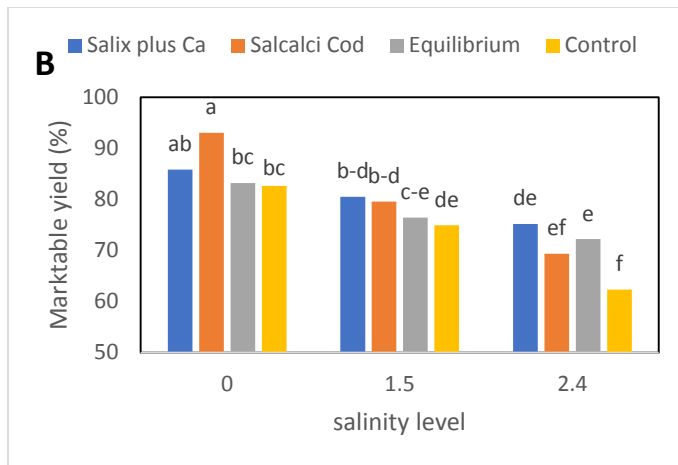


Fig.2. The combined over both seasons (2019-2020 and 2020-2021) of different anti-stressors (Salix Plus Ca, Salcalci cod, Equilibrium and control) on (A) Total yield (g/m²), (B) Marketable yield (%) and (C) pod fresh weight (g) of green bean plants grown under different concentrations of salt stress (0, 1.5 and 2.4 dS m⁻¹), 7 weeks after seed sowing. (n=3). letters in common are not indicate a significant difference between treatments (LSD test at 5%).

Fig.3 show the combined over both seasons of different anti-stressors (SPCa, SCoD, Equi and control) on (A) proline, (B) peroxidase and (C) abscisic acid of green bean plants grown under different concentrations of salt stress (0, 1.5 and 2.4 dS m⁻¹). Proline content was increased with increasing the level of salinity from 0 to 1.5 dS m⁻¹. At the second level of salinity, the treated plants with anti-stressors recorded significantly higher proline content as compared to control. The same trend was revealed at the highest level of salinity. Moreover, SPCa recorded the highest value of proline as compared to all anti-stressors and control except SCoD (Fig.3A). The content of peroxidase was duplicated around 7 times by increasing salinity stress from 0 to 1.5 or 2.4 dS m⁻¹ (Fig.3B). No significant differences were recorded between anti-stressors and control in the 0 level of salinity. However, at both levels of salinity (1.5 and 2.4 dS m⁻¹), all anti-stressor recorded higher peroxidase as compared to control without significances among them.

Abscisic acid (ABA) was increased significantly by increasing salinity stress (Fig.3C). At the second and third levels of salinity, where all treated plants with anti-stressors recorded higher ABA compared than control. Also, SPCa and Equi obtained higher ABA as compared to control and SCoD in all salinity levels.

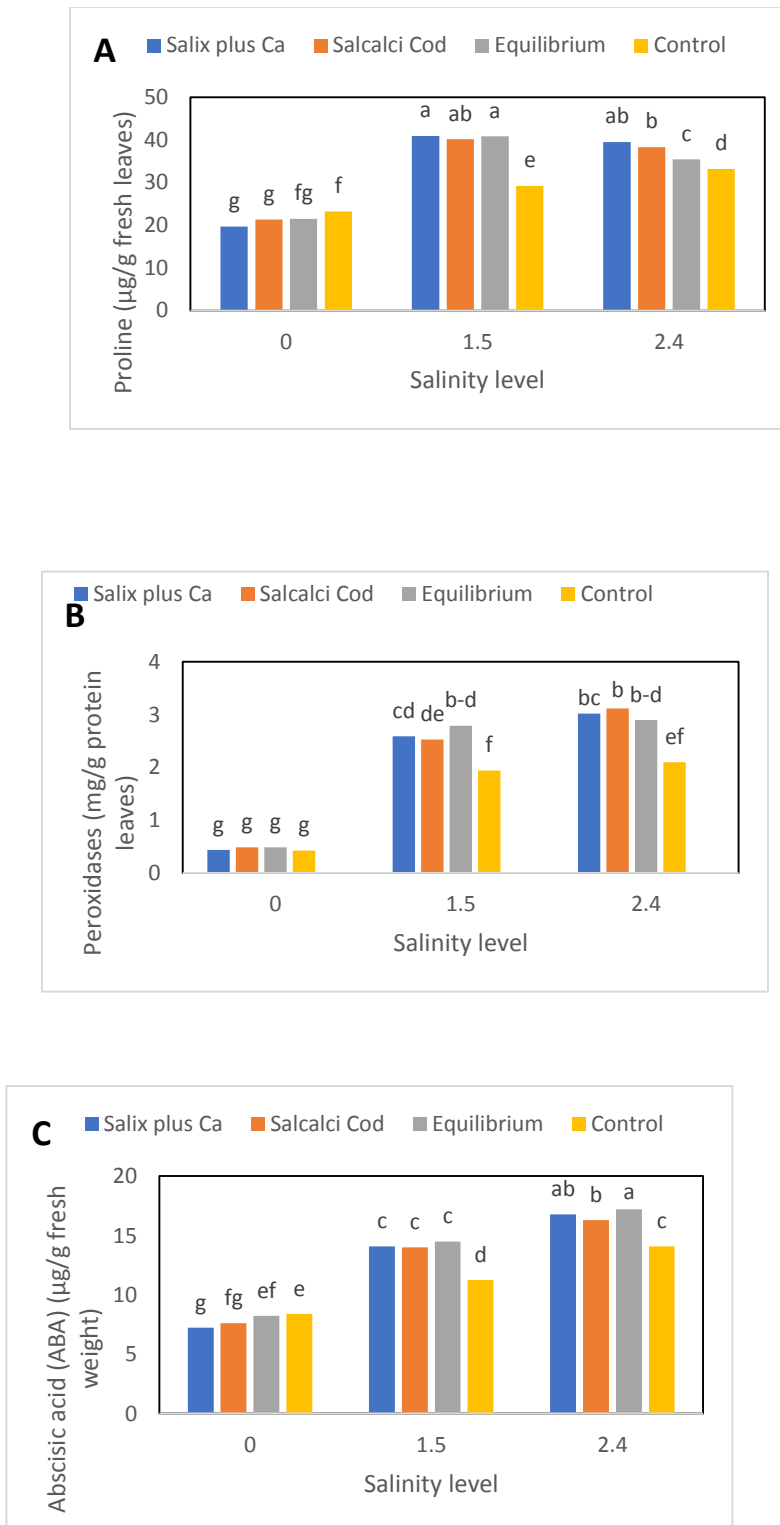


Fig.3. The combined over both seasons (2019-2020 and 2020-2021) of different anti-stressors (Saliks Plus Ca, Salcalci cod, Equilibrium and control) on (A) Proline, (B) Peroxidase and (C) Abscisic acid of green bean plants grown under different concentrations of salt stress (0, 1.5 and 2.4 dS m⁻¹), 7 weeks after seed sowing.

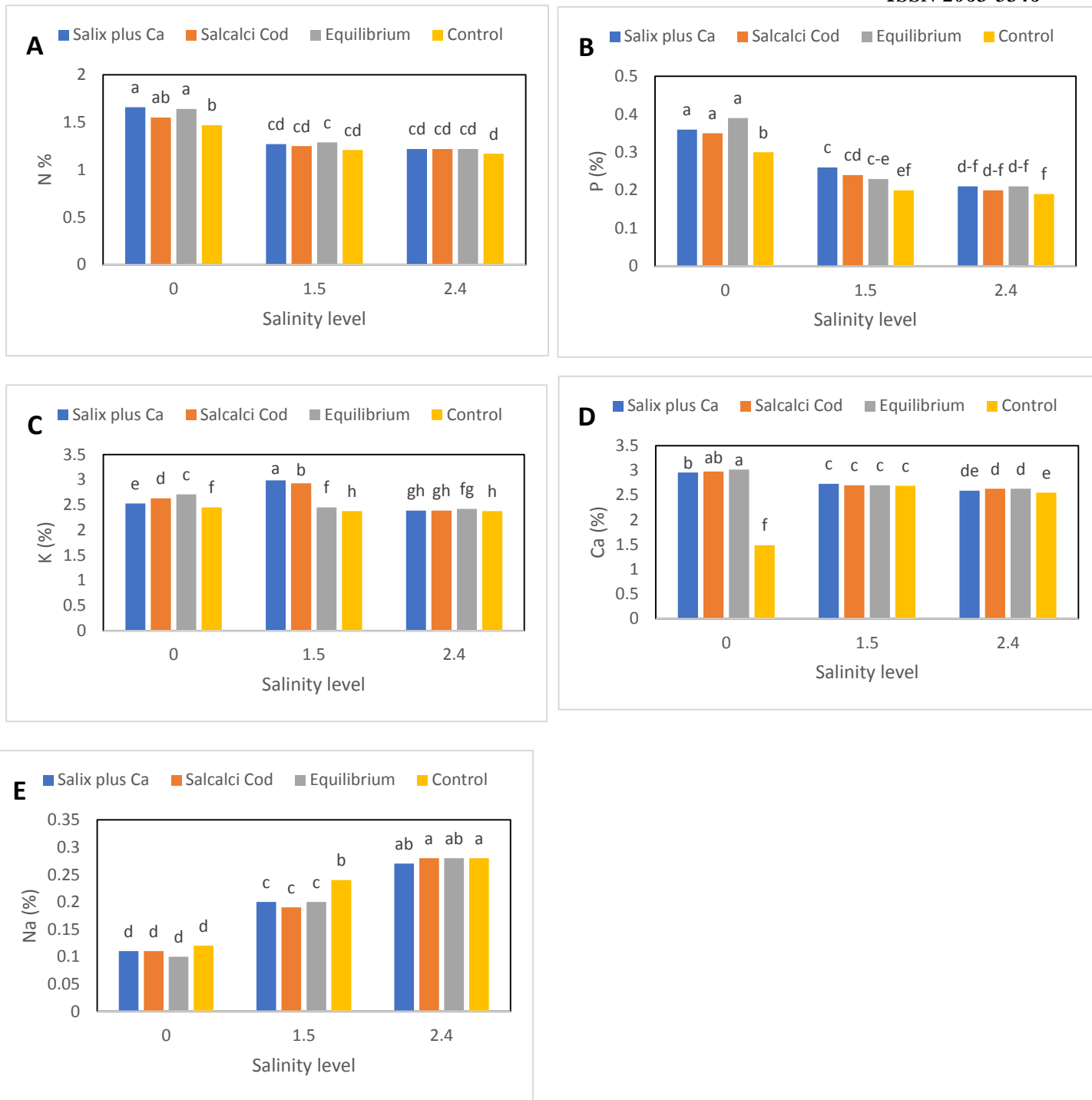


Fig.4. The combined over both seasons (2019-2020 and 2020-2021) of different anti-stressors (Salix Plus Ca, Salcalci cod, Equilibrium and control) on (A) N, (B) P, (C) K, (D) Ca and (E) Na of green bean plants grown under different concentrations of salt stress (1.2, 1.5 and 2.4 dS m⁻¹), 7 weeks after seed sowing. (n=3). letters in common are not indicate a significant difference between treatments (LSD test at 5%).

Fig.4 illustrated the combined over both seasons of different anti-stressors (SPCa, SCod, Equi and control) on (A) N, (B) P, (C) K, (D) Ca and (E) Na content of green bean plants grown under different concentrations of salt stress (0, 1.5 and 2.4 dS m⁻¹). N% decreased by increasing salinity level from 0 to 1.5 or 2.4 dS m⁻¹ (Fig. 4 A). The highest N% was obtained at the first level of salinity by SPCa and Equi. as compared to control. No significant differences were obtained among anti-stressors and control at both levels of salinity (1.5 and 2.4 dS m⁻¹). Also, P% decreased significantly by increasing salinity level (Fig. 4 B). At the first level of salinity, all anti-stressors obtained a higher value of P% as compared to control without significant differences among them. The same trend was revealed at the second level of salinity; however, no significant difference was recorded between Equi and control. At the highest level of salinity, no significant differences were obtained among anti-stressors and control. K% was varied at different salinity levels (Fig. 4C). Firstly, at the lowest level of salinity, all anti-stressors obtained a higher value of K% as compared to control. Furthermore, Equi recorded the highest value of K%. At the second level of salinity, all anti-stressors gave a higher value of K% as compared to control. Also, SPCa obtained the highest value of K% as compared to all anti-stressors and control. Contradicting at the highest level of salinity, all anti-stressors showed no significances as compared to control. Ca% was decreased significantly by increasing salinity level (Fig. 4 D). At the first level of salinity, all anti-stressors obtained a higher value of Ca% as compared to control without significant differences between SPCa and SCod or SCod and Equi. However, at the second level of salinity, no significances were recorded between all anti-stressors and control. Finally, at the third level of salinity, all anti-stressors obtained a higher value of Ca% as compared to control except SPCa. Na % was increased significantly by increasing salinity level (Fig. 4 E). No significant difference was recorded between all anti-stressors and control at the first level of salinity. At the second level of salinity, all anti-stressors caused obtaining a lower value of Na % as compared to control without significant differences among them. At the highest level of salinity, no significant differences were recorded between all anti-stressors and control.

4- Discussion

The detrimental consequences of salinity on plant development and productivity may be due to the harmed effect on cells division, elongation and plant organs development. A model of plants' biphasic reaction to salinity was reported by **Arif *et al.* (2020)**, who mentioned that the decrease in water potential of soil, leads not only to limited water-absorption capacity of plant roots, but also to a remarkable decrease in plant growth. The only determinants of osmotic stress in plants are sodium concentration and osmotic pressure. Thence, when the salt content rises, the old leaves begin to die, which indicates toxic stress and the subsequent suppression of plant growth. Salt stress stunted growth was evident in a decline in dry matter accumulation, height, leaf area, and the number of green leaves. Normal plant metabolism is hampered by salinity because it generates Na toxicity, which in turn impairs K uptake within root cells and has harmful impacts on genetically encoded enzymes (**Conde *et al.* 2011**). According to **Shahbaz *et al.* (2013)**, Increasing Na accumulation in plant cells causes K and Ca concentrations to decrease. In order to protect itself from salinity threat, a plant increases the synthesis of osmoprotectants or osmolytes and controls cellular nutrition homeostasis (**Singh *et al.*, 2015**). Osmoprotectants or Osmolytes are characterized by their low molecular weight, electrical neutrality, high solubility, and non-toxicity at molar concentrations. (**Ahn *et al.* 2011**). They have an Important function to help plants survive in extreme osmotic environments, such as salinity and drought. (**Nahar *et al.*, 2016**). At the same time, osmoprotectants are likely regulate cellular osmotic adjustment, minimize ROS-caused harm, avoid membrane damage and stabilize proteins and enzymes. (**Wani *et al.* 2013 and Singh *et al.*, 2015**). The strengthening of the antioxidant defense system and the maintenance of ion homeostasis are the two methods used by these osmoprotectants to detoxify the detrimental impacts of salinity on vegetation. (**Ashraf and Foolad 2007**). Several studies reported that the application of some additives i.e., Ca, K and humic acid on green beans or other legumes can alleviate the salt injures and enhance plants growth under salinity condition (**Aydin *et al* 2012, Kh *et al* 2012, Barakat *et al*, 2015, and Ismail and Halmy, 2018**). Ca application may mitigate the negative effects of salinity on plants by maintaining the integrity and function of plasma membranes in roots and branches. (**Lei *et al.* 2014**). Also, it is well known that Ca is an essential requirement for regulatory roles in metabolism in the plant (**El Habbasha and Ibrahim, 2015**). Na and Ca might compete for membrane-binding sites. Therefore, it has been hypothesized that elevated Ca concentrations can shield the

cell membrane from the damaging effects of salinity (Ouni *et al.*, 2014). Salinity could alter Ca²⁺ absorber and impart resulting in a Ca²⁺ deficiency in the plant and this agree with our results (Fig.4 D). Abdelhamid *et al.* (2010) confirmed that exclusion of Na⁺ ions and increased K⁺: Na⁺ ratios in salinity-stressed bean plants are essential selection criteria for salt tolerance. Important for maintaining a rapid rate of development and protecting metabolic processes from the deleterious effects of Na⁺ ions is a plant's capacity to restrict Na⁺ transport into its stem. (Wakeel 2013). Also, many studies reported that humic acid have a positive effect on plants under abiotic stress i.e., high or low temperature, drought and salinity (Aydin *et al.*, 2012) Carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone, and quinoid are the principal functional groups of humic substance. Humic acid increases membrane permeability, facilitates the transport of vital elements within the roots, and promotes respiration. Increasing cell membrane permeability, oxygen uptake, respiration, photosynthesis, phosphate uptake, and root cell elongation are however cited by some authors to explain the positive effect of humic acid on plant growth factors. (Bulgari *et al.*, 2019). All of the forementioned characteristics of humic acid may be enhanced the growth of treated plants with Ca and humic acid under salt stress at Equi anti-stressor. All plant growth parameters were enhanced by applying the anti-stressors as compared to control at moderate and high salinity levels (Fig.1 A-C). The fresh weight of pods reflects the plant's performance during previous development stages, which primarily depend on the vigor of vegetative growth and reproductive status. (Osman and Salim, 2015). Our findings revealed that pod fresh weight of all anti-stressors was significantly higher than the control at all salinity levels without significant differences among anti-stressors (Fig. 2C). The pervious result was reflected on total yield and marketable yield (Fig.2 A and B) and this was compatible with plant growth parameters. Khan and Basha (2015) mentioned that a lot of legumes are sensitive or moderately sensitive to salinity. Most of them recorded reduction in vegetative parameters and productivity after exposure to salinity stress at any stage of plant development. This agrees with our results, that increasing salinity level caused decreases in total yield and marketable yield. This reduction refers to lower average weight of marketable pods, and this reduction was increased by increasing salt concentration. In contrast, our findings showed that the anti-stressors enhanced the yield of green beans under salinity stress compared to control (Fig. 2 A). This ameliorates in the yield may be related to Ca and K which has a role as adjuncts to certain enzymes (such as peroxidases) which caused a breakdown of the toxic free radicals. This is very clear that the peroxidase values were increased by applied the anti-stressors as compared to control (Fig. 3B). Moreover, Antioxidant enzymes such as peroxidases promote ion absorption, cell division, and protein synthesis, resulting in an improvement of plant growth parameters. (Zayed *et al.* 2017). In the same context, Sharma *et al.* (2012) mentioned that changes in antioxidant activity and an imbalance in ROS generation are what cause the oxidative damage produced by salinity condition. Plants have developed a variety of antioxidant defense mechanisms to prevent the harm effect by oxidative stress; among the enzymatic ones, SOD serves as the first line of advocacy against ROS via converting the O₂ radical to H₂O₂. Numerous enzymes, can use hydrogen peroxide as a substrate. hydrogen peroxide concentrations are the lowest in cytosol, H₂O₂ is then excreted by peroxidases (Smirnoff and Arnaud, 2019). An important physiological cursor of a plant's reaction to salt condition is proline accumulation (Li *et al.*, 2010). In our study, accretion of proline was increased in plants under terms of moderate and severe salinity (Fig.3A) In addition to the treated plants with anti-stressors recorded significantly higher proline content as compared to control. Moreover, SPCa recorded highest value of proline as compared to all anti-stressors and control except SCod (Fig.3A). These results may be attributed to existence of K in SCod content. These outcomes are consistent with the findings of Yousuf *et al.* (2015) who stated that Indian mustard (*Brassica juncea*) plants received 150mM NaCl + 6mM K + 5.6mM Ca as compared with recived150mM NaCl only. High levels of proline working as a shield in plants to protect plant from salt/ osmotic stressors through stabilization multiple functional components, including complex II electron transport, membranes, proteins, and RUBISCO enzymes, as well as regulating osmotic pressure. (Lauer, 2023). Plants benefit from the higher concentration of proline under conditions of salt stress because proline regulates leaf osmotic potential of and thus contributes to osmotic adjustment. In addition, proline could be shield enzymes and enhance membrane stability under diverse terms. Abscisic acid also increased by salinity stress and all anti-stressor recorded the higher peroxidase and ABA as compared to control without significances among them (Fig. 3C). Tolerant plants frequently activate cell signaling pathways, such as those that lead to the synthesis of ABA, osmoprotectants (amino acids, carbohydrates, and polyamines), specific proteins, and certain free radical scavenging enzymes, in response to salinity stress.. (Sengupta *et al.*, 2016).

5- References

- Abbas, G.; Rehman, S.; Siddiqui, M.H.; Ali, H.M.; Farooq, M.A.; Chen, Y. 2022. Potassium and Humic Acid Synergistically Increase Salt Tolerance and Nutrient Uptake in Contrasting Wheat Genotypes through Ionic Homeostasis and Activation of Antioxidant Enzymes. *Plants*, 11, 263. <https://doi.org/10.3390/plants11030263>
- Abdelhamid, M. T., Shokr, M. and Bekheta, M. A. 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. *Communications in Soil Science and Plant Analysis*, 41, 2713-2728.
- Aebi H. Catalase in vitro. In: *Methods in enzymology*. Elsevier; 1984. p.121–6.
- Ahn C, Park U, Park .PB. 2011. Increased salt and drought tolerance by D-ononitol production in transgenic *Arabidopsis thaliana*. *Biochem Biophys Res Commun* 415:669–674.
- Akladios, S.A. and Mohamed, H.M. 2018. Ameliorative effects of calcium nitrate and humic acid on the growth, yield, component and biochemical attribute of pepper (*Capsicum annum*) plants grown under salt stress). *Scientia Horticulturae*, 236 :244–250.
- Al-Naggar, A.M.M., Sabry, S.R.S., Atta, M.M.M., El-Aleem, O.M.A., 2015. Effects of salinity on performance, heritability, selection gain, and correlations in Wheat (*Triticum aestivum* L.) doubled haploids. *Scientia* 10 (2), 70–83.
- Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. and Hayat, S., 2020. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry*, 156, pp.64-77.
- Asfaw, K.G. 2021. Effects of salinity on seedling biomass production and relative water content of some haricot bean (*Phaseolus vulgaris*) varieties. *Asian Journal of Agricultural Sciences*. 3(4): 267-274.
- Ashraf M and Foolad M.R.2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:206-216.
- Association of Official Analytical Chemistry (AOAC). *Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, USA, 1990.
- Aydin, A., Kant, C. and Turan, M., 2012. Humic acid application alleviates salinity stress of bean (*Phaseolus vulgaris* L.) plants decreasing membrane leakage. *African Journal of Agricultural Research*, 7(7), pp.1073-1086.
- Barakat, M.A.S., Osman, A.S., Semida, W.M. and Gyushi, M.A.H., 2015. Influence of potassium humate and ascorbic acid on growth, yield and chemical composition of common bean (*Phaseolus vulgaris* L.) grown under reclaimed soil conditions. *International journal of academic research*, 7(1), 193-199.
- Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* 1973, 39, 205–207.
- Bulgari, R., Franzoni, G. and Ferrante, A., 2019. Biostimulants application in horticultural crops under abiotic stress conditions. *Agronomy*, 9(6), p.306.
- Bulgari, R., Franzoni, G. and Ferrante, A., 2019. Biostimulants application in horticultural crops under abiotic stress conditions. *Agronomy*, 9(6), p.306.
- Bustan, A., Sagi, M., De Malach, Y., Pasternak, D. 2014. Effects of saline irrigation water and heat waves on potato production in an arid environment. *Field Crops Research*, 90: 275-285.

Can-Chulim, A.; Cruz-Crespo, E.; Escobar, H.M.; Sánchez-Bernal, E.I.; Madueño- Molina, A.; Bojórquez-Serrano, J.I. and Mancilla-Villa, O.R. 2017. Phaseolus vulgaris response to salinity generated by NaCl, Na₂SO₄ and NaHCO₃. Revista Mexicana de Ciencias Agrícolas. 8 (6):1287-1300

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T. and Tokar, C., 2018. The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. *Agronomy*, 8(9), p.166.

Chakraborty, K.; Bhaduri, D.; Meena, H.N. and Kuldeepsingh Kalariya, K. 2016. External potassium (K⁺) application improves salinity tolerance by promoting Na⁺- exclusion, K⁺-accumulation and osmotic adjustment in contrasting peanut cultivars. *Plant Physiology et Biochemistry*. doi: 10.1016/j.plaphy.

Chen, M., Yang, Z., Liu, J., Zhu, T., Wei, X., Fan, H. and Wang, B., 2018. Adaptation mechanism of salt excluders under saline conditions and its applications. *International Journal of Molecular Sciences*, 19(11), p.3668. <http://www.mdpi.com/journal/ijms>

Conde A, Silva P, Agasee A, Conde C, Gero's H., 2011. Mannitol transport and mannitol dehydrogenase activities are coordinated in *Olea japonica* under salt and osmotic stress. *Plant Cell Physiol* 52:1766–1775.

Fales H, Jaouni T, Babashak J. 1973. Simple device for preparing ethereal diazomethane without resorting to codistillation. *Anal Chem*. 1973;45(13):2302–3.

FAO statistics division, 2023. Available online: <https://www.fao.org/faostat/en/#data>.

Garcia, C.L., Dattamudi, S., Chanda, S. and Jayachandran, K., 2019. Effect of salinity stress and microbial inoculations on glomalin production and plant growth parameters of snap bean (*Phaseolus vulgaris*). *Agronomy*, 9(9), p.545.

Hamaiel A.F., Hamada, M.S. ; Ezzat, A.S. and El-Habashy ,H.A. 2020. Mitigating of salinity stress and amelioration productivity of potato (*Solanum tuberosum* L.) using soil conditioners and foliar application of osmoprotectants. *Middle East Journal of Agriculture Research*, 09 |4|:737-748.

Helrich, K. *Official Methods of Analysis*, 15th ed.; Association of Official Agricultural Chemist: Arlington, VA, USA, 1990; Volume 1, p. 673.

Ibrahim, F.M. and El Habbasha, S.F., 2015. Chemical composition, medicinal impacts and cultivation of camelina (*Camelina sativa*). *International Journal of Pharm Tech Research*, 8, pp.114-122.

Ismail, E.E.M. and Halmy, M.M., 2018. Effect of proline and potassium humate on growth, yield and quality of broad bean under saline soil conditions. *Journal of Plant Production*, 9(12), pp.1141-1145.

Jackson, M.L. *Soil Chemical Analysis*; Text book; Printice-Hall of India, Privat Limited: New Delhi, India. 1973, 144–197, 381.

Jan, M.; ul Haq, M.A.; Tanveer ul Haq, Ali, A.; Hussain, S. and Ibrahim, M. 2020. Protective effect of potassium application on NaCl induced stress in tomato (*Lycopersicon esculentum* L.) genotypes, *Journal of Plant Nutrition*, DOI: 10.1080/01904167.2020.176607

Kh, T., Taïbi, F. and Belkhodja, M., 2012. Effects of external calcium supply on the physiological response of salt stressed bean (*Phaseolus vulgaris* L.). *Gen Plant Physiol*, 2(2-4), pp.177-186.

Khan, P.S.S.V., Basha, P.O., 2015. Salt stress and leguminous crops. In: Azooz, M.M., Ahmad, P. (Eds.), *Legumes under Environmental Stress*. John Wiley & Sons, Ltd., pp. 21–51.

Kronzucker, H.J., Britto, D.T., 2011. Sodium transport in plant: a critical review. *The New Phytologist* 189, 54–81.

Lauer, N., 2023. Linking whole-plant responses to cell physiology in glycophytes exposed to NaCl stress. *Acta Physiologiae Plantarum*, 45(2), p.36.

Lei, B., Huang, Y., Xie, J.J., Liu, Z.X., Zhen, A., Fan, M.L. and Bie, Z.L., 2014. Increased cucumber salt tolerance by grafting on pumpkin rootstock and after application of calcium. *Biologia plantarum*, 58(1), pp.179-184.

Li, G., Wan, S., Zhou, J., Yang, Z. and Qin, P., 2010. Leaf chlorophyll fluorescence, hyperspectral reflectance, pigments content, malondialdehyde and proline accumulation responses of castor bean (*Ricinus communis* L.) seedlings to salt stress levels. *Industrial crops and products*, 31(1), pp.13-19.

Mahdy, H.A., Zaki, M.F., Abd El-Rheem Kh, M. and Ibrahim, H.A., 2022. Physiological Studies on the Effect of Seaweed Extract and Potassium Humate on the Tolerance of Green Bean Plants to Saline Water Irrigation .. *Middle East J*, 11(4), pp.1307-1316.

Montoya, C.A., Lallès, J.P., Beebe, S. and Leterme, P., 2010. Phaseolin diversity as a possible strategy to improve the nutritional value of common beans (*Phaseolus vulgaris*). *Food Research International*, 43(2), pp.443-449. Osman, H.S., 2015. Enhancing antioxidant–yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. *Ann. Agric. Sci.* 60 (2), 389– 402.

Mori, M., Di Mola I. and Quaglietta Chiarandà, F. 2011. Salt stress and transplant time in snap bean: growth and productive behaviour. *International Journal of Plant Production* 5 (1), 1735-8043.

Nahar, K., Hasanuzzaman, M. and Fujita, M., 2016. Roles of osmolytes in plant adaptation to drought and salinity. *Osmolytes and plants acclimation to changing environment: Emerging omics technologies*, pp.37-68.

Osman, H.S. and Salim, B.B., 2016. Influence of exogenous application of some phytoprotectants on growth, yield and pod quality of snap bean under NaCl salinity. *Annals of Agricultural Sciences*, 61(1), pp.1-13.

Ouni, Y., Ghnaya, T., Montemurro, F., Abdelly, C. and Lakhdar, A., 2014. The role of humic substances in mitigating the harmful effects of soil salinity and improve plant productivity. *International Journal of Plant Production*, 8(3), pp.353-374.

Peleg, Z., Apse, M.P. and Blumwald, E. , .2011). Engineering salinity and water-stress tolerance in crop plants: getting closer to the field. In *Advances in Botanical Research*. Academic Press, 405-443.

Rizwan, M., Ali, S., Ibrahim, M., Farid, M., Adrees, M., Bharwana, S.A., Zia-ur-Rehman, M., Qayyum, M.F. and Abbas, F., 2015. Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. *Environmental Science and Pollution Research*, 22, pp.15416-15431.

Saidimoradia, D.; Ghaderia, N. and Javadi T. 2019. Salinity stress mitigation by humic acid application in strawberry (*Fragaria x ananassa* Duch.). *Scientia Horticulturae* 256 :108594. www.elsevier.com/locate/scihorti

Sengupta, A., Chakraborty, M., Saha, J., Gupta, B. and Gupta, K., 2016. Polyamines: osmoprotectants in plant abiotic stress adaptation. *Osmolytes and plants acclimation to changing environment: Emerging omics technologies*, pp.97-127.

Shahbaz M, Mushtaq Z, Andaz F, Masood A. 2013. Does proline application ameliorate adverse effects of salt stress on growth, ions and photosynthetic ability of eggplant (*Solanum melongena* L.)? *Sci Hortic* 164:507–511

Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany*, <https://doi.org/10.1155/2012/217037>

Singh, M., Kumar, J., Singh, S., Singh, V.P. and Prasad, S.M., 2015. Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Reviews in Environmental Science and Bio/Technology*, 14, pp.407-426.

Smirnof, N. and Arnaud, D., 2019. Hydrogen peroxide metabolism and functions in plants. *New Phytologist*, 221(3), pp.1197-1214

Snedecor, G. W. and Cochran, W. G. 1982. *Statistical Methods*. 7th Edition, Iowa State Univ., Press, Ames, Iowa, U.S.A., pp.325-330.

Soetan, K.O., Olaiya, C.O. and Oyewole, O.E., 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *African journal of food science*, 4(5), pp.200-222.

Suddarth, S.R.P. and J.F.S. Ferreira, L.F. Cavalcante, V.S. Fraga, R.G. Anderson, J.J. Halvorson, F.T.C. Bezerra, S.A.S. Medeiros, C.R.G. Costa, and N.S. Dias, 2019. Can humic substances improve soil fertility under salt stress and drought conditions? *J. Environ. Qual.*, 48:1605-1613.

Tobe, K., Li, X., Omasa, K., 2002. Effect of sodium magnesium and calcium salts on seed germination and radicle survival of a halophyte, *Kalidium caspicum* (Chenopodiaceae). *Australian Journal of Botany* 50, 163–169.

Tobe, K., Li, X., Omasa, K., 2004. Effect of five different salts on seed germination and seedling growth of *Haloxylon ammodendron* (Chenopodiaceae). *Seed Science Research* 14, 345–353.

Tuteja, N., Peter Singh, L., Gill, S.S., Gill, R., Tuteja, R., 2012. Salinity stress: a major constraint in crop production. In: *Improving Crop Resistance to Abiotic Stress*. Wiley-VCH Verlag GmbH & Co. KGaA, pp. 71–96.

Wakeel, A., 2013. Potassium–sodium interactions in soil and plant under saline- sodic conditions. *Journal of Plant Nutrition and Soil Science*, 176(3), pp.344-354.

Wang, M.; Zheng, Q.; Shen, Q and Guo, S. .2013. The Critical Role of Potassium in Plant Stress Response. *Int. J. Mol. Sci.*, 14, 7370-7390

Wani S.H and Gosal S.S., 2011. Introduction of OsglyII gene into Indica rice through particle bombardment for increased salinity tolerance. *Biol Plant* 55:536–540

Wani SH, Singh NB, Haribhushan A, Mir JI., 2013. Compatible solute engineering in plants for abiotic stress tolerance-role of glycine betaine. *Curr Genomics* 14:157–165

Yousuf, P.Y.; Ahmad, B.A.; Hemant, Ganie, A.H.; Aref, I.M. and Iqbal, M. 2015. Potassium and calcium application ameliorates growth and oxidative homeostasis in salt-stressed Indian mustard (*Brassica juncea*) plants. *Pak. J. Bot.*, 47(5): 1629-1639,

Yu GH, Li W, Yuan ZY, Cui HY, Lv CG, Gao ZP, Han B, Gong YZ, Chen GX., 2013. The effects of enhanced UV-B radiation on photosynthetic and biochemical activities in super-high-yield hybrid rice Liangyoupeijiu at the reproductive stage. *Photosynthetica* 51:33–44

Zayed, M.M., S.H. Elkafafi, M. Amina, G. Zedan and Sherifa and F. M. Dawoud, 2017. Effect of nano chitosan on growth, physiological and biochemical parameters of *Phaseolus vulgaris* under salt stress. *J. Plant Production, Mansoura Univ.*, 8(5): 577 – 585.

Zhang, J.L., Flowers, T.J. and Wang, S.M., 2010. Mechanisms of sodium uptake by roots of higher plants. *Plant and soil*, 326, pp.45-60.