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Influence of Salinity (NaCl and Na_2SO_4) Treatments on Growth Development of Broad Bean (*Vicia faba* L.) Plant

Hameda El Sayed Ahmed El Sayed

Department of Biology, Faculty of Applied Science for Girls,
University of Umm Al Qura, Makkah Al Mukaramah, Kingdom of Saudi Arabia

Abstract: Salinity treatments, (NaCl and Na_2SO_4) reduced plant growth and increased the accumulation of Na^+, P^{3+}, Fe^{3+}, Mn^{2+} and Cl^- in root, stem, leaf and legume. The uptake of K^+ reduced in the presence of both salts whereas uptake of Ca^{2+} retarded mainly by Na_2SO_4. Chlorophyll content was affected mainly by NaCl, while Na_2SO_4 treatment lowered the rate of photosynthetic activity. Both salt compounds increased the accumulation of free amino acids including free proline but reduced the protein content in the leaves. Salt tolerance capacity of the broad beans plant in which the observations of the effect of salinity determined by different concentrations of NaCl and Na_2SO_4 on growth, chloroplast pigments, photosynthetic, free amino acids, free proline, protein and inorganic elements on plants are combined with observations on growth and yield, in order to arrive at a better understanding of their salt tolerance, has been studied and discussed in this paper. The results for the latter only (fruiting stage) are presented.

Key words: Broad bean · *Vicia faba* L. · Salinity · NaCl · Na_2SO_4 · Growth · Pigments · Photosynthetic Activity · Elements · Free Amino Acids · Free Proline and Protein

INTRODUCTION

Salinity is known to retard plant growth through its influence on several facets of plant metabolism including osmotic adjustment [1], ion uptake [2] enzyme activities [3], protein and nucleic acid synthesis [4], photosynthesis [5] and hormonal balance [6]. Although much work has been done on the effect of salinity on various aspects of crop plant growth and development, little information is available regarding salt tolerance of bean (*Vicia faba*, L.). Metabolic studies in chloroplasts from salt stressed plants of peanut performed by Sanjeeva Reddy [7] revealed the sensitive nature of the plant. Several workers have noticed a decrease in net photosynthetic rate due to salt stress [8]. According to Nieman and Clark [9] this is mediated through the effect of salinity on photophosphorylation. Salinity is reported to affect the strength of the forces binding the complex of pigment-protein-lipid in the chloroplast structure [10].

Salinity of irrigation water as a cause of yield reduction has been the subject of many investigations. Salinity effect seems to depend also on other factors such as soil properties, climate, cultural practices and water management. Whereas many data are available with regard to the effect of salinity on crop yield, a great deal less is known about the physiological processes during growth. Studies concerning the effect on crop yield are generally not combined with studies on crop physiology, the first group being conducted in the field and the second mainly in the laboratory. The salinity effect on the water stress of the plant, its gaseous exchanges and its metabolism has been analyzed over short periods see for example [11-16].

The physiological responses of three different bean cultivars (cv. Lody, cv. Gina and cv. Tara) to salt stress under laboratory conditions grown in pots as hydroponic cultures in a half-strength Hoagland nutrient solution [17]. The plants were treated for 7 days with NaCl and Na_2SO_4 (100 mM), starting at the appearance of the first trifoliate leaf unfolded. It was established that the applied dose of both salt types caused stress in the young bean plants, which found expression in the suppression of growth and photosynthesis activity. The bean cultivars showed different reaction to salinity and the type of salt. It was evident that cv. Lody was most sensitive to salt stress [17]. The applied Na_2SO_4 caused stronger inhibition in all

Corresponding Author: Hameda El Sayed Ahmed El Sayed, Department of Biology, Faculty of Applied Science for Girls, University of Umm Al Qura, Makkah Al Mukaramah, Kingdom of Saudi Arabia.
E-mail, heelsayed@uqu.edu.sa / d.hameda@hotmail.com.

cultivars than those treated with NaCl. The amount of proline in the tissues of the salt-treated plants increased, while the cell water potential reduced.

MATERIALS AND METHODS

Seeds of broad bean (*Vicia faba*, L. Cv. Giza 2), were obtained from the Ministry of Agriculture, Egypt. Seeds were sown in pots (25 cm x 35 cm) in the greenhouse. When the seedlings were well established (after 15 days) salt treatment were commenced. These involved the application of NaCl and Na_2SO_4 to produce a concentration range (0.0, 50, 100, 150 & 200 mM). The treatments were applied twice a week alternating with watering the plants with equal amounts of water to compensate for the evapo-transpiration of water and avoid excessive salt accumulation in the plant. After approximately three months of treatment, the plants were harvested at the fruiting stage and analyzed for various growth parameters, inorganic and nitrogenous constituents and pigments with affect on photosynthetic activity. The analytical procedures were as follows:

Growth Parameters: At harvest plants of broad bean (*Vicia faba*, L. Cv. Giza 2) were divided into root and shoot, to measure the shoot height, root depth, shoot and root dry weights (D.W.).

Chloroplast Pigment Analysis: An 85% aqueous acetone extract of a known F.W. of leaf was assayed Spectrometrically (*LKB NOVASPEC*) at 664, 645, 420 nm [18]. The following equations were used to determined the concentration of the pigment fractions as ã/ml.

$$\text{Chlorophyll a} = 10.3\ E_{664} - 0.918\ E_{645} \quad (1)$$

$$\text{Chlorophyll b} = 19.7\ E_{645} - 3.870\ E_{664} \quad (2)$$

$$\text{Carotenoids} = 403\ E_{452} - [0.0264\ \text{Chl. a}\ (1) + 0.426\ \text{Chl. b}\ (2)] \quad (3)$$

The pigment fractions were calculated as µg Chl./mg D.W.

Photosynthetic Activity: Chloroplasts were prepared by the method of Aronoff [19] and Osman *et al.* [20]. Fresh leaves were shredded, ground for one min in a blender, using a buffered solution of 0.4 M sucrose, 20 mM HEPES-KOH (pH 7.8), 3 mM $MgCl_2$, 4 mM sodium ascorbate and 0.1% bovine serum albumin (BSA) [20]. The much was strained through cheese-cloth, filtered and the suspension centrifuged (1 min at 8,000 X g). The pellet was resuspended in the isolation medium, centrifuged (5 min at 300 X g) and the supernatant recentrifuged (10 min at 1,000 X g). The sediment was resuspended in a 2 ml buffer solution at pH 6.8 and the aggregates dispersed. The levels of chlorophyll a & chlorophyll b were determined by the method described by [21]. An aliquot of 0.2 ml of the chloroplast suspension was extracted with 3.8 ml of 85% cold aqueous acetone and the density of the extract measured at 652 nm. The chlorophyll content was calculated according to the following equation:

$$C = E_{652} \text{ X } 1{,}000/34.5 \text{ mg chl./L} \quad (4)$$

Where c = chlorophyll a & b.

The photosynthetic activity of the isolated chloroplasts was measured using potassium ferricyanide (5 x 10-4M) as an electron acceptor. Reduction of ferricyanide was monitored spectrophotometrically (*LKP NOVA SPEC*) at 420 nm at room temperature. The reduction mixture contained 0.2 ml of chloroplast suspension, (0.2 – 0.8 mg chl. ml-1), 3.8ml HEPES buffer (pH 7.8) and 5 X 10-4M potassium ferricyanide. The mixture was illuminated at 300 Wm-2 using a slide projector provided with a heat filter with a 24 v, 250 w quartz halide bulb, 15-45 cm from the well. The photosynthetic activity of the isolated chloroplasts was calculated from the standard curve and expressed as µmol fericyanide mg chl^{-1} h^{-1} [22].

Nitrogenous Components

Proteins: Dry samples collected during the growth study were analyzed for protein content according to Lowry *et al.* [23]., after precipitating the protein with 15% TCA at 4°C.

Total Free Amino Acids: Were determined by the method described by Ya and Tunekazu [24]. An aliquot of 0.1 ml plant extract was heated in a test tube with 1.9 ml of ninhydrin citrate buffer-glycerol mixture in a boiling water bath for 12 min and cooled at room temperature. Then the tube was well shaken and the optical density read at 570 nm. A blank was determined with 0.1 ml of distilled water and a standard curve obtained with 0.005 to 0.2 mM g glycine.

Free Proline: This was estimated using the acid ninhydrin method described by Bates *et al.* [25]. Two ml of water extract were mixed 10 ml of 3% aqueous sulfosalicylic acid. Two ml of this mixture was allowed to react with 2 ml acid ninhydrin-reagent and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C; the reaction was

terminated by cooling the mixture in an ice bath. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15-20s. The chromatophore-containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene as a blank. The praline concentration was determined from a standard curve.

Inorganic Mineral Elements: Ions content measurements were carried out after extraction with 0.1 nitric acid of the ashed (powdered) milled samples at 500°C obtained after combustion in a muffle furnace, the milled samples were estimated following the "wet ashing procedure" [26]; the acid digests of the oven dried samples were analysed. Oven dried plants were subjected to acid digestion and Sodium (Na^+), potassium (K^+) & calcium (Ca^{2+}) contents were estimated photo-metrically using a corning-400 flame photometer [27, 28]. The levels of manganese (Mn^{2+})and iron (Fe^{3+}) contents were determined using an atomic absorption spectrophotometer and using that described by Durie *et al*. [29], Phosphorous (P^{3+}) was estimated by the Molybdenum-blue method [28], while chlorides (Cl^-) were determined by the $AgNO_3$ titration method as described by Jackson and Thomas [30].

RESULTS AND DISCUSSION

Growth: Results showing the effect of treatments on shoot and root growth are presented in Figs 1 a and b. High concentrations of both NaCl and Na_2SO_4 greatly reduced growth in length of shoot and root and D.W. of all plant parts. Similar results have been reported by Gauch and Wadleigh [31]. Growth suppression seems to be a non specific effect of salt, depending more on the total concentration of soluble salts than on species ions [32]. In broad bean however, while both salts caused a reduction in growth, NaCl is more detrimental than Na_2SO_4. The effect of five irrigation water salinities (1,200 – 5,250 ppm) on different bean cultivars under greenhouse conditions studied by Fallth *et al.*[33]. Crop emergence and germination were not affected by any of the water salinity levels. Crop growth, green matter (G.M.) and dry matter (D.M.) components were significantly affected with increasing water salinity. As the salinity was increased the G.M. production ranged from 81034 g/pot to 7306 g/pot and D.M. production ranged from 38.58 g/pot to 21.53 g/pot. For centuries, agriculture in arid and semi-arid environments has faced an increase in soil salinity. Salinity is one of the most important a biotic stress factors limiting plant growth and productivity [34].

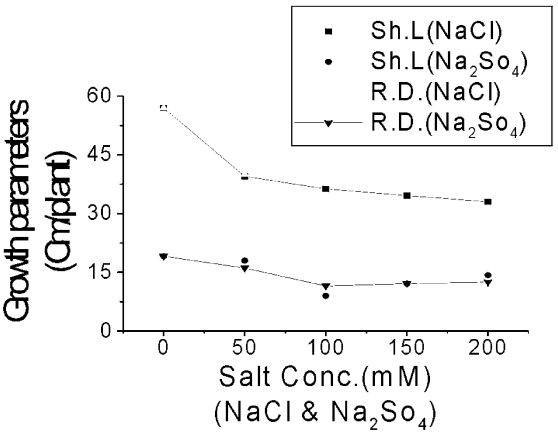

Fig. 1a: Effect of (a)- NaCl and (b)- Na_2SO_4 Salinity Treatments On Shoot Height, Root Depth, (cm/plant) of Broad Bean (*Vicia faba*, L. Cv. Giza 2) plant

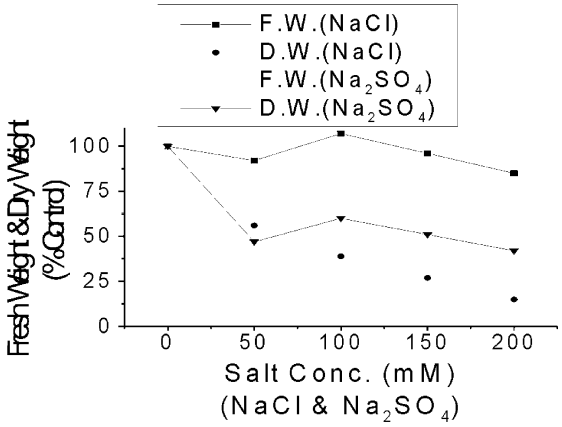

Fig. 1b: Effect of (a)- NaCl and (b)- Na_2SO_4 Salinity Treatments On Fresh and Dry Weights (g/plant) of Broad Bean (*Vicia faba*, L. Cv. Giza 2) plant

Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, water deficit, cause ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress [34, 35]. It was found the reduction of the biomass in beans under saline condition was indicative of several growth limitations, so, the salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio [17]. The salinity effect on leaf area and dry matter appeared 20 to 40 days later and finally caused a decrease of about 15%. The decrease in yield of grains was about 28%, although the average soil salinity, expressed as ECe, only equaled 2.4 d S/m for the most saline treatment [36]. The yield depression confirms

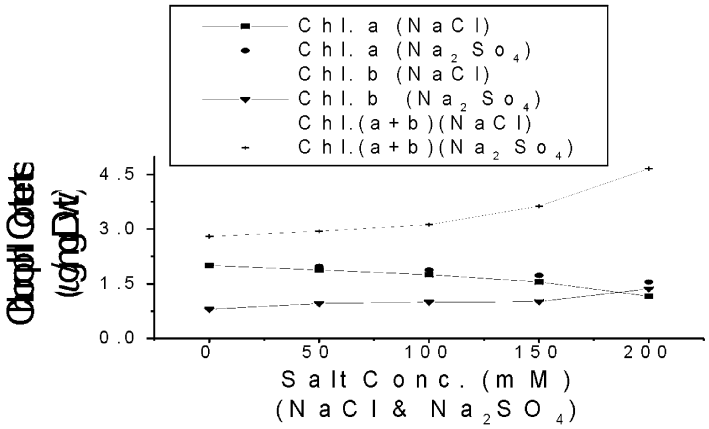

Fig. 2a: Effect of (a) - NaCl and (b) - Na_2SO_4 Salinity Treatments chlorophyll (a & b) and Chlorophyll Contents (a + b) (µg/mg F. Wt.) of Broad Bean (*Vicia faba*, L. Cv. Giza 2) plant.

Appendix Table 1: Statistical Analysis treatments, where relevant, the experimental data were subjected of One-Way analysis of variance (*ANOVA*)

NaCl			Na_2SO_4		
Chl. a	Chl. b	Chl. a+b	Chl. a	Chl. b	Chl. a+b
N.S.	N.S.	N.S.	N.S.	N.S.	*

Note*: F values* *= P> 0.05, ** = P >0.01, ***= P >0.001 and N.S..= Not Significant.*

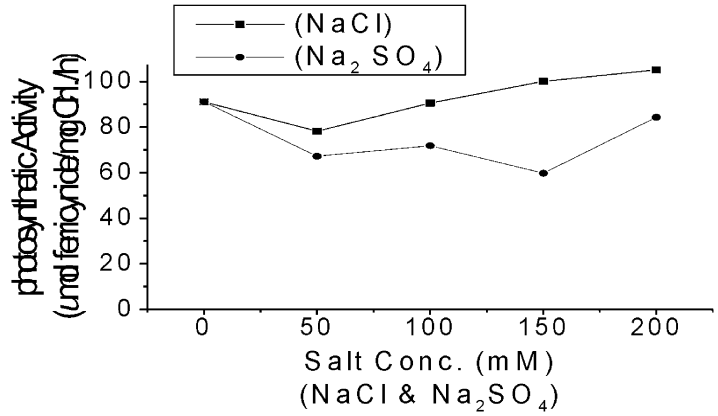

Fig. 2b: Effect of (a)- NaCl and (b)- Na2SO4 Salinity Treatments on Photosynthetic Activity (µmol ferricynide / mg chlorophyll / h.) of Broad Bean (*Vicia faba*, L. Cv. Giza 2) plant.

Appendix Table 2: Statistical Analysis treatments, where relevant, the experimental data were subjected of One-Way analysis of variance (*ANOVA*)

NaCl	Na_2SO_4
Photosynthetic Activity	Photosynthetic Activity
*	*

*Note: F values *= P> 0.05, ** = P >0.01, ***= P > 0.001 and N.S..= Not Significant.*

the low salt tolerance of broad beans [37]. The observation that the decrease in yield is mainly caused by a difference in the weight of the grains corresponds with the observation that the water stress was not significantly affected before the stage of flowering and fruit setting. It is possible that the effect of salinity would have been more pronounced if the soil salinity had been different from the start of the experiment [38].

Chloroplast Pigments and Photosynthetic Activity: The effect of salinity treatments on chlorophyll content is shown in Fig. 2 a & b. While NaCl tended to reduce chlorophyll content they were greatly increased by Na_2SO_4 levels. It was observed that plant species differed in their response to salinity with respect to chlorophyll content [39, 40]. In turnip and cabbage, chlorophyll content increased due to salinity.

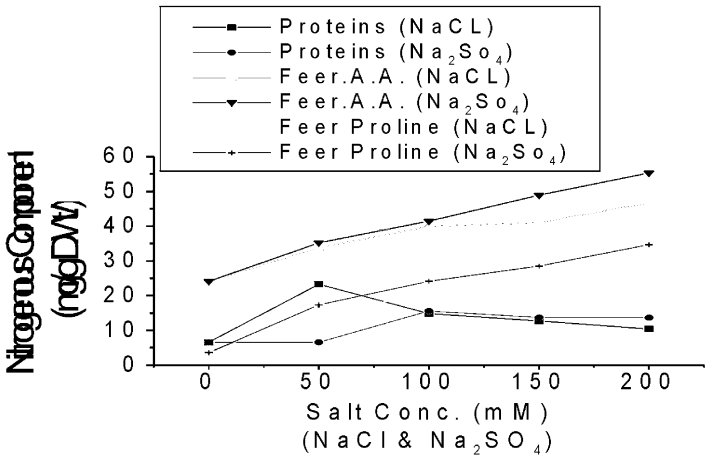

Fig. 2c: Effect of (a)- NaCl and (b)- Na_2SO_4 Salinity Treatments on Nitrogenous Components (Proteins, Total Free Amino Acids and Free Proline as mg/g D.Wt.) of Broad Bean (*Vicia faba*, L. Cv. Giza 2) plant.

Appendix Table 3: Statistical Analysis treatments, where relevant, the experimental data were subjected of One –Way analysis of variance (*ANOVA*)

NaCl			Na_2SO_4		
Proteins	Free amino acid	Free Proline	Proteins	Free amino acid	Free Proline
*	*	**	*	**	**

*Note: F values *= P> 0.05, ** = P >0.01, ***= P >0.001 and N.S..= Not Significant.*

In wheat, chloride salinity lowered total chlorophyll content while sulphate salinity did so only at higher concentrations. It would appear that salinities imposed by NaCl and Na_2SO_4 may influence chlorophyll metabolism differently. Photosynthetic activity is considerably affected by Na_2SO_4 treatment while NaCl produced no consistent trend. These results agree with those of [41] who noticed only a small effect of NaCl treatment (-20 bars) on photosynthetic rate of *Atriplex helium* while Na_2SO_4 reduced it even at 1 bar concentration. Conversely, in corn photosynthesis was found to be more susceptible to NaCl than Na_2SO_4 [42]; in barley, wheat and beans, however, there was no significant difference between the effects of NaCl and Na_2SO_4 on photosynthesis [43]. Thus there appears to be a variation in species response in photosynthetic rate to different types of salinities. The most important process that is affected in plants, growing under saline conditions, is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids [44]. Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes [45].

Nitrogenous Components: The effect of NaCl and Na_2SO_4 treatments on products of photosynthesis in *Vicia faba,* L. leaves is depicted in Fig 2 c. It is evident from the Fig. 2c that NaCl and Na_2SO_4 have affected nitrogenous component. NaCl and Na_2SO_4 both increased protein content, the former to a greater degree than the latter optima occurring at 50 and 100 mM, respectively. Total free amino acid, especially proline, tended to increase with salinity concentration especially with NaCl. These results are in agreement with those obtained by Matar *et al*. [46] that even a low sodium content in the plant can induce considerable changes in organic metabolism; specific anion effects however, should not be overlooked while considering this aspect. The increase in total free amino acid is significant and this may be one of the adaptive features particularly in the case of proline [47]. Proline tended also to increase with concentration particularly with NaCl more than Na_2SO_4. Stimulation of proline synthesis may contribute to the salt tolerance of *Vicia faba*, L. [47, 48]. A large number of plant species accumulate prolinå in response to salinity stress and that accumulation may play a role in combating salinity stress. However, data do not always indicate a positive correlation between the osmolyte accumulation and the adaptation to stress [49-51]. Salt stress induces cellular accumulation of damaging active oxygen species, which can damage membrane lipids, proteins and nucleic acids

Table (1:a): Effect of (a)- NaCl and (b)- Na_2SO_4 Salinity Treatments on Macronutrient Inorganic Elements (meq. /100 g D. Wt.) of Broad Bean (*Vicia faba*, L. Cv. Giza 2) plant

		NaCl (Mm)						Na_2SO_4 (Mm)					
Conc.													
Plant Parts		0.0	50	100	150	200	*F* Value	0.0	50	100	150	200	*F* Value
Root System	Na^+	5.65	33.93	41.75	46.30	54.37	**	5.65	42.62	50.02	40.10	27.84	**
	k^+	17.39	20.46	26.08	23.00	16.37	*	17.39	8.18	13.81	10.90	7.16	*
	Ca^{2+}	39.90	49.90	39.92	40.30	41.92	*	39.90	23.95	24.95	20.30	15.47	*
	K^+/Na^+	3.08	0.60	0.62	0.50	0.30	N.S..	3.08	0.19	0.28	0.27	0.26	N.S.
Shoot System	Na^+	3.48	9.13	50.02	45.10	38.27	***	3.48	23.05	9.13	25.8	41.75	**
	k^+	25.57	15.34	23.53	20.30	18.41	*	25.57	35.80	31.71	32.00	32.73	*
	Ca^{2+}	35.43	67.86	73.85	67.90	64.87	**	35.43	28.94	35.93	30.10	23.95	*
	K^+/Na^+	7.35	1.68	0.47	0.49	0.48	*	7.35	1.55	3.47	2.10	0.78	*
Leaves	Na^+	9.48	8.26	6.96	8.10	9.13	**	9.48	12.61	11.79	15.91	22.18	*
	k^+	25.57	22.51	16.37	19.70	21.99	N.S.	25.57	25.06	20.46	20.30	20.46	N.S.
	Ca^{2+}	279.44	239.52	259.48	225.10	189.62	**	279.44	99.80	169.66	176.30	179.64	**
	K^+/Na^+	7.35	2.72	2.35	2.39	2.41	*	7.35	1.99	1.74	1.30	0.92	*
Legumes	Na^+	4.78	11.74	26.53	34.90	43.93	**	4.78	20.88	15.22	25.70	36.97	*
	k^+	56.26	50.13	36.12	41.60	47.06	*	56.26	48.08	45.01	50.10	52.17	*
	Ca^{2+}	43.41	44.41	50.90	51.00	51.90	*	43.41	20.46	27.44	20.30	14.47	*
	K^+/Na^+	11.77	4.27	1.37	1.20	1.07	*	11.77	2.30	2.96	2.00	1.47	*

Statistical Analysis treatments, where relevant, the experimental data were subjected of One-Way analysis of variance (*ANOVA*).

*Note: F values *= P> 0.05, ** = P >0.01, ***= P >0.001 and N.S. = Not Significant.*

Table 1: b: Effect of (a)- NaCl and (b)- Na_2SO_4 Salinity Treatments on Micronutrient Inorganic Elements (meq /100 g D.Wt.) of Broad Bean (*Vicia faba*, L. Cv. Giza 2) plant.

		NaCl (Mm)						Na_2SO_4 (Mm)					
Conc.													
Plant Parts		0.0	50	100	150	200	*F* Value	0.0	50	100	150	200	*F* Value
Root System	Cl^-	3.38	22.48	32.71	22.30	13.25	**	3.10	17.70	21.80	23.10	10.80	**
	P^{3+}	3.23	8.07	6.46	6.40	6.46	*	3.23	5.65	5.97	6.90	5.97	N.S.
	Mn^{2+}	0.25	0.29	0.29	0.27	0.25	N.S.	0.25	0.29	0.29	0.31	0.29	Ns.
	Fe^{3+}	2.15	5.37	5.91	7.30	8.59	*	2.15	3.22	6.98	10.90	13.96	*
Shoot System	Cl^-	15.51	30.18	45.58	43.90	45.12	**	10.30	19.70	24.90	27.10	27.90	**
	P^{3+}	3.23	4.84	6.46	7.20	8.07	*	3.23	8.88	4.84	5.01	5.97	N.S.
	Mn^{2+}	0.22	0.22	0.22	0.22	0.22	N.S.	0.22	0.25	0.22	0.24	0.25	N.S.
	Fe^{3+}	2.15	12.35	6.44	11.30	15.04	*	2.15	8.06	5.91	0.59	5.91	*
Leaves	Cl^-	20.59	22.84	42.58	40.90	37.79	*	12.17	17.30	27.30	23.10	19.90	**
	P^{3+}	6.46	8.07	8.07	9.90	11.30	*	6.46	10.49	10.49	10.39	10.49	N.S.
	Mn^{2+}	0.25	0.26	0.27	0.28	0.29	N.S..	0.25	0.22	0.29	0.27	0.29	N.S.
	Fe^{3+}	5.91	5.91	8.06	8.20	8.59	*	5.91	8.59	8.59	8.10	7.52	N.S.
Legumes	Cl^-	8.18	8.18	47.38	37.00	20.59	*	7.30	7.10	29.20	27.30	12.30	*
	P^{3+}	11.30	12.91	11.30	15.90	19.37	*	11.30	11.30	12.11	12.50	13.72	N.S.
	Mn^{2+}	0.23	0.24	0.26	0.42	0.62	N.S.	0.23	0.25	0.25	0.26	0.25	N.S.
	Fe^{3+}	5.91	18.26	5.91	10.00	17.72	*	5.91	14.50	8.59	9.00	9.13	*

Statistical Analysis treatments, where relevant, the experimental data were subjected of One-Way analysis of variance (*ANOVA*).

*Note: F values *= P> 0.05, ** = P >0.01, ***= P >0.001 and N.S = Not Significant.*

[52, 53]. Lipid peroxidation, induced by free radicals, is also important in membrane deterioration [34, 54-56]. In most cases, salinity problems are linked to an excess of NaCl in the irrigation water, but sometimes other salts like Na_2SO_4 are present. There are few studies on the effect of Na_2SO_4 on plant growth.

Proline accumulation is an important physiological index for plant response to salt stress, as well as to other types of stress. Salinity increased markedly the Proline content in different salt sensitive and tolerant species/cultivars: with greater Proline accumulation in salt tolerant ones, which is supposed to correlate with the adaptation to salinity [39, 51]. The results presented in Fig. 4 implicated that NaCl and Na_2SO_4 stress increased Proline accumulation compared to the control in plant shoots. It was found that sulfate stress increased prîline accumulation higher than chloride type [17]. This fact suggested that the induction of proline synthesis is related not only to changes in the water potential and to the salinity type – chloride and sulfate, but also resulted

from metabolism interruption by high-stress intensity or from an adaptive response with special physiological function.

It was found the increased levels of proline, under salt stress, have been reported in two wheat cultivars [36]. It was suggested that proline accumulation may be caused by increasing proteolysis or by decreasing protein synthesis. The highest concentration of proline under salt stress is favorable to plants as proline participates in the osmotic potential of leaf and thus in the osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition. Proline accumulation may also help in non-enzyme free radical detoxifications [35, 57]. As lipid per-oxidation is the symptom mostly ascribed to oxidative damage, it is often used as an indicator of increased damage [34, 54, 58, 59]. In conclusion, salinity (NaCl and Na_2SO_4) induced a decrease in CO_2 fixation, due probably to both stomatal and non-stomatal limitations, however, with a differential sensitivity to the salt source. The different sensitivity of plant to the salt source can be attributed, at least in part, to a direct ionic effect. The growth processes in the bean plants were suppressed which was a result of the disturbed osmotic processes and the toxic effect of Cl^-, SO_4^{2-} and Na^+. The equimolar concentrations of NaCl and Na_2SO_4 suppressed, each to a different extent, the physiological processes in the plants. The weaker toxic effect in the plants treated with NaCl was probably a result, on the one hand, of the weaker inhibition of the water potential and the stomata closure and on the other hand, lower concentration of Na^+ in the tissues of the plants treated with chloride, compared to that in the plants treated with Na_2SO_4.

Mineral Nutrition: The effect of treatments on mineral constituents in root, stem, leaf and legume is shown in Table 1 a & b. With increasing salinity, Na^+ accumulated in all parts of the plant, to a greater extent with NaCl than Na_2SO_4. Sodium accumulation was more pronounced in root, stem and legume than in leaves; chloride content was increased in all parts, generally being greater than the Na^+ level. Osmotic effects are due to salt induced decrease in the soil water potential. Salinity results in a reduction of K^+ and Ca^{2+} content and an increased level of Na^+, Cl^- and SO_4^{2-}, which forms its ionic effects [51]. Reduction in biomass, photosynthetic capacity changes in leaf water potential and leaf turgor have been reported to have a cumulative effect attributed to salinity stress [60, 61], it is also cleared that several soil and other environmental factors do influence plant growth under salinity conditions. They reported differential absorption of Na^+ and Cl^- ions from the medium where their concentrations were equal [10]. It was also observed that no stochiometry was maintained between Na^+ and Cl^- content in NaCl treated Rhodes grass [62]. They reported that Cl^- content varied in various salt-stressed crops and except for the most salt tolerant sugar beet, it was always higher than Na^+ [63]. It was proposed that the high uptake of Cl^- relative to Na^+ in salt stressed plants could be responsible for growth inhibition by depressing uptake of other anions such as nitrates [64]. This may be one of the reasons for the differential influence of NaCl and Na_2SO_4 on growth in bean plants.

The K^+ content in various parts of salt stressed bean plants appears to be differentially influenced by NaCl and Na_2SO_4 (Table1 a). NaCl reduced the K^+ content in leaf, legume and stem, whereas it was increased in the root. Conversely with Na_2SO_4 the K^+ content was decreased in root, leaf and legume and increased in the stem. The K^+/Na^+ ratio is considerably decreased in all parts of the plant due to salinity, indicating that K^+ uptake is competitively reduced due to both salts. Such disturbance of K^+ uptake has been demonstrated by Lessani and Marschner [63]. Great importance has been attributed to K^+ in the process of salt tolerance by Bernstein [1] and Larsen [65]. According to Maas and Nieman [32], K^+ is important not only in osmotic adjustment to salinity but also to a wide range of turgor-mediated responses in plants including stomatal and leaf movements. The decrease in K^+ content in various parts of the bean plant in response to salinization, reflects the salt sensitive nature of this species.

Calcium content in the leaves was also reduced by both salts (Table 1a), to a greater degree by Na_2SO_4, which causes a decline in Ca^{2+} content in root, leaf, stem and legume; NaCl caused this reduction only in the leaves. Similarly Ca^{2+} uptake was inhibited by Na_2SO_4 to a greater degree than NaCl [66, 74]. While Ca^{2+} is reported to play a major role in salt tolerance [67, 68], most worker have observed a depressive effect of Na^+ on Ca^{2+} uptake [63]. According to Lessani and Marschner [63] Na^+ and Ca^{2+} ions probably compete much more for common uptake sites. From the present results it appears that Na^+ in combination with SO_4^{--} is more effective than with Cl^- in this respect.

Phosphorus content was increased due to salt stress in all plant parts (Table 1 b). The influence of salinity on phosphorus uptake, however, is controversial. A suppression of (P) uptake due to salt stress has been recorded by Strogonov [47] whereas increased P^{3+} content

due to salt stress has been reported by [69]. Indeed symptoms of P^{3+} toxicity induced by salinity have been recognized by Nieman and Shannon [70]. According to Wilson *et al.* [71] resistance to secondary salt induced stress in Glycine falcate, is due to its ability to maintain a high P^{3+} content in the presence of salt stress. It is possible that a high P^{3+} content in various parts of salinized bean plants may play a similar role.

Fe^{3+} and Mn^{2+} contents were increased in all parts of the salt treated bean plants (Table 1b), the increase in Fe^{3+} was more pronounced than that of Mn^{2+}. Similarly increased levels of Fe^{3+} resulting from salinity treatments have been reported for tomato, squash, soybean, [72] and pea [73], though Strogonov [47] observed a decreased in Fe^{3+} content due to NaCl salinity. Interspecies differences in response to salinization have been reported for uptake of Mn^{2+}. Maas *et al.*[72] reported that Mn^{2+} content increased in the shoots of tomato and soybean but decreased in tops of squash due to NaCl salinization. Dahiya and Singh [73] found suppression of Mn^{2+} uptake due to salinity in pea, while both NaCl and Na_2SO_4 exert a similar influence on Fe^{3+} and Mn^{2+} contents. A similar trend was also noticed by Maas *et al.* [72] in Tomato, soybean and squash. Such effects may be due to restricted growth of the tops [1]. If excessive accumulation of Fe^{3+} and Mn^{2+} crosses the physiological limits necessary for growth they may cause toxic effects.

It is evident from the foregoing discussion that both NaCl and Na_2SO_4 exert profound influence on the path of carbon and nitrogen assimilation during photosynthesis. NaCl and Na_2SO_4 however differ in their effect on carbon metabolism and only extensive enzymatic studies will throw more light on these effects. Such studies are in progress. It is apparent from the previous discussion that NaCl and Na_2SO_4 influence the uptake and distribution of ions like Na^+, K^+, P^{3+}, Mn^{2+} and F^{3+} in an almost similar manner though the values differ to some degree. Ione imbalance rather than restricted ion uptake may be involved in growth inhibition. Further in legumes ionic imbalance due to salt stress appears to influence the legume development process in broad bean plants.

CONCLUSIONS

The comparison between the effect of NaCl and Na_2S0_4 stresses on metabolites and mineral salts in broad bean plants (*Vicia faba* L.) showed some similarities and differences in response to these stresses. Both salts reduced plant growth through reduction of both chlorophyll content by NaCl and the rate of photosynthesis by Na_2S0_4 treatment. Both salts stimulated the accumulation of free amino acids and free proline, but reduced the protein content in the leaves. They also increased the accumulation of Na, P, Fe, Mn and CI in leaf, stem, root and legumes. Potassium reduced in both salts whereas uptake of Ca^{2+} was mainly reduced by Na_2S0_4. The relationships between the growth of Broad bean and salts (NaCl and Na_2SO_4) treatment were discussed.

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