



Research article

Comparative effect of sodium chloride and sodium sulfate on the biomass and quantitative changes in starch, protein and total soluble sugar in pea seeds (*Pisum sativum* L.)

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[Accepted: 07 December 2020]

Abstract: The nature of the soil is a very important factor in the growth and development of a crop. Crop plants suffer a decline in growth and yield, when exposed to the saline condition. Pea considers one of the main leguminous crops, due to its ability to produce significant quantities of protein, carbohydrates and nutrient-rich seeds. Plants were subjected to four salt treatments, 4, 8, 12 and 16 mmhos cm⁻¹ of sodium chloride and sodium sulfate and the biomass and biochemical responses were measured. All growth attributes such as stem, root and leaf fresh and dry weight decrease with the increased salinities doses. Salt treatments were no significant effects on the biomass and quantitative changes in starch, protein and soluble sugar in seeds of pea. But it was noted that the starch contents were much reduced in 16 mmhos cm⁻¹, the salinity level of sodium chloride and sodium sulfate as compared to control. The protein content and sugar content value were increased in a higher concentration of sodium chloride and sodium sulfate, when compared to control in Pea, CV. Azad P-1. The proline content increased with salt stress up to 8 mmhos cm⁻¹ in CV-Azad P-1. It was also observed that the high dose of sodium sulfate is declined biomass and quantitative changes in starch, than that of sodium chloride solution in pea seeds.

Keywords: Quantitative - Biomass - Soluble sugar - Na₂SO₄, - Azad P-1.

[Cite as: Gulafshan, Rehman F, Khan SJ, Tahir IK & Shaheen A (2020) Comparative effect of sodium chloride and sodium sulfate on the biomass and quantitative changes in starch, protein and total soluble sugar in pea seeds (*Pisum sativum* L.). *Tropical Plant Research* 7(3): 638–644]

INTRODUCTION

Garden pea (*Pisum sativum* L.) is a very popular nutritious vegetable crop, belong to family Fabaceae and grown in many of the middle-eastern countries. Pea is an important legume vegetable, consumed both as grain legumes and as a vegetable. Its seeds contain 18–20% dry matter whose 10–12% carbohydrate and 5–8% is protein (Vural *et al.* 2000). The plant grown under salinity conditions are stressed in three ways; phytotoxicity of Na⁺ and Cl⁻ ions, decreased water potential in the rhizosphere which caused water deficit and nutrient imbalance by the reduction in the uptake and/or shoot transport (Marschner *et al.* 1995). Sowires *et al.* (1997) reported biochemical changes associated with salt stress in Pea and Bean tissue culture. They found the concentration of sodium, carbohydrates and free proline generally increased with an increasing salinity level of sodium chloride. Negrao *et al.* (2017) observed that the soil salinity is a major abiotic constraint affecting crop yields such as relative growth rate, water relation, transpiration, transpiration use efficiency, ionic relations, photosynthesis, senescence, yield and yield components. Alam *et al.* (2015) reported that the salinity effect was evaluated based on of biomass yield reduction, physiological attributes. Ghosh *et al.* (2016) studied that the salinity has been a key abiotic constraint devastating crop production worldwide. According to Zribi *et al.* (2009) and Giuffrida *et al.* (2013), salinity reduced root bio mass in Broccoli and Cauliflower. Sodium, chlorine and boron are some elements, which have specific toxic effects on plants. Excessive accumulation of sodium in cell walls can rapidly lead to osmotic stress and cell death (Munns 2002). Salinity reduces yields and effects physiology and biochemistry of plants. Seed germination, water deficit, ion balance of the cellular ions and

osmotic stress is affected by salinity (Khan *et al.* 2002, Khan & Panda 2008). Ion toxicity and osmotic stress cause metabolic imbalance, which in turn leads to oxidative stress (Chinnusamy *et al.* 2006). Sodium sulphate (Na_2SO_4) is the dominant salt in alkaline soils, therefore sodium exists in the soil solution as well as on clay surfaces. Consequently, salinity stresses the plants in two ways, i. higher concentrations of salts in the soil make it harder for roots to extract water (osmotic stress), and ii. high salt level within the plant may be harmful (specific ion toxicity) (Munns & Tester 2008, Hussain *et al.* 2008). The salt ions like present in the Na^+ , SO_4^{2-} soil compete with the uptake of other nutrient ions like K^+ , Ca^{2+} , etc. which results in the nutritional disorder and eventually leads to reduce the quality and yield of plants (Grattan & Grieve 1999). Salinity can also be affected protein content negatively or positively when treated with different doses of salt concentration (Tort & Turkyilmaz 2004, Beltagi *et al.* 2006, Chen *et al.* 2007, Kapoor & Srivastava (2010). Thus, in the present study, an attempt has been made to understand the comparative effects of sodium chloride and sodium sulfate on the biomass and quantitative changes in soluble sugar, protein and starch of pea seeds (*Pisum sativum*) in cultivar Azad P-1.

MATERIAL AND METHOD

Plant material and culture

Investigate the comparative effect of salt stress on biomass and chemical parameter of Pea CV Azad P-1 will be screened out in Pod condition. Pea plants were grown in green houses and irrigated three time a week. After one month the plants were treated with different salinity level (4, 8, 12 and 16 mmhos cm^{-1}) of NaCl and Na_2SO_4 , according to Richard (1954). Treatments were completed during three consecutive weeks and the pots irrigated with distilled water every three days. After 95 days, the plants were selected and taken out of the soil carefully without damaging the root system, measured and analyzed. A total of three replicates were chosen for analysis of growth and biochemical measurement. All the data were statistically analysed using Completely Randomized Design (CRD) with three replications.

Growth measurement

The relative stress induced inhibition of biomass was estimated by determining the number of growth parameters in the control and the stressed plants, after 95 days after sowing. Plants were harvested for growth measurement. The fresh weight of root, stem and leaves were measured immediately. The dry weight were measured by drying the root, stem and leaves of the plants at 80°C to give a constant weight. Weight of the dry samples were recorded with the help of an electronic balance.

Determination of proline

Proline was determined by using ninhydrin reagent and measured according to Bates *et al.* (1973). Proline was extracted from 0.5 g of fresh leaf in 10 ml of 3% sulfosalicylic acid. 2 ml. of the extract was reacted with two ml. of glacial acetic acid for 75 min at 100°C and incubated in an ice bath and the reaction mixture was extracted with adding 4 ml. of toluene. The extract was vortexed for 20 second. The absorbance of toluene layer was spectrophotometrically determined at a wave length of 520 nm. Concentration was determined from a standard curve and calculated on a fresh weight basis ($\text{meo mol proline g}^{-1} \text{fw}^{-1}$).

Soluble sugar estimation

95 days old plants were carefully drag out from the pots. Pod of pea were collected and then taken out of the seeds in the pods. For soluble sugar contents, the sample was oven dried for 24 hrs. at 80°C . Soluble sugar was estimated in the ethanol soluble fraction as described by Nelson (1944). 100 mg of oven-dried sample (pea seed) was extracted in 80% ethanol. 0.5 ml aliquot was taken in a test tube and volume was made to 1 ml with double distilled water. After adding 1 ml copper reagent, the tube were bath for 20 minutes and then cooled at room temperature. Added 1 ml arsenomolybdate reagent than to each test tube. The final volume was made to 10 ml with distilled water. The absorbance was measured at 570 nm and expressed as mg g^{-1} dry weight.

Protein estimation

Protein (mg g^{-1} fresh weight of seed) was measured by the method of Lowry *et al.* (1951). (I) 5 ml of the copper solution was added to tubes containing 0.1 ml of the protein extract. The copper solution composed of (a) 100 ml of sodium chloride (0.1 N) in which were dissolved, 2 g of anhydrous sodium carbonates and 1 ml. of sodium tartrate (2.7%) (b) 1 ml of copper sulphate (1%). (a & b) were mixed immediately before use and the tubes were left for 15 minutes, then the optical density (o.D.) was measured at 570 nm. (II) The same steps were repeated with the standard solution (of Known conc.) of Bovine Serum Albumin. (III) Step (I and II) were

repeated thrice and the mean value of the three readings were compared with the standard curve of Bovine Serum Albumin.

Starch estimation

Starch content was estimated using the method of Paleg (1960). Fresh plant material (pea seed) was weight and transferred to pyrex stopper tubes containing 15ml. of 80% ethanol and placed in a boiling water bath .The sample was homogenized and centrifuged. The residue was dissolved in 20 ml. 0.7% KOH and it was gelatinized in boiling water bath for 40 minutes, cooled and centrifuged. Standard solution of 2% starch was prepared in 0.7% KOH. The final volume was made to 10 ml with distilled water and the absorbance was measured at 600 nm.

RESULTS AND DISCUSSION

Biomass

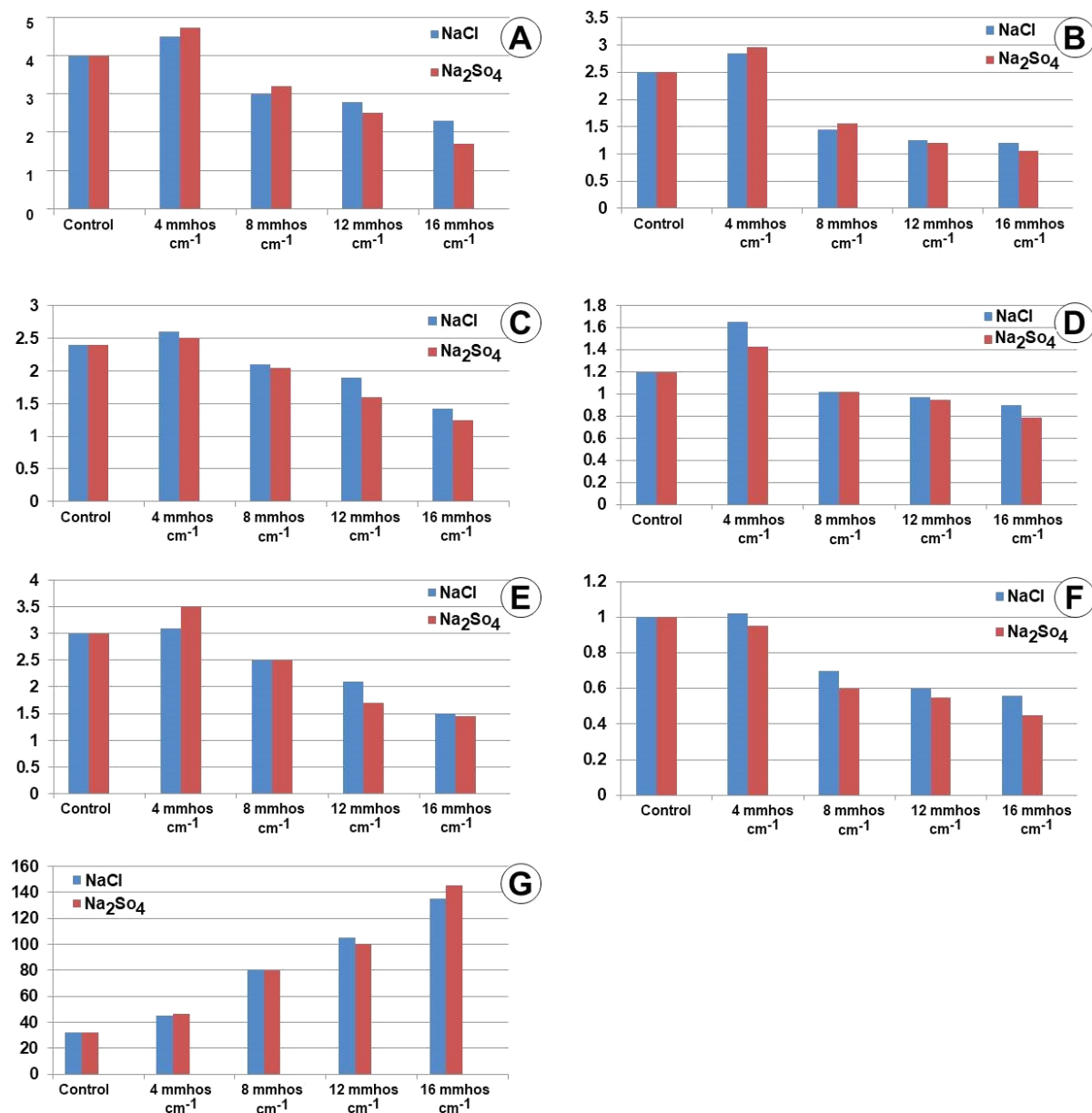


Figure 1. Effect of treatment with different concentration of NaCl and Na₂SO₄: **A**, Stem f.w. (g); **B**, Stem d.w. (g); **C**, Root f.w. (g); **D**, Root d.w. (g); **E**, Leaf f.w. (g); **F**, Leaf d.w. (g); **G**, Proline (µg g⁻¹ f.w. of leaf) of pea.

Going through the data in table 1 and figure 1, it is obvious that there is a negative effect for salt stress on the fresh and dry weight Stem, Root and Leaf of the Pea CV. Azad P1. The data indicates that control had a more fresh and dry weight of stem in comparison to salinity level of 8, 12 and 16 mmhoscm⁻¹ of sodium chloride and sodium sulfate. The maximum increase in fresh and dry weight of stem were achieved by using the minimum concentration (4 mmhos cm⁻¹). These result for fresh and dry weights in this study , agree with the result presented by Dantus *et al.* (2005), in their study on cowpea (*Vigna unguiculata* L.), where they report that www.tropicalplantresearch.com

using 10 mM of sodium chloride increased fresh and dry weights of shoot system of their seedlings. The salinity level of 16 mmhos cm^{-1} was most effective in decreasing the fresh and dry weight of stem in pea under both salt treatments. The result showed that excess of salt significantly reduced root fresh and dry weight due to reduced osmotic potential. In general excess salt, root zone has harmful effects on growth in plants. Fresh weight and dry weight of root gradually decreased with an increase in both salt treatments. Gomes *et al.* (2015) reported that excess salinity reduces growth and development and may even cause plant death because of the greater osmotic effect outside the root and the resulting restriction in water flow from the soil into plants, which plant need if they are to survive and grow under salt stress conditions. The reduction were more pronounced at higher concentration of Na_2SO_4 , due to the increased toxicity of sulfate over chloride rather than a promotion of sodium toxicity by sulfate. It was noted that salt stress also decreased the total fresh and dry weight of leaves in comparison to control. The fresh and dry weight of leaves significantly more decreased under the different concentration of Na_2SO_4 in comparison to different concentration of NaCl in Pea. The salinity level of 16 mmhos cm^{-1} of NaCl and Na_2SO_4 significantly reduced fresh and dry weight of leaf in CV. AzadP1, to respective control.

Table 1. Effect of treatment with different concentration of NaCl and Na_2SO_4 on Biomass (g per plant) of pea.

Treatment (mmhos cm^{-1})	Stem f.w. (g)		Stem d.w. (g)		Root f.w. (g)		Root d.w. (g)	
	NaCl	Na_2SO_4	NaCl	Na_2SO_4	NaCl	Na_2SO_4	NaCl	Na_2SO_4
Control	4.0	4.0	2.4	2.4	2.5	2.5	1.2	1.2
4	4.5	4.75	2.60	2.5	2.85	2.96	1.65	1.43
8	3.0	3.2	2.1	2.05	1.45	1.56	1.02	1.02
12	2.8	2.5	1.9	1.6	1.25	1.2	0.97	0.95
16	2.3	1.7	1.42	1.25	1.2	1.05	0.9	0.79
CD at 5%		=1.47		=0.90		=0.86		=0.78
SE=		=0.64		=0.39		=0.37		=0.33

Proline

The data given in table 2 represents the proline content as affected by chemical treatments. Proline content ($\mu\text{g g}^{-1}$ fresh weight of leaf) increased with both salt concentrations ranging from 4, 8, 12, & 16 mmhos cm^{-1} , but the moderate and high salinities induced a significant increase in proline content in the fresh leaves of cultivar Azad P1. In salt tolerant and salt sensitive cultivars of many crops, proline accumulation is a primary defense which response to maintain the osmotic pressure in a cell (Misra & Gupta 2005). There was a linear increase in free proline accumulation with increasing severity and duration of stress. Our results are in agreement with those previously reported for *Pisum* variety (Hokmabadi *et al.* 2005, Karimi *et al.* 2014).

Table 2. Effect of treatment with different concentration of NaCl and Na_2SO_4 on Leaf fresh and dry weight (g per plant) and Proline ($\mu\text{g g}^{-1}$ f.w. of leaf) of pea.

Treatment (mmhos cm^{-1})	Leaf f.w. (g)		Leaf d.w. (g)		Proline ($\mu\text{g g}^{-1}$ f.w. of leaf)	
	NaCl	Na_2SO_4	NaCl	Na_2SO_4	NaCl	Na_2SO_4
Control	3.0	3.0	1.0	1.0	32.25	32.25
4	3.1	3.5	1.02	0.95	45.35	46.12
8	2.5	2.5	0.7	0.6	80.51	79.85
12	2.1	1.7	0.6	0.55	105.25	100.20
16	1.5	1.45	0.56	0.45	135.07	145.54
CD at 5%		=1.29		=0.26		=25.18
SE=		=0.56		=0.11		=9.05

Soluble sugar

Salinity stress influenced soluble sugar in pea seed of CV. Azad P1, shown in table 3 and figure 2. A significant increase in the soluble sugar contents was observed in dry seeds of Pea at moderate and high salinities of NaCl and Na_2SO_4 . Soluble sugar contents were increased gradually by increasing both salt concentrations. The data indicates that a higher concentration of sodium sulfate had more increase (13.25 mg g^{-1}) soluble sugars in comparison to a high concentration of sodium chloride (12.57 mg g^{-1}). According to (Chelli-Chaabouni *et al.* 2010), stress plants accumulate compatible solutes such as soluble sugars and proline, which are known for their osmoprotection activity.

Protein

In table 3, results indicate a positive effect for NaCl and Na_2SO_4 using various concentration on the total protein of pea seeds. It appears from the data that there was a general increase in protein content that corresponded with the increase in both salt concentrations. Salinity level of 16 mmhos cm^{-1} was most effective

in increasing the protein content in pea seeds under both salt treatments. Kapoor & Srivastava (2010) observed an increase in protein content when increasing salt concentration on *Vigna mungo* L.

Table 3. Effect of treatment with different concentration of NaCl and Na₂SO₄ on Soluble sugar (mg g⁻¹ d.w.), Protein (mg g⁻¹ f.w.) and Starch (mg g⁻¹ f.w.) of pea.

Treatment (mmhos cm ⁻¹)	Soluble sugar (mg g ⁻¹ d.w)		Protein (mg g ⁻¹ f.w.)		Starch (mg g ⁻¹ f.w.)	
	NaCl	Na ₂ SO ₄	NaCl	Na ₂ SO ₄	NaCl	Na ₂ SO ₄
Control	8.22	8.22	28.04	28.04	42.23	42.23
4	9.15	8.97	30.15	31.29	39.27	35.26
8	11.05	10.05	35.50	36.15	32.18	30.05
12	11.51	12.67	40.45	40.95	27.37	24.15
16	12.57	13.25	45.25	47.28	20.14	18.15
CD at 5%		=1.05		=4.06		= 5.24
SE=		=0.34		=1.67		=2.09

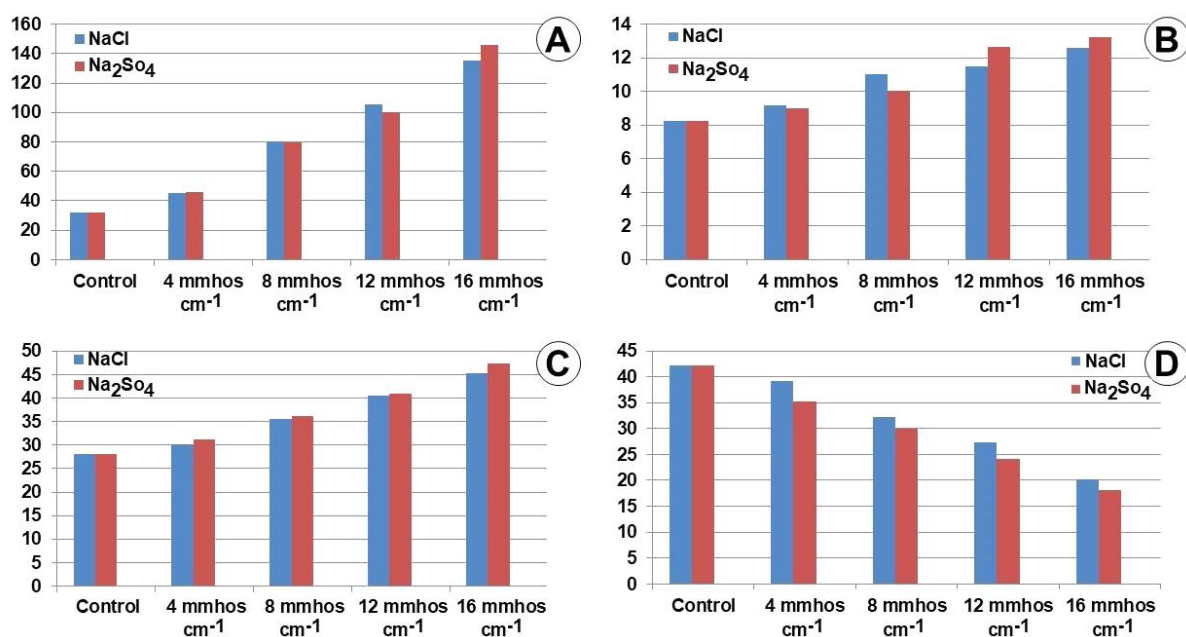


Figure 2. Effect of treatment with different concentration of NaCl and Na₂SO₄: **A**, Proline (µg g⁻¹ f.w. of leaf); **B**, Soluble sugar mg g⁻¹ d.w.; **C**, Protein (mg g⁻¹ f.w.); **D**, Starch (mg g⁻¹ f.w.) of pea.

Starch

Table 3 and figure 2 indicate the data on starch contents (mg g⁻¹fw.) as affected by NaCl and Na₂SO₄ salts. The starch content value was 42.23 mg g⁻¹fw in control. On the other side, values were 20.14 mg g⁻¹ and 18.15 mg g⁻¹ in salinity level of 16 mmhos cm⁻¹ of sodium chloride and Sodium sulfate treatments. It was noted that the starch content was much reduced in a higher concentration of NaCl and Na₂SO₄.

CONCLUSION

In the present study the adverse effect of salt stress decreased the biomass of pea plants. The reduction of biomass could be attributed to the osmotic effect of salinity stress which caused an increase of growth inhibitors, decreased growth promoter and disturbance of water in plant grown under salt stress. The reduction were more in a higher concentration of Na₂SO₄, due to the increased toxicity of sulfate over chloride rather than a promotion of sodium toxicity by sulfate. Starch contents were much reduced in high salinity level of sodium chloride and sodium sulfate, but the protein content and sugar content value were increased in a higher concentration of both salt stress. Salt stress registered a significant increase in proline content which support the hypothesis that proline acts as a protective compound during salt stress. These organic solutes could be used as a biochemical indicator for increased salt tolerance in the pea.

ACKNOWLEDGEMENTS

The authors are thankful to the head, department of botany, Mohammad Ali Jauhar University, for providing necessary facilities.

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