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Chitosan modulated antioxidant activity, inorganic ions homeostasis and endogenous melatonin to improve yield of *Pisum sativum* L. accessions under salt stress

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ABSTRACT

Effects of NaCl stress and foliar treatment of chitosan (CTS) on growth, antioxidant activity, inorganic ions and yield attributes of two pea (*Pisum sativum* L.) accessions were analyzed in this study. Experimentation was done in total 36 pots with 2 accessions and in three way completely randomized design (CRD) with 3 replicates. *P. sativum* plants were supplied with three salt concentrations (0, 60 and 120 mM) and two concentrations of CTS (0 and 120 mg/L). Both *P. sativum* accessions performed notably different from each other under salt stress. On the basis of remarkable reduction in biomass and yield parameters of ccession 200–03 under NaCl stress, it is assumed that this accession might be sensitive against salt stress. Whereas accession 200–06 under NaCl stress showed non-significant reduction in yield and biomass indicating that this accession might be resistant against salt stress. Foliar treatment of CTS boosted antioxidant enzyme activities, enhanced secondary metabolites (leaf proline and total phenolics), lowered the level of H_2O_2 and improved the RMP and yield in both ccessions. Salt stress resulted in enhanced content of endogenous melatonin. Chitosan treatment further boosted melatonin synthesis. It is concluded that foliar treatment of CTS mitigated the deleterious effects of salt stress and modulated growth in *P. sativum* plants under salt stress. Therefore, it is recommended that chitosan induced growth modulation in plants may be exploited.

1. Introduction

P. sativum is an important legume vegetable crop in the world. It belongs to Fabaceae family. It is cultivated all around the world as an essential component of human nutrition as well as animal fodder (Dahl et al., 2012). After soybean and common beans, *P. sativum* is 3rd utmost important legume crop (Timerman-Vaughan et al., 2004). It is consumed as fresh, canned or frozen vegetable. It is cool season crop and gives maximum yield in humid and cold regions. 1–6 °C is the minimum range of temperature required for *P. sativum* seed germination and *P. sativum* plant survives at 5 °C (Duzdemir et al., 2009). It can grow in various soil types ranging in texture from sandy loam to heavy clay (Debicz and Wróblewska, 2011).

It is a key source of high-quality vegetable proteins, minerals, vitamins, complex carbohydrates and fiber in the human diet. (Dahl et al., 2012; Tulbek et al., 2017). Proteins of *P. sativum* and their hydrolysates have many benefits regarding health like antihypertensive, antioxidant, lowering cholesterol, anti-inflammatory and modulation of intestinal bacterial activities, additionally *P. sativum* protein has various functional properties contributing to food product's texture and structure, including its oil holding capacity, water holding capacity, solubility, and foaming, emulsifying, and gelling properties (Ge et al., 2020). Few biological activities of proteins or protein hydrolysates in *P. sativum* can be increased by using some chemical or different combinational

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treatment approaches (Dominika et al., 2011; Wang et al., 2017; Zha et al., 2019; Li et al., 2020). Hence intake of *P. sativum* protein in daily diet is good to improve health of human beings as they have the potential of reducing risk of some chronic diseases (Li et al., 2011; Dahl et al., 2012).

In soil, accumulation of soluble salts upto an extent that agricultural and ecological well-being gets affected is referred to as salinity (Rengasamy, 2006). Soil salinity is an utmost threat to agriculture as it limits the crop productivity and growth of plant (Habib et al., 2016). Primary effects of salt stress on crops include osmotic stress whereas secondary effects include poor germination or delay in germination as well as growth abnormalities regarding post germination (Läuchli and Grattan, 2007; Munns and Tester, 2008). Effect of osmotic stress is prompt and plants manifest quick responses to osmotic stress whereas secondary effects of salt stress are comparatively slower and detrimental effects become evident at later stages when tissues of plants have accumulated sodium ions in such excessive amounts that ultimately leads to abnormalities regarding photosynthesis, cause cell damage and malfunctioning of various metabolic processes (Munns and Tester, 2008; Parihar et al., 2015; Hanin et al., 2016). In plant tissues, when accumulation of sodium ion increases upto 100 mM, cell division, structure of cell membrane and functions of enzymes are badly affected which ultimately leads to reduction in plant growth (Tuteja, 2007). In leaves which is the site for transpiration, toxic levels of ions cause cell injury and consequently leads to growth retardation (Parihar et al., 2015).

Due to salt toxicity, nearly 99 % of salt sensitive plant species die (Panta et al., 2014). Essentially in semi-arid and arid regions, soil salinity is most significant environmental factor as it reduces the productivity of crops and badly affects the soil porosity. Crop plants show variety of responses to soil salinity that results in poor yield. Salt stress disturbs many physiological, biochemical and metabolic processes like, photosynthesis, transpiration and respiration (Barnawal et al., 2014; Usman et al., 2023). Higher concentrations of salts in soil also damage photosynthetically active leaves, thus leads to early leaf senescence and chlorosis (Hanin et al., 2016). In addition to specific ion toxicity (Na^+), salinity stress further induces oxidative and osmotic stress. Indirect elevations in salt concentration disturbs the osmotic balance, that in turn obstructs plant's mechanism of water uptake (Farooq et al., 2017).

P. sativum is sensitive to oxidative, drought and salinity stress. Major reason behind infertile soil is aggregation of excessive salts, glycophytes such as *P. sativum* are not able to tolerate harsh environmental conditions, ultimately have reduced productivity. Furthermore, excessive salts result in growth inhibition, effects photosynthate availability, grounds infection and badly affect formation of nodules (Yousef et al., 2020). Elevation in salinity levels remarkably reduced *P. sativum* yield (Najafi et al., 2007). Upon exposure to salinity level of 100 mM, *P. sativum* productivity may get reduced upto 50 % (Subbarao et al., 1990). Salt stress severely effects yield, growth and development of *P. sativum* by restricting metabolic activities like osmotic potential, activities of enzymes and imbalance of ions (Vinocur and Altman, 2005). Gaseous exchange is also negatively affected by salinity as supply of CO_2 is restricted in leaves that ultimately results in lower degree of photosynthesis, production of H_2O_2, ^{-}OH and [O] and limited reaction of electron transport chain (Mateo et al., 2004). For cells of plants nascent oxygen is damaging because it causes necrosis (Shaukat et al., 2019).

Chitosan (CTS) is a simple linear polysaccharide made up of α, 1–4 linked D-glucosamine and N-acetyl-D-glucosamine (Sandford et al., 1992). It is a chitin derivative obtained by the de-acetylation of chitin present in the exoskeletons of arthropods that include crustaceans such as shrimps, crabs and lobsters, mollusc radula, insects, scales of lissamphibian and cephalopod's beak (Kurita, 2006). CTS is found in agriculture as an important elicitor and potential biostimulant. It is biodegradable, biocompatible and nontoxic that's why it's potentially broad application is favored (Hidangmayum et al., 2019). Along with changing climatic conditions and rapid increase in food demand that results in unendurable use of synthesized chemicals, CTS application as an evoker has the potential to address issues regarding both biotic and abiotic stress adaptation. Allan and Hadwiger (1979) studied application of chitosan for the first time. CTS reduces the detrimental effects of abiotic stresses via stress transduction pathway through secondary messengers and increases the physiological responses in plants (Hidangmayum et al., 2019). Treatment of chitosan provokes rate of photosynthesis, closure of stomata by abscisic acid synthesis; increase antioxidant enzymes through signaling pathway of hydrogen peroxide and nitric oxide and incites production of sugars, amino acids, organic acids, and many other metabolites required for energy metabolism, stress signaling and osmotic adjustment under stress. This is further used as antitranspirant compound in numerous plants via foliar application and provides protection against other negative effects (Hidangmayum et al., 2019).

Due to adverse climatic conditions, soil salinity is increasing drastically day-by-day that negatively affected various plants. There is urgent need to identify molecular, physiological, and biochemical salt tolerance markers to improve performance of crops under saline conditions. 20 % of arable land is salt affected and it is predicted to increase over 50 % by 2050. Our experiment will enable farmers to grow *P. sativum* crop in the saline soil without any damage to plants and reduction in yield. *P. sativum* is a cash crop and is essential constituent of human diet for having high quality protein contents. Its improved growth and yield even in saline areas will strengthen the world's economy and meet the food demand of fast-growing population. Objectives of this study was to examine the morphological, biochemical and physiological attributes of *P. sativum* plants under salt stress, to compare the performance of two *P. sativum* accessions (*Pisum sativum* L.) under salinity stress and to assess the potential role of CTS in mitigation of salt stress.

2. Research methodology

2.1. Research design and plant material

Two accessions of *P. sativum* 200–03 and 200–06 obtained from Ayub Agriculture Research Institute (AARI) Faisalabad were used for experimentation. Research area of botanical garden (Department of Botany) University of Education, Township campus Lahore was used to grow the plants for experiment. Experiment was conducted to investigate the impact of exogenously applied chitosan to address salt stress in ccessions 200–03 and 200–06. Experimentation was done in 36 pots of 2 accessions using complete randomized design (CRD) along with 3 replicates. 3 levels of salinity and 2 levels of chitosan treatment were given as following: Seeds were planted in prewashed sand in the first week of November 2022. For seed plantation plastic pots having a hole of one inch covered with cotton fabric were used to fill the sand. At one-inch depth of sand 6 seeds were planted in each pot. Within one week of seed plantation, seed germination was completed. After seed germination seedling thinning was done that resulted in 4 seedlings per pot. Hoagland's nutrient stock solutions were prepared in the laboratory. By mixing specific quantity of all these nutrient stock solutions with tap water 36 L Hoagland's solution was prepared in a plastic bucket having capacity of 40 L in the research area of botanical garden and one liter of this prepared solution was supplied to each pot from seedling to yield stage using a break of 2–3 weeks. Three treatments of salt (0 mM, 60 mM &120 mM) along with Hoagland's solution were applied after 28 days of seed germination by gradually increasing the salt levels from 30 mM and 60 mM until reached to the final levels of salt 60 mM and 120 mM. Foliar application of chitosan treatment (120 mg/L) was done on the three sets

Table 1
Levels of Salinity & Chitosan Treatment.

Sr. #	Treatment	Levels of Treatment
1	Salinity	0 mM, 60 mM & 120 mM
2	Chitosan	0 mg/L & 120 mg/L

of plants manually (see Tables 1 & 2). Low molecular wt. CTS (50,000–190,000 Da) having viscosity 20–300 cP was used.

2.2. Sample collection

After two weeks of treatment, one representative plant was uprooted from each of the 36 pots as a sample. Roots of sample plants were then rinsed with municipal water to remove the sand. After washing, extra water from the roots was removed using fluffy cloth. Roots of every sample plant were separated using a pointed blade.

2.3. Growth attributes

Data of following morphological parameters was recorded using weighing balance and measuring scale. Fresh weight of shoot (g); Dry weight of shoot (g); Fresh weight of root (g); Dry weight of root (g); Shoot length (cm); Root length (cm); No. of leaves; Total leaf area per plant (cm^2).

2.4. Stability of membrane

2.4.1. Malondialdehyde (MDA)

Lipid peroxidation was determined by making some modifications in (Cakmak and Horst, 1991) method in terms of the concentration of the thiobarbituric acid-reactive substances (TBARS). 0.2 g fresh leaves from each of the 36 pots were homogenized at 4 °C using 1 % trichloroacetic acid (3 mL). The homogenate was then transferred into the falcon tubes and centrifuged at 2000 rpm for fifteen min and supernatant was isolated from residue. 0.5 % TBA was prepared in 20 % TCA (trichloroacetic acid). Half mL supernatant was added into three mL of prepared TBA solution in a test tube. These test tubes were then incubated in shaking water bath (Model: HSW-1/06) at 95 °C for fifty minutes. After incubation the test tubes having reaction samples were placed in an ice water bath as to terminate reaction via cooling. After reaction termination, these reaction samples were centrifuged at ten thousand rpm for 10 min. Absorbance was read at 532 nm wavelength using spectrophotometer. For non-specific absorption, absorbance at 600 nm was subtracted from absorbance at 532 nm. By using the absorption coefficient 155 $mmol^{-1}$ cm^{-1} concentration of TBARS was figured out.

$$MDA\ (nmol) = [(A532nm - A600nm) / 1.56 \times 105] \times V/W \times 106$$

2.4.2. Relative membrane permeability (RMP)

RMP was determined by following (Yang et al., 1996) method. Young leaves of uniform size were excised from each of the 36 pots. Each leaf was cut into small pieces in a test tube containing 20 mL distilled H_2O_2. These test tubes having the sample leaves were then vortexed using Corning® LSE™ Vortex Mixer for 5 s and initial electrical conductivity (EC_o) of the solution was determined by Milwaukee MW805 MAX pH/EC/TDS/Temperature Portable Meter. After that these test tubes were kept in fridge at 4 °C for twenty-four hours and then EC_1 was recorded. After measuring EC_1 these test tubes having leaf samples were autoclaved at 121 °C for 20 min using autoclave model: SH-AC-60 M to determine EC_2.% of RMP was determined using formula as follows:

$$RMP\ (\%) = [EC1 - EC0 / EC2 - EC0] \times 100$$

2.4.3. Hydrogen peroxide determination (H_2O_2)

Following Velikova et al. (2000) method, 0.1 g fresh leaves were collected from each of the 36 pots for the determination of H_2O_2 level in plant tissues. Using pre-chilled pestle and mortar fresh leaves were grinded with 5 mL of 0.1 % trichloroacetic acid. Then homogenate was transferred into the falcon tubes and centrifuged at twelve thousand rpm for 15 min at 4 °C using HERMLE Z 326 K Universal Centrifuge. After that supernatant was separated from the residue. 500 μL of supernatant was added in 500 μL potassium phosphate buffer (pH 7) and 1 mL potassium iodide (1 M). Absorbance was read at 390 nm wavelength by spectrophotometer.

2.5. Biochemical parameters

2.5.1. Protein and antioxidant determination

0.5 g fresh leaves from each of the 36 pots were homogenized in 10 mL of chilled 50 mM potassium phosphate buffer (pH 7.8). The homogenate was then centrifuged at 6000 rpm and 4 °C temperature for 20 min. After centrifugation, supernatant was separated from the residue and kept in deep freezer for the estimation of TSP & antioxidant enzyme activities.

2.5.1.1. Total soluble protein content (TSP). TSP of extract was figured out by obeying (Bradford, 1976) method. The Bradford solution was prepared by dissolving 0.1 g Coomassie brilliant blue in fifty mL of 95 % ethanol. After that hundred mL of 85 % O-phosphoric acid was added and using distilled H_2O_2 made the volume upto 1 L. 0.1 mL of prepared extract was added into five mL of Bradford solution and vortexed. Absorbance was read at 595 nm wavelength using spectrophotometer. Bovine serum's standard curve was prepared by diluting the stock solution of bovine serum albumin as 100 mg, 200 mg, 300 mg, 400 mg and 500 mg from stock solution of 7.5 mg per fifteen mL. That curve was utilized for figuring out proteins in unknown samples.

2.5.1.2. Superoxide dismutase activity. SOD activity was figured out by obeying (Giannopolitis and Ries, 1977) method with some modifications. SOD activity is calculated based on the antioxidant's ability to prevent nitroblue tetrazolium's (NBT) photoreduction. A single unit of SOD activity was described by the concentration of the enzyme which inhibited fifty percent nitroblue tetrazolium's photoreduction. Reaction solution was comprised of 4 ingredients: NBT, riboflavin, EDTA and methionine. 0.0307 g NBT and 0.00038 g riboflavin were dissolved in hundred mL of distilled H_2O_2 in dark. 0.0019 g EDTA and 0.9699 g methionine were dissolved in 200 mL of distilled H_2O_2 in light. Then mixed both the solutions in dark and made the volume upto 500 mL using distilled water. For enzyme activity assay took 2.725 mL of reaction solution, distilled water (0.25 ml) and enzyme extract (0.025 ml) in a test tube. Four controls (two light and two dark controls) containing 2.725 mL reaction solution and 0.25 mL distilled water were prepared. Two light controls and all the test tubes containing reaction solution, distilled water and enzyme extract were placed under light conditions (60 W light) for 20 min. Whereas two dark controls were placed in dark for 20 min. Read the absorbance of samples at wavelength 560 nm via spectrophotometer.

2.5.1.3. Catalase and peroxidase activity. POD and CAT activity was determined through (Chance and Maehly, 1955) method with some modifications.

Activity of catalase. 3 mL catalase reaction mixture have one mL of fifty mM potassium phosphate buffer having pH 7, 1.9 mL of 5.9

Table 2
Application of Salt and Chitosan (CTS) treatments.

Sr. #	Plant sets	Treatments	Levels of Treatments
1	$L_1S_1C_1$	Control	0 mM + 0 mg/L
2	$L_1S_1C_2$	CTS	120 mg/L
3	$L_1S_2C_1$	NaCl	60 mM
4	$L_1S_2C_2$	NaCl + CTS	60 mM + 120 mg/L
5	$L_1S_3C_1$	NaCl	120 mM
6	$L_1S_3C_2$	NaCl + CTS	120 mM + 120 mg/L
7	$L_2S_1C_1$	Control	0 mM + 0 mg/L
8	$L_2S_1C_2$	CTS	120 mg/L
9	$L_2S_2C_1$	NaCl	60 mM
10	$L_2S_2C_2$	NaCl + CTS	60 mM + 120 mg/L
11	$L_2S_3C_1$	NaCl	120 mM
12	$L_2S_3C_2$	NaCl + CTS	120 mM + 120 mg/L

millimolar H_2O_2 and 100 μL enzyme extract. Enzyme extract was put in the reaction mixture while placing cuvette in spectrophotometer. Read the absorbance at 240 nm wavelength in every thirty *sec* within a time limit of 150 s.

Activity of peroxidase. 2 mL peroxidase reaction solution contained 700 microlitre of fifty millimolar potassium phosphate buffer having pH 5, six hundred microlitre guaiacol (20 mM), 600 microlitre of forty millimolar H_2O_2 and enzyme extract (100 μL). Using spectrophotometer read the absorbance at wavelength 470 nm with 30 s intervals in a time limit of 150 s.

2.5.2. Leaf proline

Proline content was measured by following (Bates et al., 1973) method. 0.2 g fresh leaf sample from each of the 36 pots was homogenized in 5 mL of 3 % sulfo-salicyclic acid. Homogenate was then filtered using whatman filter paper. Acid ninhydrin was prepared by dissolving 2.5 g of ninhydrin into sixty mL glacial acetic acid and forty mL O-phosphoric acid (6 M). 2 mL of homogenate filtrate was mixed with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin in a test tube. These test tubes having reaction solution were then incubated in oven (POL-EKO-APARATURA SP.J. Type: SLN 32 IG SMART) at 100 °C for 60 min. After incubating in oven these test tubes containing reaction solution were placed in an ice bath as to terminate the reaction via cooling. After that four mL of toluene was added in reaction solution mixture and mixed for one to two min by passing a continuous stream of air. Chromophore (containing toluene) was isolated from aqueous phase. Read the absorbance at 520 nm wavelength against blank (toluene) via spectrophotometer.

2.5.3. Total phenolics

Total phenolic content was determined by obeying (Kamath et al., 2015) method with some modifications. Leaf samples from each of the 36 pots were oven dried at 80 °C for overnight. 0.250 g grounded dry leaf sample was homogenized for one minute in 5 mL of 80 % acetone. This homogenate was centrifuged at 3000 rpm for fifteen minutes at 4 °C. After centrifuge let the supernatant of each sample to dry. Once supernatant was dried, added 5 mL of methanol in solid residue and vigorously mixed it for 1 and 2 min. After mixing let it stand for 2 min and then methanol layer was separated from the residue. Folin-Ciocalteu procedure was used for the determination of total phenolic content. Reaction mixture contained 800 microlitre of 7.5 % Na_2CO_3, thousand microlitre of Folin-Ciocalteu reagent and 2000 microlitre of sample. All these ingredients of reaction mixture were thoroughly mixed and let it stand for thirty minutes. After that read the absorbance of samples at wavelength 765 nm by spectrophotometer. Phenolic content determined as gallic acid equivalents (mg) per dry material (g).

2.6. Inorganic ions

Plant samples from each of the 36 pots were oven dried at 70 °C. After drying samples were digested using (Wolf, 1982) method. 0.1 g of dry grounded sample was kept in 2 mL of sulphuric acid in digestion flask for 24 h. These samples were then heated on Magnetic Heating Stirrer (Model: 85–2). During heating when fumes started to produce added H_2O_2 until digestion mixture became transparent. Once digestion was completed filtered the mixture and using distilled water made the volume of filtrate upto 50 ml.

2.6.1. Determination of cations (Na^+ and K^+)

Using flame photometer (Sherwood model 360) Na^+ and K^+ cations were measured from the digested mixture.

2.7. Endogenous melatonin assessment

Byeon and Back (2014) published a method for measuring endogenous melatonin in plant leaves. To begin, 0.2 g of plant leaves were pulverised with liquid nitrogen, followed by 1 mL of methanol. The resulting solutions were transferred to centrifuge tubes and stored overnight at 4 °C. After centrifugation at 10,000 g for 10 min, the supernatant was collected in new tubes. The remaining residues were dissolved in 0.5 mL of methanol after the supernatant was evaporated. The solution was filtered using a 0.22 m needle filter to get a clean sample. Melatonin concentrations were determined using high-performance liquid chromatography with a fluorescence detector system (Rigol L3000). A Kromasil C18 column (250 mm 4.6 mm particle size, Eka Nobel, Bohus, Sweden) was used for the experiment. The mobile phase was a 40:60 blend of methanol and ultra-pure water. The injection volume was 10 L, and the flow rate was 0.8 mL per minute1. The column temperature was held constant at 30 °C, and fluorescence detection was performed at excitation wavelengths of 280 nm and emission wavelengths of 340 nm. Melatonin concentrations in the samples were determined by comparing their peak regions to a standard curve generated using known melatonin values.

2.8. Yield parameters

After four months of germination yield from the mature plants was obtained and seeds were stored. Following yield parameters were recorded; no. of pods plant^{-1}; No. of seeds pod^{-1} and weight of 100 seeds (g).

2.9. Statistical analysis

Applied ANOVA on all parameters by using Cohort software, 1988–2004 (CoStat statics program of version 6.303). LSD mean compare test was performed with $p < 0.05$ significance level.

3. Results

3.1. Effect of salt stress and chitosan treatment on growth attributes

3.1.1. Fresh weight of shoot

Both accessions of *P. sativum* were highly remarkably distinct from each other with respect to shoot fresh weight. Table 3 shows that salt stress and foliar treatment of CTS have highly significant effect on shoot fresh weight in *P. sativum* plants. In accession 200–03 shoot fresh weight was reduced by 19 and 34 % under 60 and 120 mM NaCl stress respectively as compared to control. However, in accession 200–06 reduction under both levels of salt stress in shoot fresh weight was non-significant as compared to control. CTS's foliar treatment ameliorated the effect of salt stress and increased the shoot fresh weight in both accessions with respect to their controls. Maximum increase in shoot fresh weight due to chitosan application was observed at 120 mM NaCl stress in both accessions. However, this increase was upto 19 % in accession 200–03 and 14 % in accession 200–06 (Fig. 3-A). The interaction of Accession × Salt Stress was highly significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 3).

3.1.2. Dry weight of shoot

In case of shoot dry wt. both accessions of *P. sativum* behaved significantly different from each other under salt stress and foliar application of Chitosan (Table 3). There was significant reduction in shoot dry wt. of both accessions under NaCl stress. In accession 200–03 due to NaCl stress shoot dry weight get reduced by 41 % (60 mM) and 60 % (120 mM) whereas in accession 200–06 reduction was 12 % at both salinity levels as compared to control. Foliar treatment of CTS increased the shoot dry weight in accession 200–03 upto 24 % at 60 mM and 33 % at 120 mM salt stress as compared to untreated salt stressed plants. However, in accession 200–06 this increase was 13 % at 60 mM and 15 % at 120 mM salt stress as compared to untreated salt stressed plants (Fig. 3-B). Two interactions Accession × Salt Stress and Accession ×

Table 3
ANOVA of shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW) and root dry weight (RDW) of two Pea (*Pisum sativum* L.) accessions under salt stress and foliar application of Chitosan.

Source of Variance	df	SFW		SDW		RFW		RDW	
		MS	P	MS	P	MS	P	MS	P
Accession	1	28.6011***	.0000	1.9363***	.0000	2.6349***	.0000	0.0715***	.0000
Salt stress	2	13.0536***	.0000	2.1631***	.0000	0.0294***	.0000	0.0022***	.0000
Chitosan	1	9.7677***	.0000	0.6478***	.0000	0.0273***	.0002	0.0026***	.0000
Accession * Salt stress	2	8.1421***	.0000	1.3453***	.0000	0.0158***	.0004	0.0005**	.0027
Accession * Chitosan	1	0.1100ns	.3894	0.0974**	.0100	0.0003ns	.6525	0.0004*	.0231
Salt stress * Chitosan	2	0.1170ns	.4534	0.0105ns	.4432	0.0007ns	.5976	0.0003*	.0245
Accession * Salt stress * Chitosan	2	0.0243ns	.8447	0.0064ns	.6069	0.0014ns	.3971	0.0000ns	.9146
Error	24	0.1432<-		0.012<-		0.0014		0.0001	

Chitosan were highly significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 3).

3.1.3. Fresh weight of root

Both accessions of *P. sativum* were highly significantly different from each other with respect to root fresh weight. Salt stress and foliar application of CTS have highly significant effect on root fresh weight in *P. sativum* plants (Table 3). In accession 200–03 root fresh weight was declined by 7 and 14 % under 60 mM and 120 mM NaCl stress, respectively, as compared to control. However, in accession 200–06 decline in root fresh weight was minimum as 4 % at 60 mM and 9 % at 120 mM NaCl stress as compared to control. CTS treatment mitigated the deleterious effect of sodium chloride stress and improved the root fresh weight in both accessions with respect to their controls. In accession 200–06 maximum improvement in root fresh weight due to chitosan application was upto 19 % at 60 mM NaCl stress whereas in accession 200–03 increase in root fresh weight due to chitosan application was non-significant under salt stress (Fig. 3-C). The interaction Accession × Salt Stress was highly significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 3).

3.1.4. Dry weight of root

In case of root dry weight, both accessions of *P. sativum* behaved significantly different from each other under salt stress & foliar treatment of Chitosan (Table 3). There was significant reduction in root dry wt. of both accessions under salt stress. In accession 200–03 due to NaCl stress root dry weight was lowered by 15 % (60 mM) and 26 % (120 mM) whereas in accession 200–06 reduction was 10 % (60 mM) and 26 % (120 mM). CTS treatment increased the root dry weight in accession 200–03 upto 11 % at 60 mM and 27 % at 120 mM salt stress as compared to untreated salt stressed plants. However, in accession 200–06 this increase was 8 % at 60 mM and 31 % at 120 mM salt stress as compared to untreated salt stressed plants (Fig. 3-D). The interaction Accession × Salt Stress was highly significant. Two interactions Accession × Chitosan and Salt Stress × Chitosan were significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 3).

3.1.5. Shoot length

Under salt stress both accessions of *P. sativum* showed significantly distinct behavior from each other with respect to shoot length. The interaction Accession × Salt Stress was highly significant. However, interaction among all three factors Accession × Salt Stress × Chitosan was non-significant (Table 4). Under salt stress, reduction in the shoot length of accession 200–03 was 18 % (60 mM) and 25 % (120 mM) as compared to control (Fig. 1). Whereas maximum reduction observed in the shoot length of accession 200–06 under salt stress was 6 % (60 mM) as compared to control (Fig. 2). Foliar treatment of CTS has non-significant effect on the shoot length of both accessions under salt stress (Fig. 4-A).

3.1.6. Root length

Both accessions of *P. sativum* were highly significantly different from each other with respect to root length. Salt stress had highly significant effect on root length in both accessions (Table 4). In accession 200–03 salt stress increased the root length upto 7 % (60 mM) and 12 % (120 mM) as compared to control. Whereas in accession 200–06 this increase was 6 % at both levels of salt stress. CTS treatment has non-significant effect on root length in both accessions (Fig. 4-B). Interaction among all the factors Accession × Salt stress × Chitosan was non-significant (Table 4).

3.1.7. Number of leaves per plant

Both accessions of *P. sativum* were highly significantly different from each other with respect to number of leaves per plant. Table 4 shows that salt stress and foliar application of CTS have highly significant effect on number of leaves in *P. sativum* plants. In accession 200–03 no. of leaves get reduced by 9 % and 18 % under 60 and 120 mM NaCl stress, respectively as compared to control. However, in accession 200–06 this reduction was upto 6 % (60 mM) and 10 % (120 mM) as compared to control. Under salt stress foliar treatment of chitosan have non-significant effect on number of leaves in both accessions (Fig. 4-C). The interaction of Accession × Salt Stress was highly significant. However, interaction among all the three factors Accession × Salt Stress ×

Table 4
ANOVA of shoot length (Sl), root length (RL), no. of leaves (NL) and leaf area (LA) of two Pea (*Pisum sativum* L.) accessions under salt stress and foliar application of Chitosan.

Source of Variance	df	SL		RL		NL		LA	
		MS	P	MS	P	MS	P	MS	P
Accession	1	996.4544***	.0000	2864.0336***	.0000	1877.7778***	.0000	1018.6736***	.0000
Salt stress	2	105.2708***	.0000	21.4608***	.0000	131.5833***	.0000	18.7344***	.0000
Chitosan	1	1.7778ns	.5951	1.1025ns	.3652	100.0000***	.0005	10.5625***	.0000
Accession * Salt stress	2	75.2153***	.0002	1.1536ns	.4232	42.5278**	.0043	8.5144***	.0000
Accession * Chitosan	1	2.3511ns	.5415	0.0025ns	.9653	0.4444ns	.7906	1.6469*	.0330
Salt stress * Chitosan	2	18.0469ns	.0718	0.1158ns	.9147	1.0833ns	.8400	0.3700ns	.3334
Accession * Salt stress * Chitosan	2	5.9136ns	.3953	0.1875ns	.8658	2.6944ns	.6510	0.1878ns	.5655
Error	24	6.1281<-		1.2939<-		6.1667<-		0.3217<-	

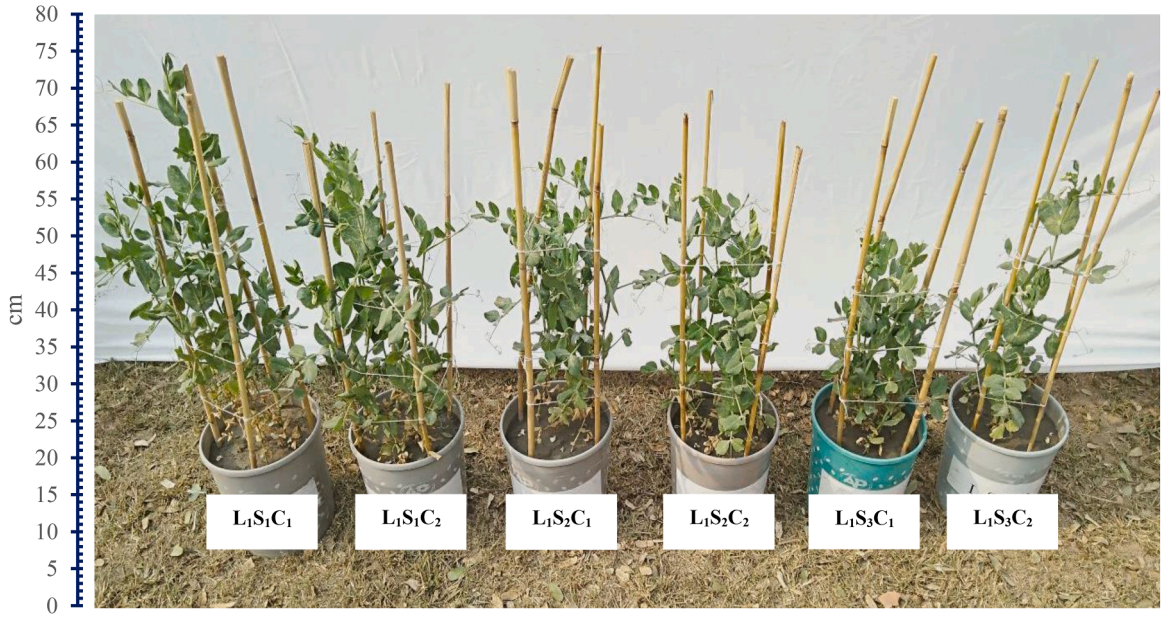

Fig. 1. Plants of pea accession 200–03. Control plant ($L_1S_1C_1$), Chitosan treated plant ($L_1S_1C_2$), 60 mM Salt stressed plant ($L_1S_2C_1$), 60 mM Salt stress + Chitosan treated plant ($L_1S_2C_2$), 120 mM Salt stressed plant ($L_1S_3C_1$) and 120 mM Salt stress + Chitosan treated plant ($L_1S_3C_2$). Plants shown here are 45 days old.

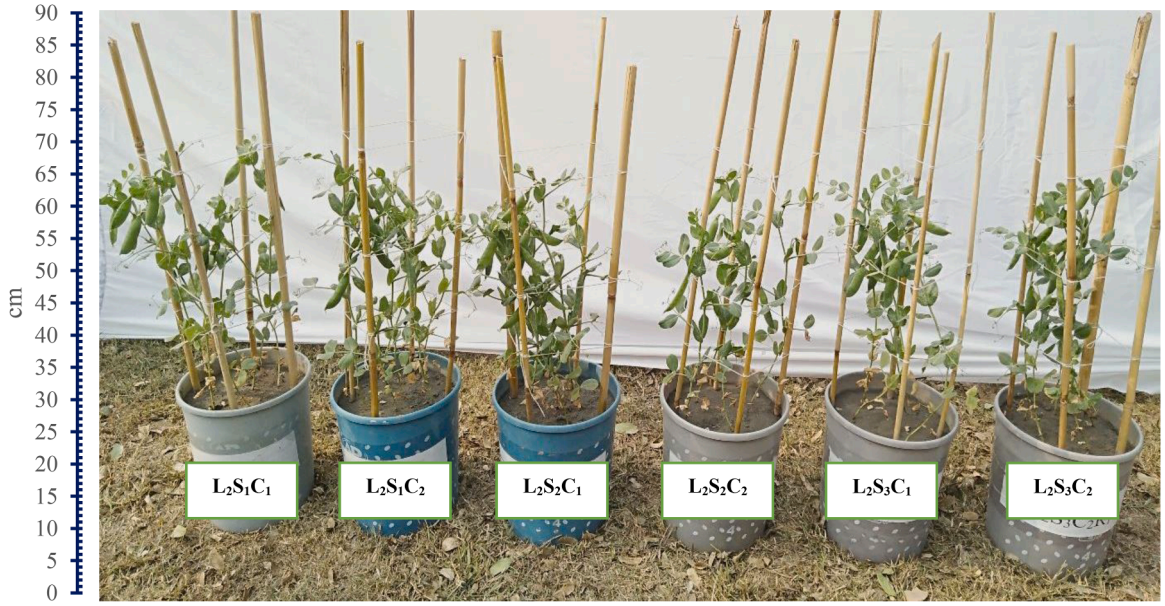

Fig. 2. Plants of pea accession 200–06. Control plant ($L_2S_1C_1$), Chitosan treated plant ($L_2S_1C_2$), 60 mM Salt stressed plant ($L_2S_2C_1$), 60 mM Salt stress + Chitosan treated plant ($L_2S_2C_2$), 120 mM Salt stressed plant ($L_2S_3C_1$) and 120 mM Salt stress + Chitosan treated plant ($L_2S_3C_2$). Plants shown here are 45 days old.

Chitosan was non-significant (Table 4).

3.1.8. *Total leaf area plant*$^{-1}$

In case of leaf area, both accessions of *P. sativum* behaved significantly different from each other under salt stress and foliar application of Chitosan (Table 4). In accession 200–03 due to NaCl stress leaf area get reduced by 7 % (60 mM) and 19 % (120 mM) whereas in accession 200–06 maximum reduction observed at 120 mM salt stress was 9 %. CTS treatment alleviated the injurious effects of NaCl stress and significantly expanded the leaf area upto 7 % at both salinity levels in accession 200–03 whereas in accession 200–06 this expansion was non-significant (Fig. 4-D). The interaction of Accession × Salt Stress was highly significant whereas interaction of Accession × Chitosan was significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 4).

3.2. *Effect of salt stress and chitosan treatment on stability of membrane*

3.2.1. *Malondialdehyde (MDA)*

Both accessions of *P. sativum* were highly remarkably distinct from each other with respect to MDA content. Salt stress & foliar treatment of CTS has highly significant effect on MDA content in both accessions. The interaction of Salt Stress × Chitosan was highly significant. However, the interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 5). Under salt stress elevation in MDA content was observed in both accessions. In accession 200–03 due to NaCl stress upto 13 % (60 mM) and 23 % (120 mM) elevation in MDA content was recorded as compared to control. Whereas in accession

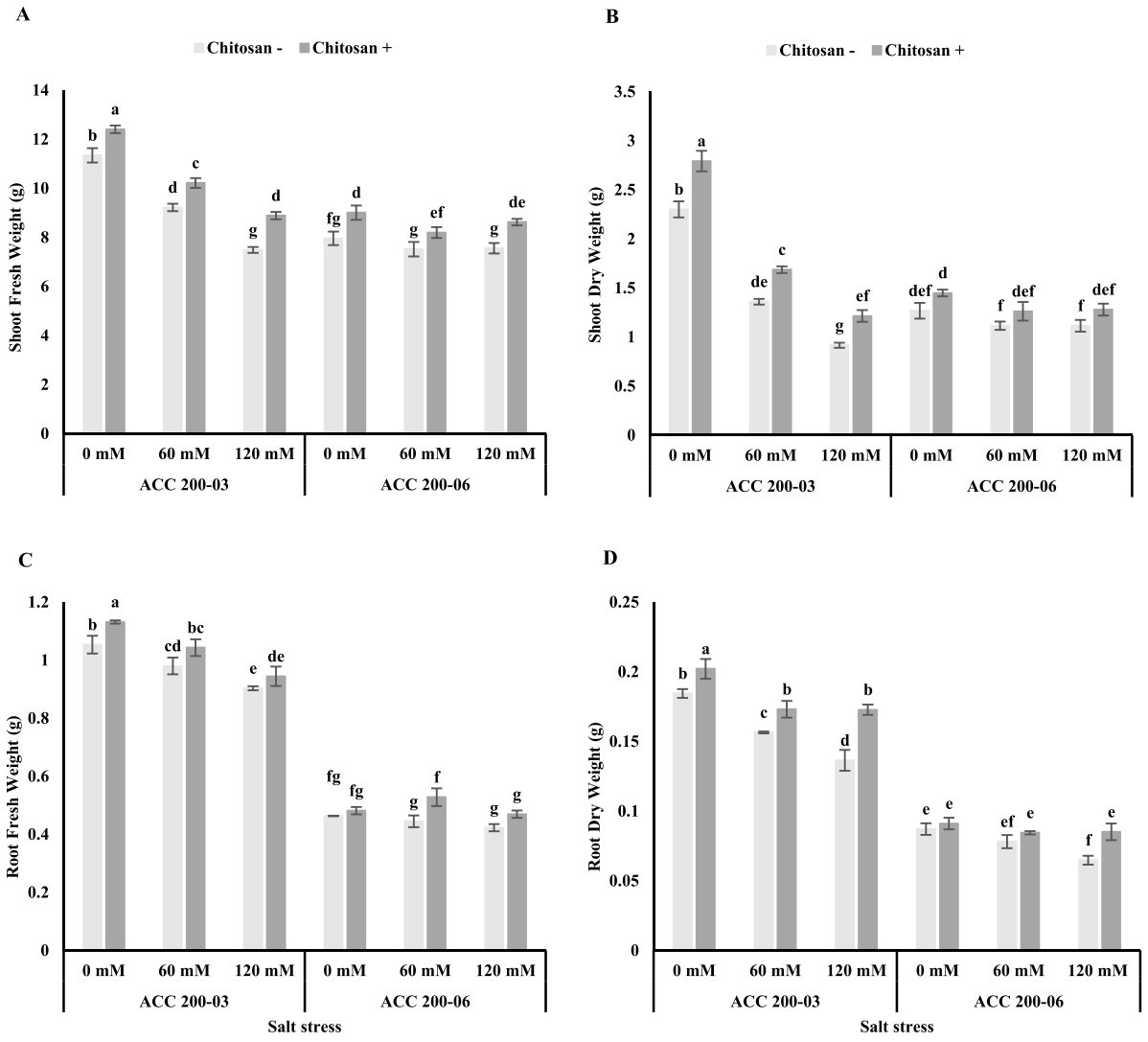

Fig. 3. Biomass of two *P. sativum* accessions under salt stress and foliar application of Chitosan. A. Shows shoot fresh weight, B. Shoot dry weight, C. Root fresh weight and D. Root dry weight. Data in (A-D) represents the average value of three replicates for every treatment ± errors bars as standard error (SE). Average values (bars in graph) with different letters (a, b, c and so on) describe statistically significant differences among the treatment groups as determined by three-way ANOVA followed by LSD test ($p < 0.05$).

200–06 elevation in MDA content was upto 19 % (60 mM) and 20 % (120 mM) as compared to control. Foliar treatment of chitosan notably lowered the MDA content in both accessions under salt stress. In accession 200–03 due to chitosan application upto 18 % (60 mM) and 32 % (120 mM) decline in MDA content was observed as compared to stressed untreated plants. Whereas in accession 200–06 this reduction was upto 15 % at both salinity levels as compared to stressed untreated plants (Fig. 5-A).

3.2.2. *Relative membrane permeability*

In case of relative membrane permeability, both accessions of *P. sativum* behaved significantly different from each other under salt stress and foliar application of Chitosan. Two interactions Accession × Salt Stress and Accession × Chitosan were highly significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 5). Salt stress increased the relative membrane permeability in both accessions of *P. sativum*. In accession 200–03 due to salt stress upto 17 % (60 mM) and 43 % (120 mM) increase in relative membrane permeability was observed as compared to control. Whereas in accession 200–06 relative membrane permeability was increased upto 23 % (60 mM) and 49 % (120 mM) as compared to control. CTS application alleviated the deleterious effect of salinity by lowering the relative membrane permeability in both accessions. In accession 200–03 due to chitosan application upto 23 % (60 mM) and 11 % (120 mM) reduction in relative membrane permeability was observed as compared to stressed untreated plants. Whereas in accession 200–06 this reduction was upto 13% (60 mM) and 14 % (120 mM) as compared to stressed untreated plants (Fig. 5-B).

3.2.3. *Hydrogen peroxide (H_2O_2)*

Under salinity stress and aerial treatment of CTS both accessions of *P. sativum* showed significantly distinct behavior from each other with respect to the level of H_2O_2. The interaction among all three factors Accession × Salt Stress × Chitosan was highly significant (Table 5). In accession 200–03 salt stress raised the level of H_2O_2 upto 6 % (60 mM) and 12 % (120 mM) as compared to control. Whereas in accession 200–06 this rise was upto 10 % at 120 mM NaCl stress as compared to control. Foliar treatment of chitosan mitigated the destroying impact of salinity and significantly lowered the levels of H_2O_2 in both accessions. In accession 200–03 chitosan application reduced the level of H_2O_2 upto 26 % (60 mM) and 17 % (120 mM) as compared to stressed untreated plants. However, in accession 200–06 this reduction was upto 11 % (60

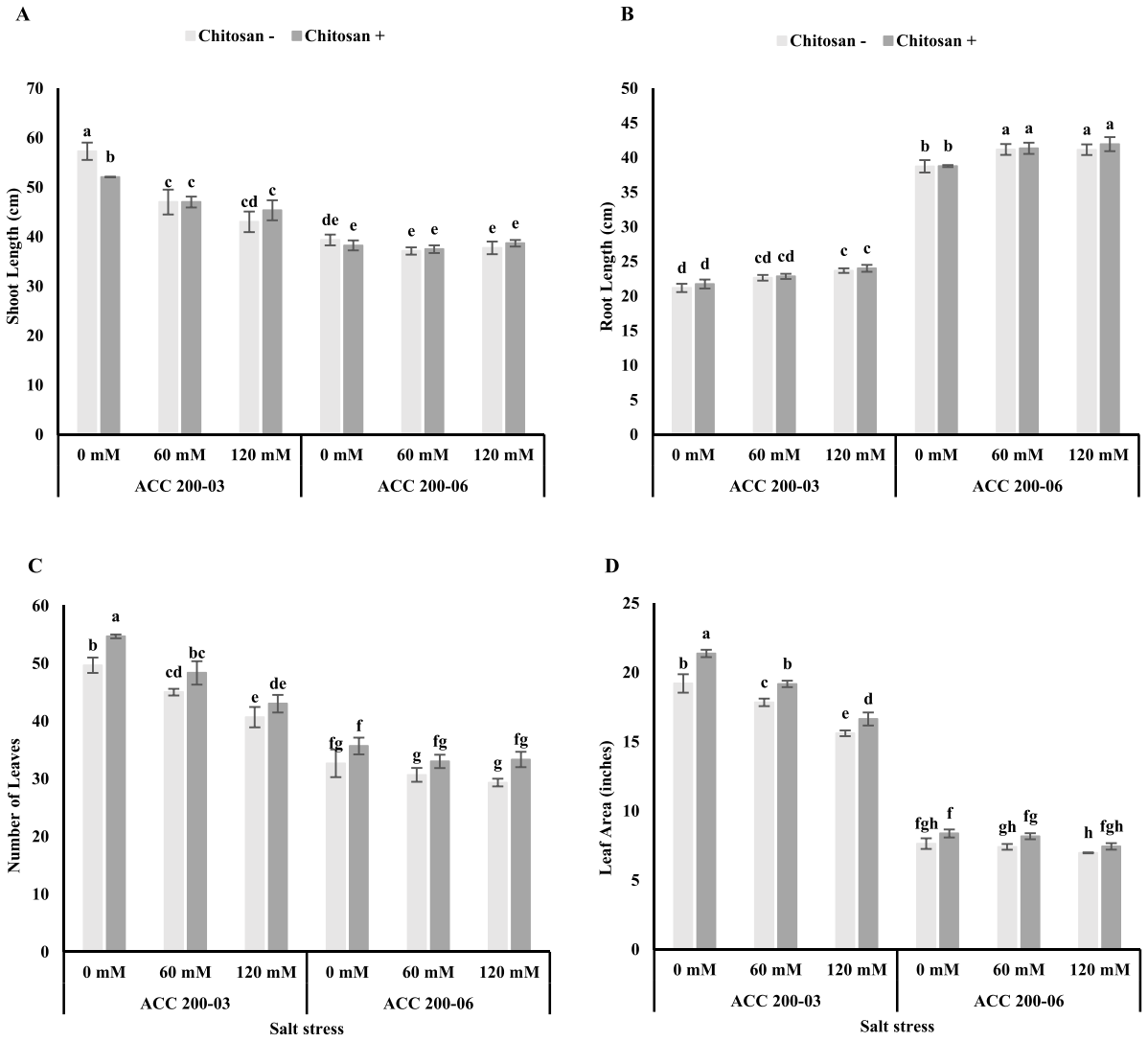

Fig. 4. Growth attributes of two *P. sativum* accessions under salt stress and foliar application of Chitosan. A. Shows shoot length, B. Root length, C. Number of leaves and D. Leaf area. Data in (A-D) represents the average value of three replicates for every treatment ± errors bars as standard error (SE). Average values (bars in graph) with different letters (a, b, c and so on) describe statistically significant differences among the treatment groups as determined by three-way ANOVA followed by LSD test ($p < 0.05$).

Table 5
ANOVA of malondialdehyde (MDA), relative membrane permeability (RMP) and hydrogen peroxide (H_2O_2) of two Pea (*Pisum sativum* L.) accessions under salt stress and foliar application of Chitosan.

Source of Variance	df	MDA		RMP		H_2O_2	
		MS	P	MS	P	MS	P
Accession	1	47.5480***	.0000	1803.6786***	.0000	445.0693***	.0000
Salt stress	2	3.3116***	.0001	186.1256***	.0000	10.9731***	.0000
Chitosan	1	24.4454***	.0000	88.3867***	.0000	116.3342***	.0000
Accession * Salt stress	2	0.5153ns	.1481	27.5825***	.0000	1.4112ns	.1424
Accession * Chitosan	1	0.7500ns	.0954	16.5412***	.0009	10.6113***	.0005
Salt stress * Chitosan	2	3.7709***	.0001	3.5174ns	.0672	3.3086*	.0157
Accession * Salt stress * Chitosan	2	0.5472ns	.1329	2.4063ns	.1480	3.8817**	.0087
Error	24	0.2489<-		1.1618<-		0.6669<-	

mM) and 18 % (120 mM) as compared to stressed untreated plants (Fig. 5-C).

3.3. Effect of salt stress and chitosan treatment on biochemical parameters

3.3.1. Total soluble proteins

Under salt stress and aerial treatment of CTS both accessions of *P. sativum* showed significantly distinct behavior from each other with respect to total soluble protein content. The interaction Accession × Salt

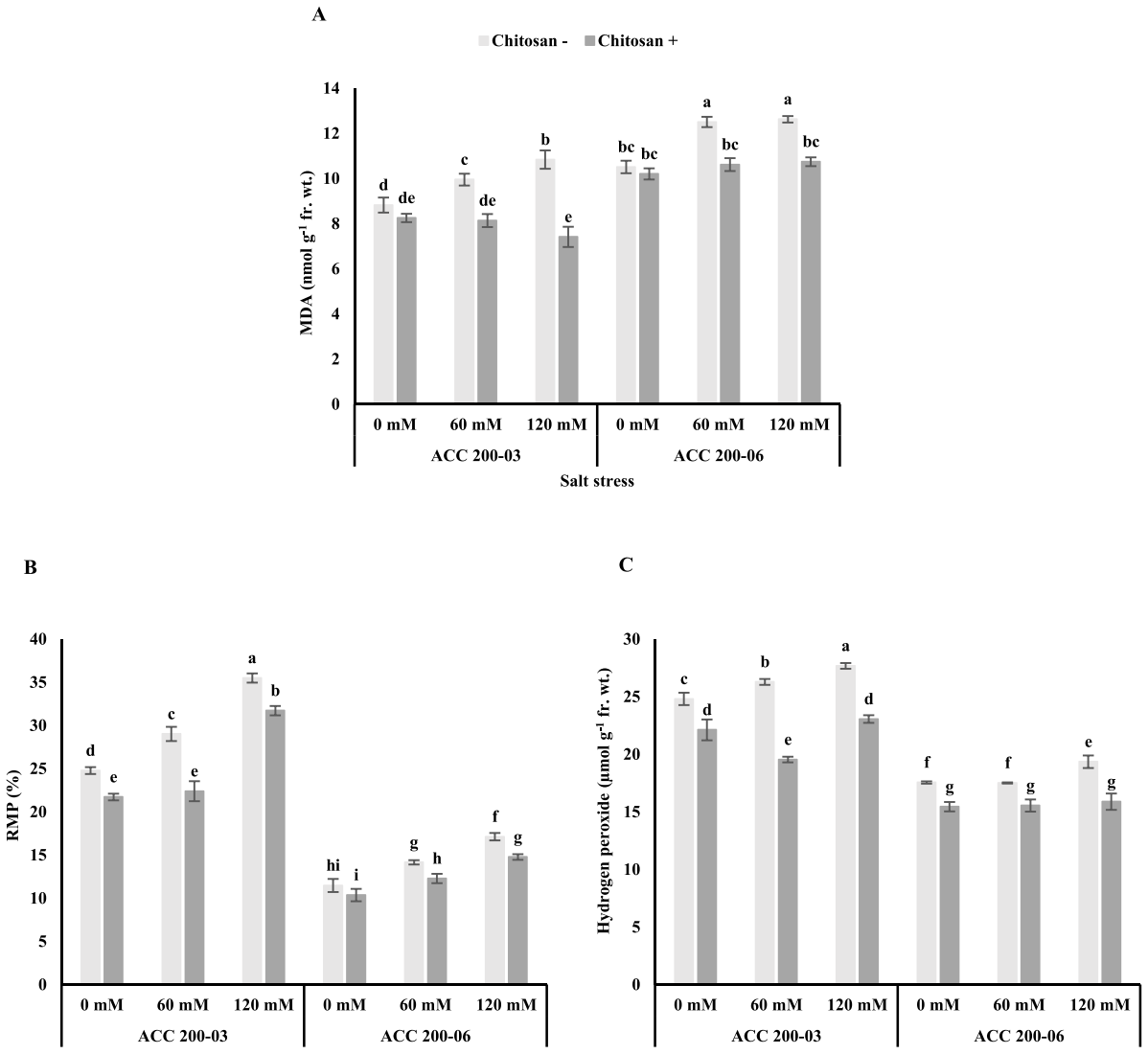

Fig. 5. Membrane stability parameters of two *P. sativum* accessions under salt stress and foliar application of Chitosan. A. Shows malondialdehyde, B. Relative membrane permeability and C. Hydrogen peroxide. Data in (A–C) represents the average value of three replicates for every treatment ± errors bars as standard error (SE). Average values (bars in graph) with different letters (a, b, c and so on) describe statistically significant differences among the treatment groups as determined by three-way ANOVA followed by LSD test ($p < 0.05$).

Stress was highly significant. However, interaction among all three factors Accession × Salt Stress × Chitosan was non-significant (Table 6). In accession 200–03 salt stress significantly raised the total soluble protein content upto 17 % at 120 mM NaCl stress as compared to control. Whereas in accession 200–06 this rise was non-significant at both salinity levels. Foliar application of chitosan significantly and non-significantly improved the TSP. In accession 200–03 chitosan application significantly increased TSP upto 9 % at 60 mM NaCl stress as compared to stressed untreated plant. However, in accession 200–06 rise in total soluble protein content due to chitosan application was non-significant at both salinity levels (Fig. 6-A).

3.3.2. *Superoxide dismutase (SOD)*

In case of superoxide dismutase activity, both accessions of *P. sativum*

Table 6
ANOVA of total soluble proteins (TSP) content, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity of two Pea (*Pisum sativum* L.) accessions under salt stress and foliar application of Chitosan.

Source of Variance	df	TSP		SOD		CAT		POD	
		MS	P	MS	P	MS	P	MS	P
Accession	1	7.0251e-4***	.0000	5.2349e10***	.0009	0.2726***	.0000	4.7065***	.0000
Salt stress	2	9.9171e-4***	.0000	3.8346e11***	.0000	0.0123***	.0000	1.8466***	.0000
Chitosan	1	5.6895e-4***	.0001	1.2645e11***	.0000	0.0241***	.0000	3.2327***	.0000
Accession * Salt stress	2	3.2878e-4***	.0003	3.28421e9ns	.4237	0.0210***	.0000	0.2507***	.0000
Accession * Chitosan	1	6.0643e-5ns	.1548	2.1406e9ns	.4537	0.0026***	.0004	0.0283ns	.0772
Salt stress * Chitosan	2	9.5069e-6ns	.7163	1.9284e10*	.0131	0.0035***	.0000	1.1957***	.0000
Accession * Salt stress * Chitosan	2	2.6675e-5ns	.4011	1.68647e9ns	.6385	0.0060***	.0000	0.0258ns	.0634
Error	24	2.81e-5<-		3.68977e9<-		0.0002<-		0.0083<-	

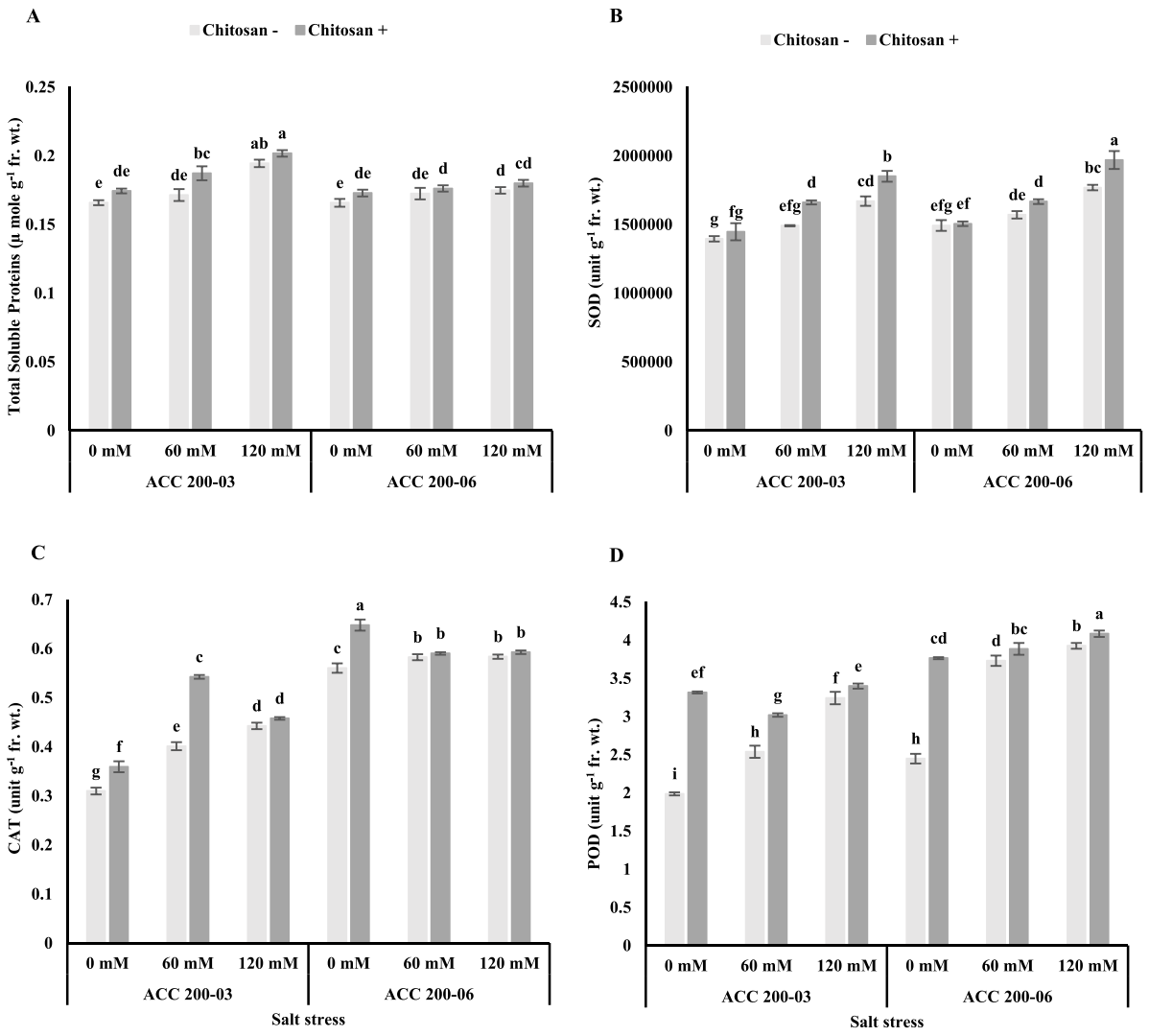

Fig. 6. Biochemical characters of two *P. sativum* accessions under salt stress and foliar application of Chitosan. A. Shows total soluble proteins, B. Superoxide dismutase, C. Catalase and D. Peroxidase. Data in (A-D) represents the average value of three replicates for every treatment ± errors bars as standard error (SE). Average values (bars in graph) with different letters (a, b, c and so on) describe statistically significant differences among the treatment groups as determined by three-way ANOVA followed by LSD test ($p < 0.05$).

behaved significantly different from each other under salt stress and foliar treatment of CTS. The interaction of Salt Stress × Chitosan was significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 6). Salt stress boosted up the activity of SOD in both accessions. In accession 200–03 due to NaCl stress upto 7 % (60 mM) and 20 % (120 mM) rise in SOD activity was observed as compared to control. Whereas in accession 200–06 SOD activity was increased upto 5 % (60 mM) and 19 % (120 mM) as compared to control. CTS treatment upregulated the activity of SOD in both accessions. In accession 200–03 maximum upregulation of SOD activity due to chitosan application was upto 11 % at both salinity levels as compared to stressed untreated plant. Whereas in accession 200–06 this upregulation was upto 6 % (60 mM) and 11 % (120 mM) as compared to stressed untreated plants (Fig. 6-B).

3.3.3. Catalase (CAT)

Both accessions of *P. sativum* were highly significantly different from each other with respect to catalase activity. Salt stress and foliar application of CTS has highly remarkable effect on CAT activity in both accessions. The interaction among all the three factors Accession × Salt Stress × Chitosan was highly significant (Table 6). Salt stress upregulated the activity of catalase in both accessions. In accession 200–03 due to NaCl stress upto 30 % (60 mM) and 43 % (120 mM) rise in CAT activity was observed as compared to control. Whereas in accession 200–06 CAT activity was increased upto 4 % at both salinity levels as compared to control. CTS treatment also upregulated CAT activity in both accessions. In accession 200–03 maximum upregulation of CAT activity due to chitosan application was upto 35 % (60 mM) as compared to stressed untreated plant. Whereas in accession 200–06 upregulation of CAT activity due to foliar application of chitosan was non-significant as compared to stressed untreated plants (Fig. 6-C).

3.3.4. Peroxidase (POD)

In case of peroxidase activity, both accessions of *P. sativum* behaved significantly different from each other under salt stress and foliar application of Chitosan. Two interactions Accession × Salt Stress and Salt Stress × Chitosan were highly significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 6). Salt stress upregulated the activity of peroxidase in both accessions. In accession 200–03 due to NaCl stress upto 28 % (60 mM) and 63 % (120 mM) rise in POD activity was observed as compared to control. Whereas in accession 200–06 POD activity was increased upto 52 % (60 mM) and 60 % (120 mM) as compared to control. CTS treatment upregulated the activity of POD in both accessions. In

accession 200–03 maximum upregulation of POD activity due to chitosan application was upto 19 % (60 mM) as compared to stressed untreated plant. Whereas in accession 200–06 this upregulation was upto 4 % at both salinity levels as compared to stressed untreated plants (Fig. 6-D).

3.3.5. Leaf proline

In case of leaf proline, both accessions of *P. sativum* behaved significantly different from each other under salinity stress and aerial treatment of Chitosan. The interaction of Accession × Chitosan was significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was highly significant (Table 7). Salt stress notably elevated the leaf proline content in both accessions. In accession 200–03 due to NaCl stress upto 127 % (60 mM) and 202 % (120 mM) elevation in leaf proline content was noted in comparison with control. Whereas in accession 200–06 proline content was increased upto 181 % at both salinity levels as compared to control. Both accessions behaved differently in response to chitosan application. In accession 200–03 CTS treatment significantly elevated the leaf proline content upto 26 % (60 mM) and 21 % (120 mM) as compared to stressed untreated plants. However, in accession 200–06 effect of chitosan application was non-significant at both salinity levels (Fig. 7-A).

3.3.6. Total phenolics

Under salt stress and aerial treatment of CTS both accessions of *P. sativum* showed significantly distinct behavior from each other with respect to total phenolic content. The interaction of Accession × Salt Stress was highly significant. However, interaction among all three factors Accession × Salt Stress × Chitosan was non-significant (Table 7). In accession 200–03 salt stress raised the total phenolic content upto 4 % at 120 mM NaCl stress as compared to control. Whereas in accession 200–06 this rise was 2 % at 120 mM NaCl stress as compared to control. Foliar application of chitosan significantly and non-significantly improved the total phenolic content. In accession 200–03 chitosan application significantly elevated the total phenolic content upto 1 % at 60 mM NaCl stress as compared to stressed untreated plant. However, in accession 200–06 this rise was 1 % at 120 mM salt stress as compared to stressed untreated plants (Fig. 7-B).

3.4. Effect of salt stress and chitosan treatment on inorganic ions

3.4.1. Shoot sodium

Under salt stress and aerial treatment of CTS both accessions of *P. sativum* showed significantly distinct behavior from each other with respect to shoot sodium level. The interaction among all three factors Accession × Salt Stress × Chitosan was highly significant (Table 8). In accession 200–03 salt stress raised the level of Na^+ upto 61 % (60 mM) and 163 % (120 mM) as compared to control. Whereas in accession 200–06 this rise was upto 77 % (60 mM) and 107 % (120 mM) as compared to control. CTS treatment lessened the detrimental effects of salinity by decreasing Na^+ levels in both accessions. In accession 200–03 chitosan application reduced the level of Na^+ upto 13 % (60 mM) and 20 % (120 mM) as compared to stressed untreated plants. However, in accession 200–06 this reduction was upto 17 % (60 mM) and 4 % (120 mM) as compared to stressed untreated plants (Fig. 8-A).

Table 7
ANOVA of leaf proline (LP) and total phenolics (TP) of two Pea (*Pisum sativum* L.) accessions under salt stress and foliar application of Chitosan.

Source of Variance	df	LP		TP	
		MS	P	MS	P
Accession	1	0.0295***	.0000	0.4597***	.0000
Salt stress	2	0.1072***	.0000	8.8544***	.0000
Chitosan	1	0.0135***	.0000	0.8257***	.0000
Accession * Salt stress	2	0.0035***	.0000	0.9530***	.0000
Accession * Chitosan	1	0.0005*	.0248	0.0608ns	.0785
Salt stress * Chitosan	2	0.0008***	.0008	0.0014ns	.9275
Accession * Salt stress * Chitosan	2	0.0028***	.0000	0.0358ns	.1587
Error	24	0.0001<-		0.0180<-	

3.4.2. Root sodium

In case of root sodium level, both accessions of *P. sativum* behaved significantly different from each other under salt stress & foliar treatment of CTS. The interaction of Salt Stress × Chitosan was significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 8). In accession 200–03 due to salt stress upto 49 % (60 mM) and 72 % (120 mM) increase in root Na^+ level was observed as compared to control. Whereas in accession 200–06 this increase was upto 41 % (60 mM) and 68 % (120 mM) as compared to control. CTS treatment mitigated the adverse effect of sodium chloride stress by lowering Na^+ level in both accessions. In accession 200–03 due to chitosan application upto 18 % (60 mM) and 13 % (120 mM) decrease in root Na^+ level was observed as compared to stressed untreated plants. Whereas in accession 200–06 this reduction was upto 8 % (60 mM) and 7 % (120 mM) as compared to stressed untreated plants (Fig. 8-B).

3.4.3. Shoot potassium

Under NaCl stress and foliar treatment of CTS both accessions of *P. sativum* showed significantly distinct behavior from each other with respect to shoot K^+ level. Two interactions Accession × Salt stress and Accession × Chitosan were significant. However, interaction among all three factors Accession × Salt Stress × Chitosan was non-significant (Table 8). In accession 200–03 salt stress lowered the K^+level upto 14 % (60 mM) and 28 % (120 mM) as compared to control. Whereas in accession 200–06 this reduction was upto 15 % (60 mM) and 30 % (120 mM) as compared to control. CTS treatment lessened the detrimental effects of salinity by elevating K^+ level in both accessions. In accession 200–03 chitosan application elevated the level of K^+ upto 15 % (60 mM) and 18 % (120 mM) as compared to stressed untreated plants. However, in accession 200–06 this elevation was upto 15 % (60 mM) and 17 % (120 mM) as compared to stressed untreated plants (Fig. 8-C).

3.4.4. Root potassium

In case of root K^+ level, both accessions of *P. sativum* behaved significantly different from each other under salt stress & aerial treatment of CTS. The interaction of Accession × Salt Stress was significant. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 8). In accession 200–03 due to salt stress upto 26 % (60 mM) and 45 % (120 mM) decrease in root K^+ level was observed as compared to control. Whereas in accession 200–06 this reduction was upto 19 % (60 mM) and 33 % (120 mM) as compared to control. CTS treatment alleviated the deleterious effect of salinity by elevating K^+ level in both accessions. In accession 200–03 due to chitosan application upto 16 % (60 mM) and 34 % (120 mM) rise in root K^+ level was observed as compared to stressed untreated plants. Whereas in accession 200–06 this rise was upto 15 % (60 mM) and 18 % (120 mM) as compared to stressed untreated plants (Fig. 8-D).

3.5. Effect of salt stress and chitosan treatment on endogenous melatonin synthesis

Melatonin concentrations rise in reaction to salt stress. Melatonin concentrations were also shown to be higher after chitosan treatment implication.

3.6. Effect of salt stress and chitosan treatment on yield parameters

3.6.1. No. of pods per plant

Both accessions of *P. sativum* were highly significantly different from each other with respect to no. of pods/plant. Table 9 shows that foliar application of chitosan has highly remarkable effect on no. of pods/plant

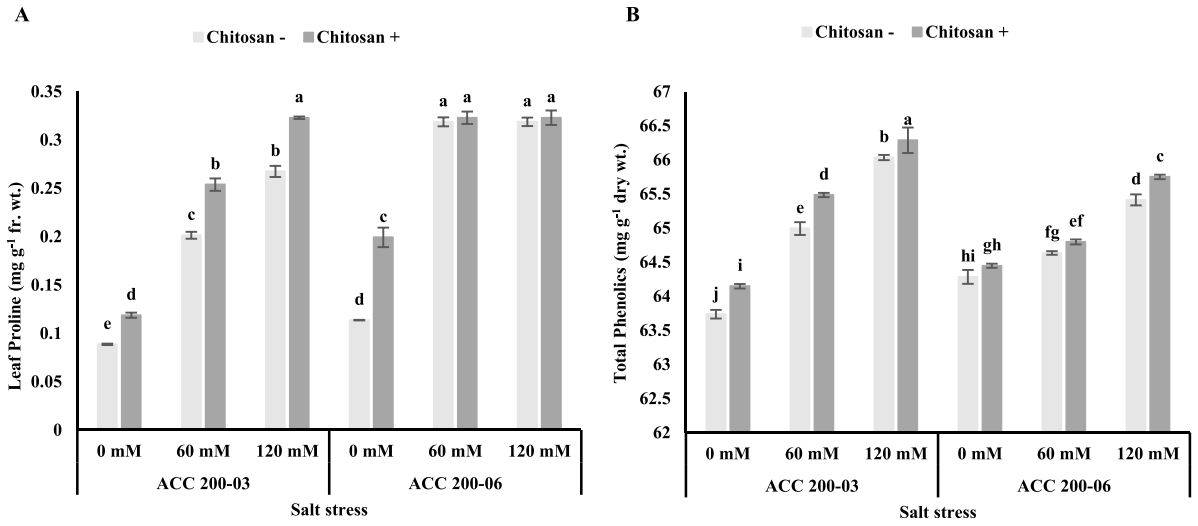

Fig. 7. Leaf proline and total phenolics content of two *P. sativum* accessions under salt stress and foliar application of Chitosan. A. Shows leaf proline and B. Total phenolics. Data in (A-B) represents the average value of three replicates for every treatment ± errors bars as standard error (SE). Average values (bars in graph) with different letters (a, b, c and so on) describe statistically significant differences among the treatment groups as determined by three-way ANOVA followed by LSD test ($p < 0.05$).

Table 8
ANOVA of levels of shoot sodium (S Na^+), root sodium (R Na^+), shoot potassium (S K^+) and root potassium (R K^+) of two Pea (*Pisum sativum* L.) accessions under salt stress and foliar application of Chitosan.

Source of Variance	df	S Na^+		R Na^+		S K^+		R K^+	
		MS	P	MS	P	MS	P	MS	P
Accession	1	18.7778***	.0000	100.0000***	.0000	348.4444***	.0000	25.8403***	.0007
Salt stress	2	328.2708***	.0000	464.2986***	.0000	214.6736***	.0000	282.1111***	.0000
Chitosan	1	23.3611***	.0000	61.3611***	.0000	121.0000***	.0000	85.5625***	.0000
Accession * Salt stress	2	7.5486***	.0004	3.2708ns	.1005	7.1319*	.0356	7.1944*	.0271
Accession * Chitosan	1	1.3611ns	.1680	4.0000ns	.0912	11.1111*	.0221	2.0069ns	.2892
Salt stress * Chitosan	2	5.9236**	.0014	5.9236*	.0206	0.5625ns	.7411	4.7500ns	.0820
Accession * Salt stress * Chitosan	2	4.4236**	.0053	1.6458ns	.2979	3.3819ns	.1831	0.7778ns	.6396
Error	24	0.6736<-		1.2916<-		1.8542<-		1.7083<-	

whereas effect of salt stress on no. of pods per plant was non-significant. In accession 200–03 no. of pods per plant get reduced upto 22 % at both levels of salt stress as compared to control. However, in accession 200–06 no. of pods per plant was not changed in NaCl stressed plants as compared to control. CTS treatment ameliorated the effect of NaCl stress and increased the no. of pods per plant in both accessions with respect to their controls. In accession 200–03 due to chitosan application upto 57 % (60 mM) and 14 % (120 mM) increase in no. of pods per plant was observed as compared to stressed untreated plants. Whereas in accession 200–06 this increase was upto 33 % (60 mM) and 17 % (120 mM) as compared to stressed untreated plants (Fig. 9-A). The interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 9).

3.6.2. *No. of seeds per pod*

In case of no. of seeds per pod, both accessions of *P. sativum* behaved significantly different from each other. Foliar application of chitosan has highly remarkable effect on no. of seeds/pod whereas effect of NaCl stress on no. of seeds per pod was non-significant. The interaction of Accession × Salt Stress was highly notable. However, interaction among all the three factors Accession × Salt Stress × Chitosan was non-significant (Table 9). In accession 200–03 due to salt stress upto 25 % (60 mM) and 38 % (120 mM) reduction in no. of seeds per pod was observed as compared to control. Whereas in accession 200–06 salt stress increased the no. of seeds per pod upto 9 % at both salinity levels as compared to control. CTS treatment improved the no. of seeds per pod in both accessions. In accession 200–03 due to chitosan application upto 33 % (60 mM) and 40 % (120 mM) increase in no. of seeds per pod was observed as compared to stressed untreated plants. Whereas in accession 200–06 this increase was upto 17 % at both salinity levels as compared to stressed untreated plants (Fig. 9-B).

3.6.3. *Weight of 100 seeds*

Under NaCl stress and aerial treatment of CTS both accessions of *P. sativum* showed significantly distinct behavior from each other with respect to weight of 100 seeds. Two interactions Accession × Salt stress and Accession × Chitosan were highly significant. However, interaction among all three factors Accession × Salt Stress × Chitosan was non-significant (Table 9). In accession 200–03 salt stress reduced the weight of 100 seeds upto 34 % (60 mM) and 68 % (120 mM) as compared to control. Whereas in accession 200–06 this reduction was upto 7 % (60 mM) and 10 % (120 mM) as compared to control. CTS treatment improved the weight of 100 seeds in both *P. sativum* accessions. In accession 200–03 chitosan application increased the weight of 100 seeds upto 39 % (60 mM) and 71 % (120 mM) as compared to untreated stressed plants. However, in accession 200–06 chitosan application has non-significant effect on weight of 100 seeds (Fig. 10).

3.7. *Pearson correlation and principle component analysis*

Pearson's correlation graphs (Fig. 11) illustrate salt uptake and its effect on multiple growth, biochemical and yield parameters of both accessions studied in the experiment. The graph showed that in accession 200–03, root and shoot Na^+ was positively correlated to root

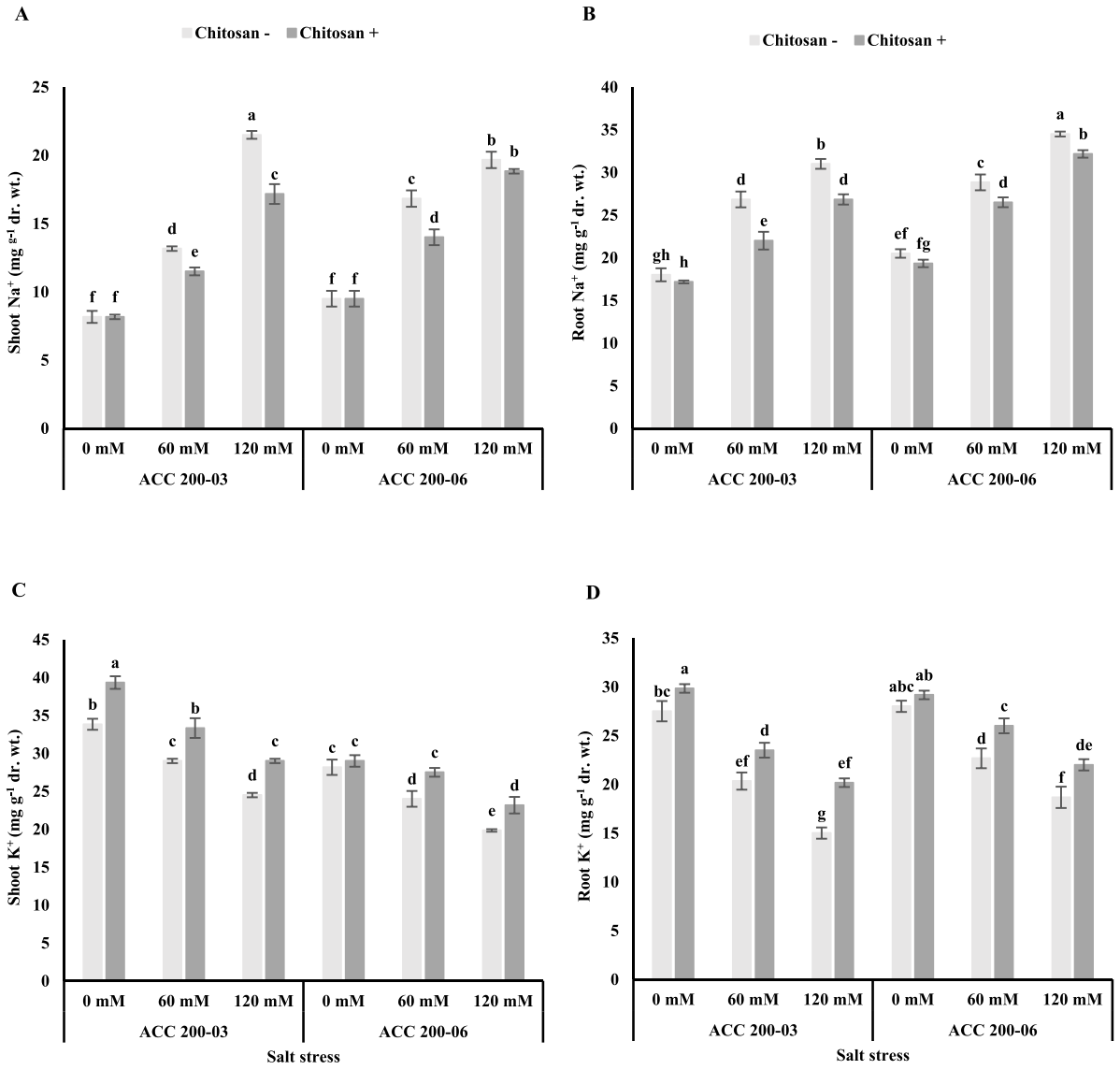

Fig. 8. Inorganic ions content of two *P. sativum* accessions under salt stress and foliar application of Chitosan. A. Shoot Na^+, B. Root Na^+, C. Shoot K^+ and D. Root K^+. Data in (A-D) represents the average value of three replicates for every treatment ± errors bars as standard error (SE). Average values (bars in graph) with different letters (a, b, c and so on) describe statistically significant differences among the treatment groups as determined by three-way ANOVA followed by LSD test ($p < 0.05$).

Table 9
ANOVA of No. of pods/plant, no. of seeds/pod and weight of 100 seeds of two Pea (*Pisum sativum* L.) accessions under salt stress and foliar application of Chitosan.

Source of Variance	df	No. of Pods $Plant^{-1}$		No. of Seeds Pod^{-1}		Wt. of 100 Seeds	
		MS	P	MS	P	MS	P
Accession	1	4.6944***	.0001	26.6944***	.0000	485.9526***	.0000
Salt stress	2	0.5833ns	.0931	0.5278ns	.1430	395.2601***	.0000
Chitosan	1	3.3611***	.0007	4.6944***	.0002	141.8005***	.0000
Accession * Salt stress	2	0.5278ns	.1145	1.8611**	.0031	264.6744***	.0000
Accession * Chitosan	1	0.2500ns	.2994	0.0278ns	.7418	106.2136***	.0000
Salt stress * Chitosan	2	0.3611ns	.2178	0.0278ns	.8953	0.2534ns	.7077
Accession * Salt stress * Chitosan	2	0.0833ns	.6912	0.0278ns	.8953	0.3793ns	.5982
Error	24	0.2222<-		0.2500<-		0.7225<-	

length, CAT, SOD, TSP, total phenolics and leaf proline and relative membrane permeability of plants. While it was negatively correlated to shoot length, no. of pods per plant, root dry weight, leaf area, number of leaves, root fresh wt., shoot fresh and dry wt., weight of 100 seeds, root K^+, shoot K^+ and no. of seeds per pod. Pearson's correlation graphs for accession 200–06 (Fig. 13), showed that Na^+ in root and shoot were positively correlated to each other and to RMP, total phenolics, SOD, POD, TSP, proline, root length, MDA and H_2O_2. While these were negatively correlated to root fresh weight, shoot length, leaf area, shoot fresh wt., shoot dry wt., no. of leaves, CAT, root and shoot K^+, weight of 100 seeds and no. of pods per plant.

Principle component analysis (PCA) (Figs. 12a, b & 14a, b) showed

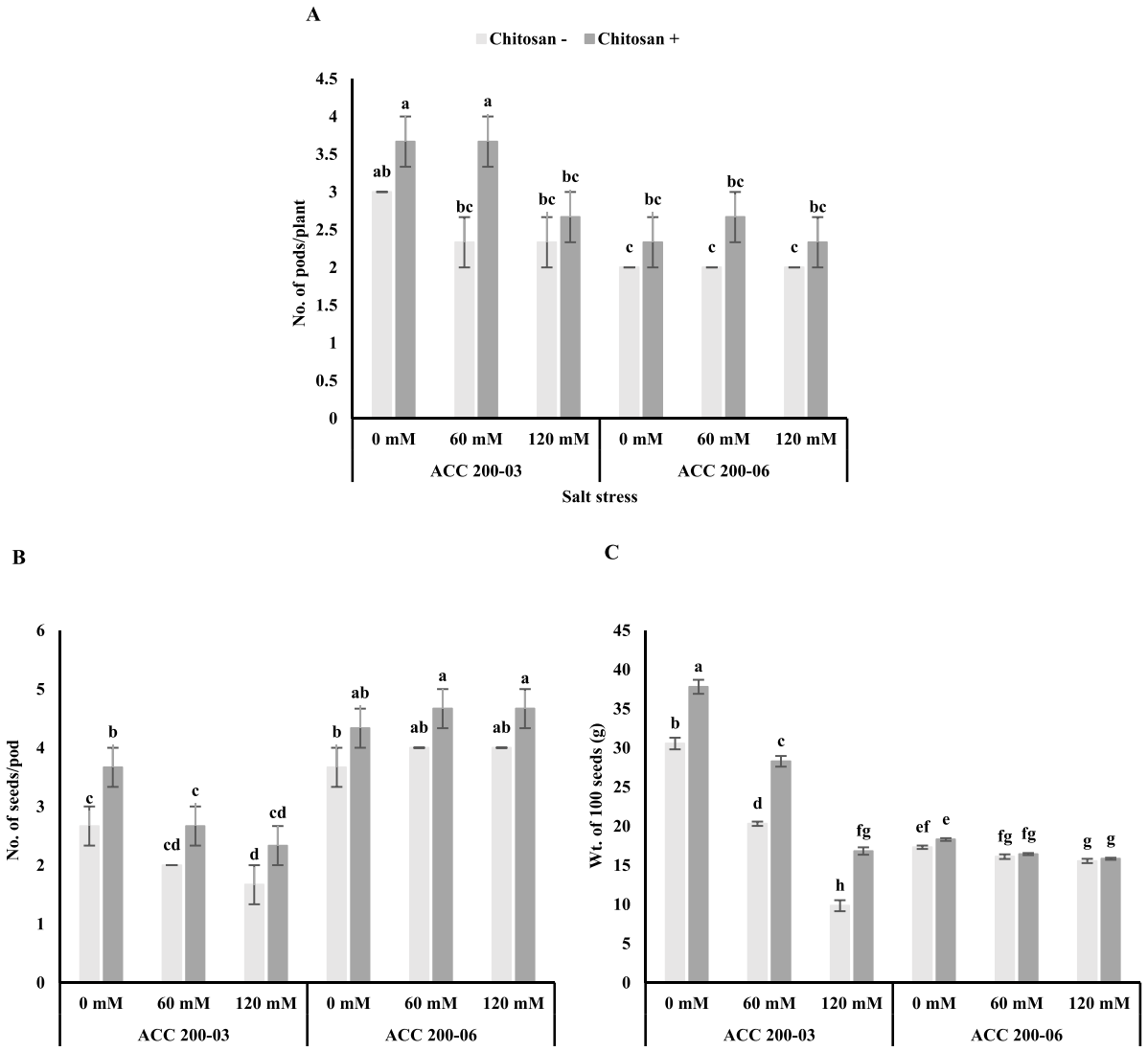

Fig. 9. Yield attributes of two *P. sativum* accessions under salt stress and foliar application of Chitosan. A. Number of pods per plant, B. number of seeds per pod and C. weight of 100 seeds. Data in (A-C) represents the average value of three replicates for every treatment ± errors bars as standard error (SE). Average values (bars in graph) with different letters (a, b, c and so on) describe statistically significant differences among the treatment groups as determined by three-way ANOVA followed by LSD test ($p < 0.05$).

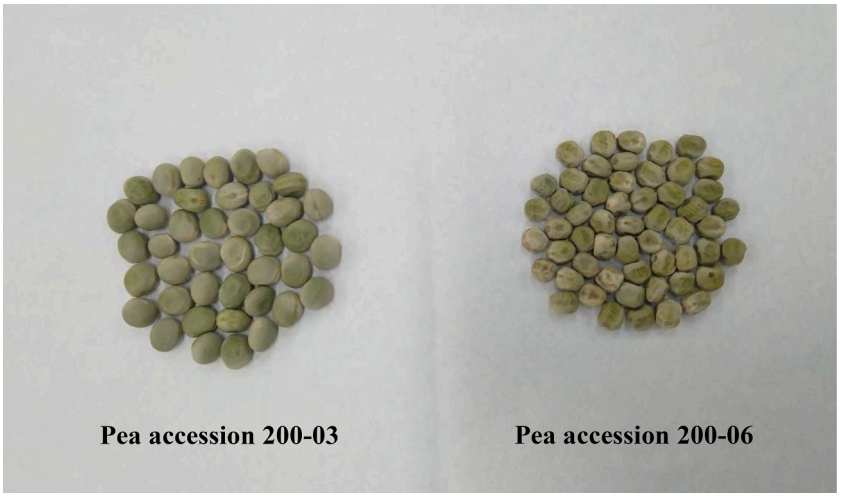

Fig. 10. Comparison of size and shape of pea seeds.

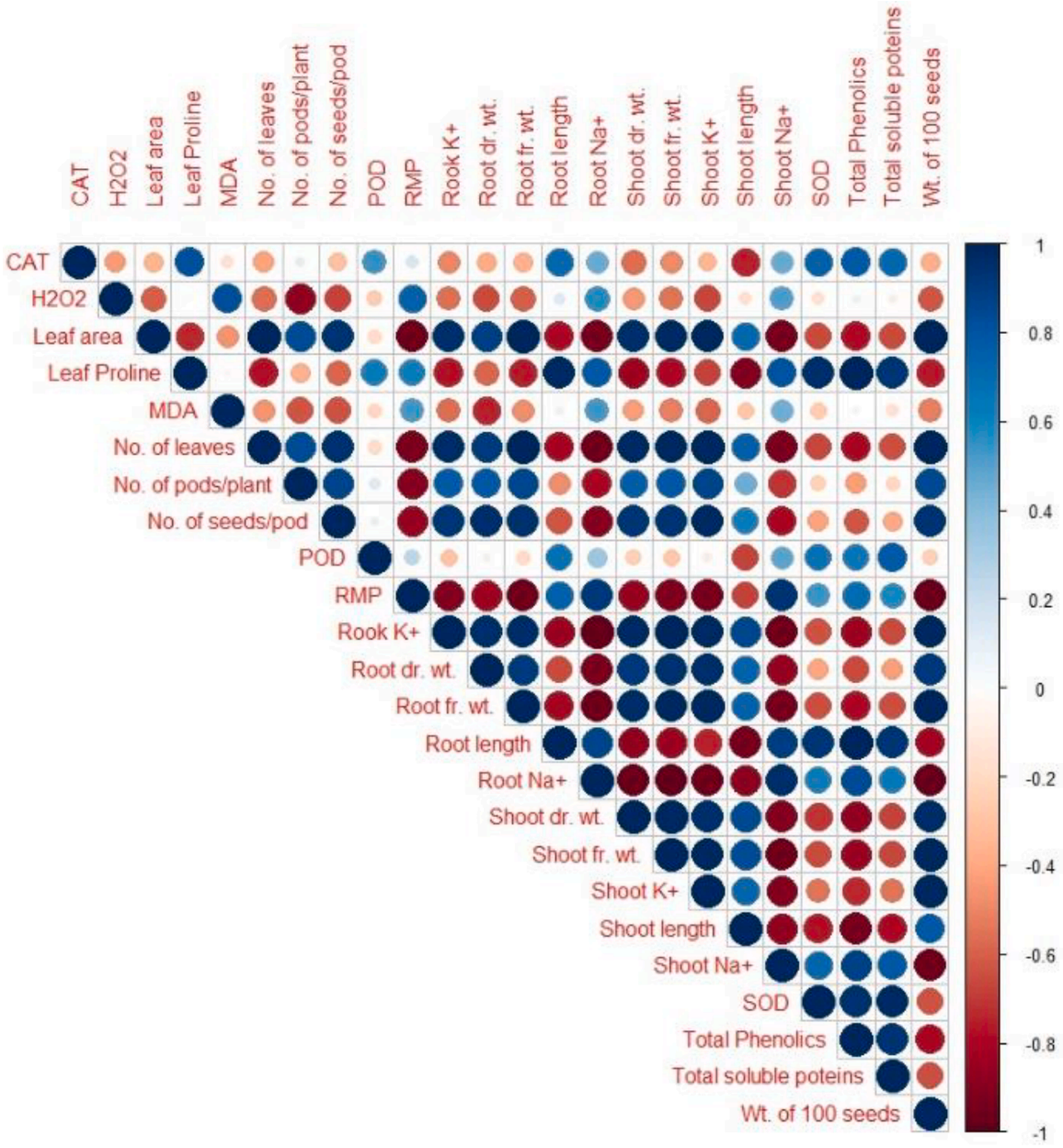

Fig. 11. Correlation between salt uptake and growth, biochemical, inorganic ions and yield attributes of accession 200–03 studied in this experiment. Various abbreviations used are as follows: CAT = catalase, POD = peroxidase, SOD = superoxide dismutase, MDA = Malondialdehyde, H_2O_2 = hydrogen peroxide, RMP = relative membrane permeability, Na^+= sodium, K^+= potassium.

effects of individual treatment on various morphological, biochemical and yield parameters of *P. sativum* accessions 200–03 and 200–06, respectively, examined in this study. The graph showed that all the treatments were distributed in PC1 and PC2 (principle components). Salt stress treatments (3 and 5) were more separated from control (1) and other treatments like 2, 4 and 6. PCA of parameters for accession 200–03 showed that all variances present in data first two components PC1 and PC2 had maximum contribution 81.35 %, where PC1 account for 62.35 % and PC2 account for 19 %. For accession 200–06 PCA of parameters showed that PC1 and PC2 had 49.73 % and 25.83 % contribution, respectively. Collectively, PC1 and PC2 account for 75.56 % of contribution among all the principle components.

4. Discussion

Salinity is a key element that affects plant growth and reduces agricultural productiveness around the world (Zhang et al., 2021). Salt stress has a deleterious impact on many plant development and production processes by generating ion toxicity, nutritional imbalance, hyperosmotic stress, oxidative injury, metabolic abnormalities and photoinhibition (Zhu, 2003; Chen et al., 2018). As plants are sessile in nature they must acquire a number of defensive mechanisms to cope with high salinity conditions. There are three categories in which the numerous physiological and biochemical mechanisms employed by plants to adapt to salt stress can be divided: ionic stress, osmotic stress, and detoxification process (Yang and Guo, 2018).

Chitosan has numerous agricultural applications since it modulates plant growth and development and boosts plant resilience to a variety of abiotic and biotic stimuli (Pongprayoon et al., 2013; Katiyar et al., 2014; Malerba and Cerana, 2016). It causes numerous responsive secondary metabolites, genes and proteins to be expressed in plants. CTS activates a signal transduction pathway that transports secondary molecules

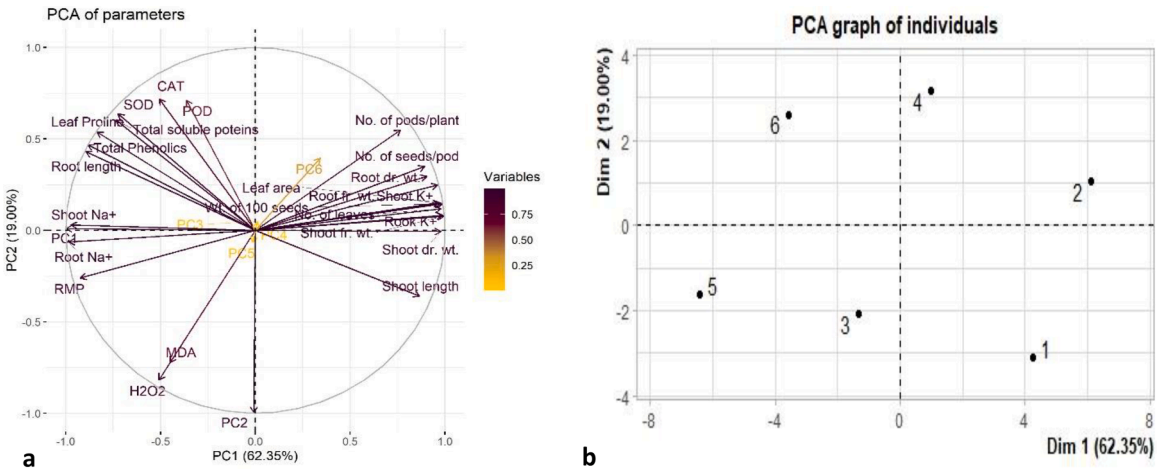

Fig. 12. Principal component analysis (PCA), PCA of parameters (a) and PCA of treatment (b). (where numerics 1,2,3,4,5,6 represents different treatments applied to accession 200–03. Various abbreviations used in this figure are similar to Fig. 11).

including H_2O_2 and NO. It alters the plant cell's molecular biology, physiology and biochemistry. Plant responses, however, vary depending on structure and concentration of chitosan of used, plant species and developmental phase (Pongprayoon et al., 2022). Present study demonstrates the influence of NaCl stress on various parameters (i.e., morphological, physiological, biochemical, inorganic ions and yield) of two *Pisum sativum* L. accessions and effect of aerial treatment of CTS to alleviate drastic effects of salinity.

This study presents that increasing supply of salt stress substantially reduced the growth parameters (shoot and root fresh and dry wt., shoot length, leaf area, no. of leaves) of *P. sativum* accession 200–03 that is consistent with what has already been described in *Pisum sativum* L. (El-Hefnawy, 2020; Dadasoglu et al., 2021) and *Phaseolus vulgaris* L. (Zayed et al., 2017). Whereas reduction observed in these growth parameters of *P. sativum* accession 200–06 was minimum. Due to salt stress root length of both accessions was increased and this increase might be related to the presence of genes that can withstand stress (Rizwana et al., 2015; Mahpara et al., 2019). Aerial treatment of CTS mitigated the adverse effects of salinity on growth parameters of both *P. sativum* accessions. These findings are in agreement with previous studies on various crops including lettuce (Zhang et al., 2021), ajowan (Mahdavi and Rahimi, 2013), wheat (Zou et al., 2015) and maize (Turk, 2019). Decline in vegetative growth was brought about by increased Na^+ uptake which was accompanied by a decrease in the plant's K^+ uptake. High sodium levels in the soil, which prevent the plant from absorbing water from the soil, subjects the plant under osmotic stress (Zeeshan et al., 2020). Cells lose turgidity as a result of inadequate water intake, resulting in poor physiological and biochemical activities, since enzymes typically function in an aqueous environment (Vighi et al., 2017). Combined outcomes of these constraints have an impact on photosynthetic apparatus and eventually on plant growth and development (Choi et al., 2014).

Due to elevation in ROS production, salt stress increase the oxidative damage and lipid peroxidation (Weisany et al., 2014). Sodium chloride stress induced reactive oxygen species are substantially alleviated by escalated activities of enzymatic and non-enzymatic antioxidants (Wang et al., 2013; Noreen et al., 2012). To relief plants under stressed conditions, enzymatic antioxidants (POD, SOD, APX and CAT) are at forefront (Ighodaro and Akinloye, 2018; Hasnain et al., 2023). Salt stress notably boosted the activity of antioxidants which is a defensive strategy in stressed plants (Kim et al., 2005). Results of our study show enhanced contents of antioxidants (POD, SOD and CAT) and TSP in both *P. sativum* accessions under salt stress. These results are in agreement with previous findings (Noreen et al., 2021). Aerial treatment of CTS further enhanced the content of total soluble proteins and antioxidants (POD, SOD and CAT) in both *P. sativum* accessions. In salt stressed chili pepper plants upregulation of SOD and CAT was recorded upon treatment with CTS-Spm nanoparticles (Ramadan et al., 2022). In salt stressed lettuce plants exogenous application of chitosan upregulated CAT activity (G. Zhang et al., 2021). Increased protein content and antioxidant activity (POD and SOD) was observed in salt stressed mung bean seedlings treated with nano-chitosan (Sen et al., 2020). Uridine diphosphate N-acetyl-D-glucosamine (UDP-GlcNAc) as nucleotide sugar is a part of the chemical makeup of chitosan. When applied to plants, UDP-GlcNAc is recognized by cells through chitin synthase and chitin deacetylase enzymes resulting in the formation of CTS oligomers that play a key role in plant cell signaling (Hadwiger, 2015; Malerba and Cerana, 2016). CTS oligomers penetrate the nucleus and trigger cascade reactions that result in the expression of antioxidant enzymes and hormones synthesis (Zeng et al., 2010; Hadwiger, 2015; Pichyangkura and Chadchawan, 2015; Rabêlo et al., 2019). Increase in TSP could be attributable to the production of osmotin-like proteins or structural proteins, specifically proteins involved in cell wall remodeling. Protein accumulation in saline-grown plants may provide a sort of nitrogen storage that is used again once stress is over and may aid in osmotic adjustments (Amini and Ehsanpour, 2005). Elevated level of total soluble proteins during stress is related to increased production and cumulation of numerous enzymes implicated in stress-induced tolerance response (Wang et al., 2013; Waris et al., 2023).

Outcomes of our study shows that under salt stress RMP, H_2O_2 and MDA level were increased in both *P. sativum* accessions. Foliar application of chitosan lowered the H_2O_2 and MDA content and stabilized the relative membrane permeability in both *P. sativum* accessions. These outcomes are in harmony with the previous findings (ALKahtani et al., 2020; Mehmood et al., 2020; Zhang et al., 2021). Increase in relative membrane permeability can be attributed to the excessive uptake of Na^+ and Cl^- ions which disrupts cell membrane integrity by causing an overabundance of ROS (Mansour, 2013). In response to stressful circumstances, plants frequently show increased H_2O_2 synthesis (Yen et al., 2013). Abiotic stress increased ROS generation in plant cells. To sustain cellular metabolic activities under stress circumstances and hence minimize oxidative damage, a balance between ROS formation and degradation is essential (Farhangi-Abriz and Torabian, 2018). MDA, a naturally occurring byproduct of lipid peroxidation has long been employed as a marker of the degree of cell damage brought on by stress (Ma et al., 2015). Chitosan treatment resulted in a considerable reduction in lipid peroxidation. This could be ascribed to the function of chitosan in protecting cells from oxidative damage under saline conditions. Similarly, chitosan greatly lowered O_2^- and H_2O_2 due to the presence of OH^- and NH_2 that react with ROS indicating that CTS can

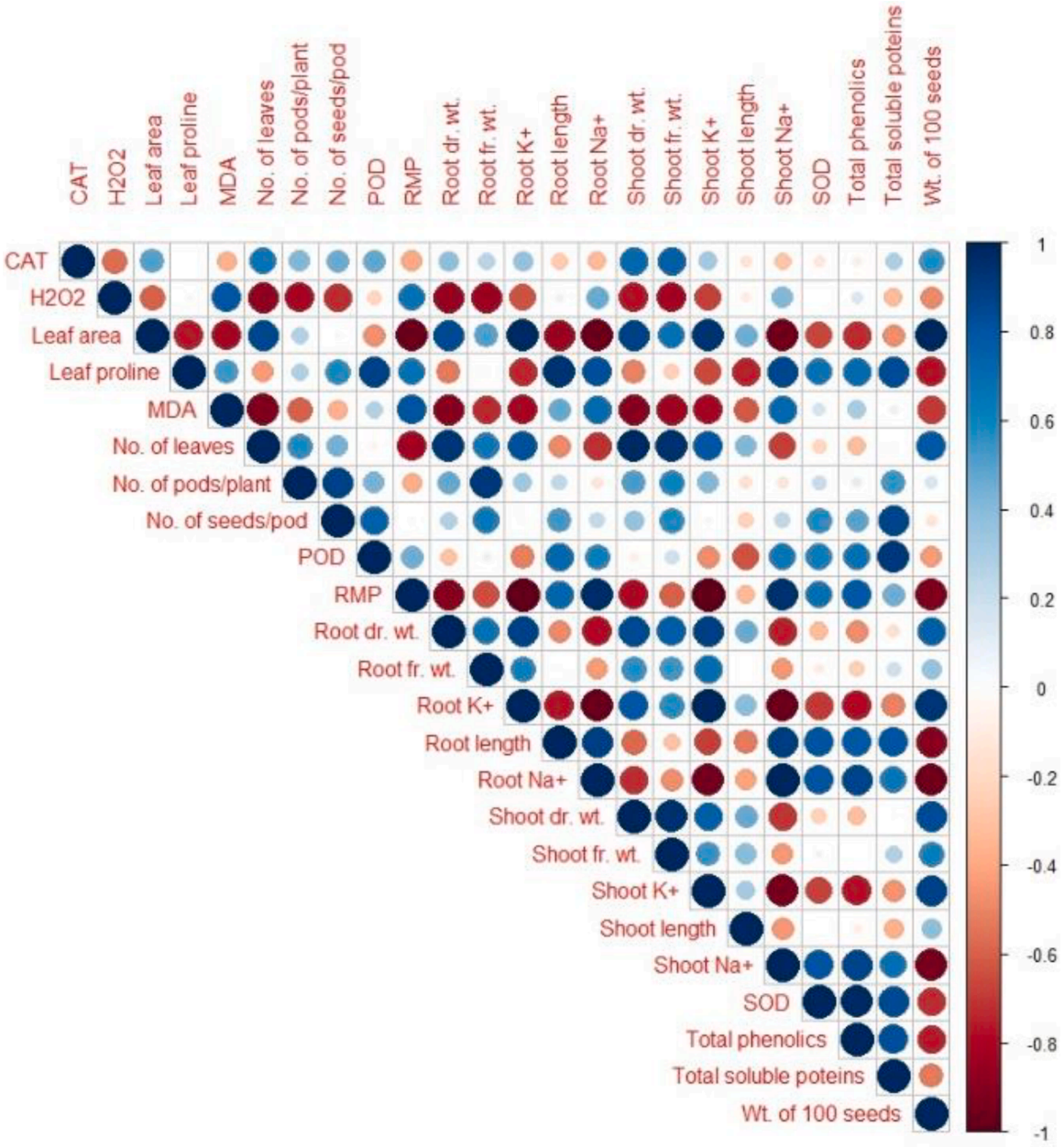

Fig. 13. Correlation between salt uptake and growth, biochemical, inorganic ions and yield attributes of accession 200–06 studied in this experiment. Various abbreviations used are as follows: CAT = catalase, POD = peroxidase, SOD = superoxide dismutase, MDA = Malondialdehyde, H_2O_2 = hydrogen peroxide, RMP = relative membrane permeability, Na^+= sodium, K^+= potassium.

scavenge O_2^- (Prashanth et al., 2007).

Results of our present study shows that in both *P. sativum* accessions salt stress increased the contents of leaf proline and total phenolics. CTS application elevated leaf proline and total phenolics in both *P. sativum* accessions. In lettuce plants, NaCl stress remarkably elevated the proline content. However, exogenously applied CTS further increased the leaf proline content in lettuce plants exposed to NaCl stress (Zhang et al., 2021). Under salt stress elevation in total phenolic content was observed in seedlings of durum wheat. However, CTS treatment also showed remarkable elevation in total phenolic content in NaCl stressed seedlings of durum wheat (Quitadamo et al., 2021). The substantial proline buildup in the NaCl stressed plants demonstrated how important this osmolyte is for the osmotic adjustment in salty conditions. This effect of salt stress could be attributed to its involvement in lowering proline oxidation to glutamate, hence raising proline concentration (Khan et al., 2019). Proline can enhance the plant's ability to adapt to salty circumstances by regulating osmotic potential, stabilizing cellular structure, minimizing injury to the photosynthetic machinery and inducing the expression of genes that respond to salt stress (Zou et al., 2015). It was previously discovered that proline buildup could be caused by a sodium chloride-induced rise in nitrogen metabolism (Da Rocha et al., 2012). An earlier investigation discovered that exogenous chitosan might significantly improve nitrogen metabolism in wheat (Zhang et al., 2017). Phenolic compounds are among the most prevalent families of secondary metabolites produced by the phenylpropanoid pathway. They are important for plant metabolism and physiology including adaptation under stressful circumstances. It is evident that abiotic stresses trigger phenylpropanoid pathway for the production of different phenolic compounds that ultimately reduce membrane lipid peroxidation and provide shield to plant cells from the harmful effects of oxidative stress by scavenging ROS (Sharma et al., 2019). Treatment of durum wheat cultivars with chitosan nanoparticles having N-acetyl

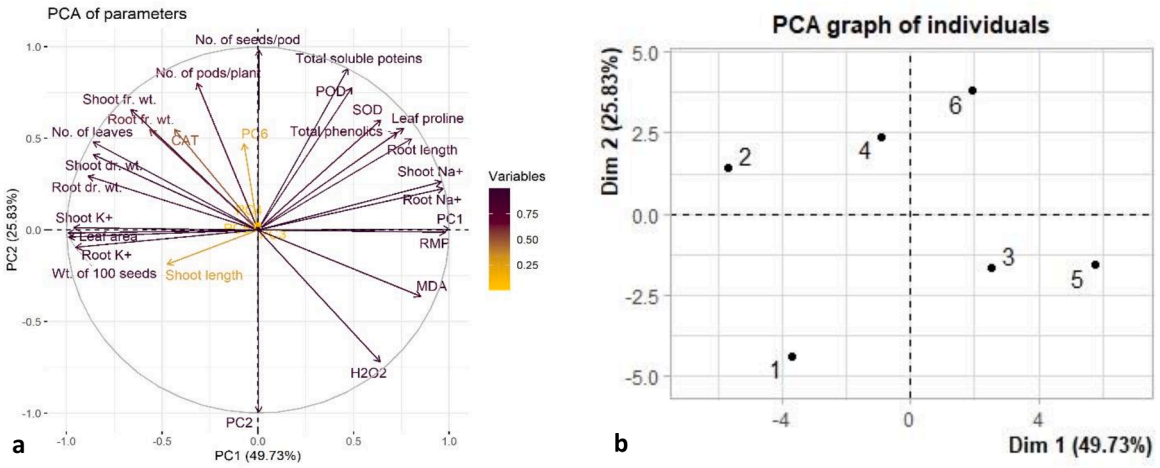

Fig. 14. Principal component analysis (PCA), PCA of parameters (a) and PCA of treatment (b). (Where numerics 1,2,3,4,5,6 represents different treatments applied to accession 200–06. Various abbreviations used in this figure are similar to Fig. 13).

cysteine triggers phenylpropanoid pathway (Picchi et al., 2021).

Our findings showed that NaCl stress remarkably elevated Na^+content while decreased the K^+content in roots and shoots of both *P. sativum* accessions. Whereas foliar application of CTS elevated the K^+ content and lowered the Na^+content in the shoots and roots of both *P. sativum* accessions. These findings are in agreement with what has already been reported (Mehmood et al., 2020; Zhang et al., 2021). Previous study on soybean plants revealed that under salt stress their roots need more photosynthetic products to acquire nutrients and enough water for the maintenance of normal growth (Ning et al., 2018). Under salty conditions only a small portion of the energy (10–40 %) produced by photosynthetic process and fixed into carbon compounds is effectively utilized by the plant for biomass accumulation. Following that, high salt levels penetrate the shoot and ultimately accumulate to hazardous levels in older leaves. This toxic condition leads to early senescence, lowering of plant's photosynthetic leaf area to a level that cannot support growth (Munns and Gilliham, 2015). Cytoplasm and organelle's metabolism is severely hampered by the cumulation of high amounts of Na^+ in plant tissues exposed to salty environment. Chloroplastic Na^+ buildup is toxic to cells in the same way that cytosolic Na^+ is (Assaha et al., 2017). Cellular sodium and potassium homeostatic disturbance caused by excessive sodium frequently results in lower K^+/Na^+ ratio (Mekawy et al., 2015). As a result of which plants experience potassium deficiency due to sodium's competitive suppression of its uptake in plants under NaCl stress. K^+ is a crucial nutrient for plant development and growth. It also plays a role in regulating stomatal closure, balancing osmotic pressure, photosynthesis and activities of enzymes (Zhang et al., 2020). An essential characteristic of plant salt resistance is the maintenance of a high shoot K^+/Na^+ ratio (Cuin et al., 2008). Aerial treatment of chitosan may promote plant development by balancing nutrient levels and lowering ion toxicity (G. Zhang et al., 2021). It was discovered that chitosan treatment prevented Na^+ions from being transported to the tissues responsible for photosynthesis by raising the expression of *AsHKT1* and genes encoding Na^+/H^+ exchangers under salt stress (Geng et al., 2020).

Our study demonstrates that salinity has drastic effect on *P. sativum* yield as in both *P. sativum* accessions crop yield gets reduced due to NaCl stress. These results are in agreement with previous findings on *P. sativum* (Ismail et al., 2022; Sapre et al., 2022; Sofy et al., 2020) and spinach plants (Ijaz et al., 2023) . Aerial treatment of CTS enhanced the yield in both *P. sativum* accession as compared to untreated stressed plants. These results are in similarity with what has already been reported in barley cultivars (Ibrahim and Arafa, 2020), chili pepper plants (Ramadan et al., 2022) and sweet pepper plants (ALKahtani et al., 2020). Reduction in crop yield could be ascribed to salinity's inhibitory impact on certain metabolic activities in plant tissues (Ghassemi-Golezani et al., 2012). It has been demonstrated that at high salt levels, salts may accumulate in the apoplast, dehydrating the cell and suppressing enzymes responsible for metabolizing carbohydrates in the cytoplasm or they can have a direct harmful effect on chloroplast and photosynthesis (Munns and Tester, 2008). Decline in photoassimilating assembly and photoassimilates's mobilization which decreased the index of harvesting (Oyetunji and Imade, 2015), viability of pollens and receptivity of stigma (Mohamed et al., 2018) were likely to blame for the decrease in yield under salt stress conditions. Decline in total seed weight caused by salinity stress can be ascribed to the suppression of mineral nutrient uptake and transfer throughout grain growth and filling stages. Furthermore, salt can cause significant damage to ovary, leading to fruit drop and decline in yield (Munns and Tester, 2008). Chitosan application boosted yield in salt-stressed plants by increasing stomatal conductance and net photosynthetic CO_2-fixation activity (Khan et al., 2002). Furthermore, chitosan's effect on flowering could be attributed to its influence on yield enhancement in salt stressed circumstances (Utsunomiya and Kinai, 1994).

Na^+ uptake by accession 200–03 significantly reduced morphological, and certain inorganic ion contents like K^+ in root and shoot. It is also evident from correlation graph (Fig. 11) which showed that Na^+ in shoot and root are highly negatively correlated to aforementioned parameters (Fig. 11). Yield parameters like 100 seeds wt., no. of pods per plant and no. of seeds per pod were highly positively correlated to morphological parameters in accession 200–03. It is thus assumed that treatment of CTS certainly improved metabolic processes in plants which ultimately resulted in improved growth and yield as compared to non-treated plants. PCA graphs also strengthen these assumptions. PCA graphs (Fig. 12a, b) showed that parameters POD, CAT, SOD, proline, total phenolics, TSP, shoot and root Na^+, MDA, H_2O_2 and RMP were significantly and positively correlated to each other and with PC1 which contribute for 62.35 %. Most of PC1 parameters are related to defense mechanism in plants or their resulting effects are induced under stress conditions. Parameters aligned with PC1 were negatively correlated to parameters of PC2, which contain most of morphological and yield characters studied in this research work.

The results of this study revealed that accession 200–06 survive and grow well under salt stress as compared 200–03. Thus, it was assumed that 200–06 can be a resistant accession of *P. sativum*. Correlation graph (Fig. 13) further support this assumption. This graph showed that Na^+in root and shoot of plants was positively correlated to root length. Plants may have certain modifications in their morpho-anatomical structures which resulted in improved root length even in stress conditions. Na^+uptake in accession 200–06 was also positively correlated with

enzymatic as well as non-enzymatic antioxidants and yield characters like no. of seeds per pod. Which indicated certain type of defensive/resistive approach in accession 200–06 that resulted in improved yield even under stress. PCA of parameters (Fig. 14a and b) also support these findings. PCA graph of parameters for accession 200–06 showed that most of the variances were present in first two principle components, contributing 75.56 % of the total. Out of which PC1 contributed 49.73 % and PC2 contributed 25.83 %.

5. Conclusion

Effects of NaCl stress and foliar treatment of CTS on growth, antioxidant activity, inorganic ions and yield attributes of two *P. sativum* accessions were analyzed in this study. *P. sativum* plants were treated with three salt concentrations (0, 60 and 120 mM) and two concentrations of CTS (0 and 120 mg/L). Both *P. sativum* accessions performed notably different from each other under salt stress. On the basis of remarkable reduction in biomass and yield parameters of accession 200–03 under NaCl stress, it is assumed that this accession is sensitive against salt stress. Whereas accession 200–06 under NaCl stress showed non-significant reduction in yield and biomass under stress conditions, indicating that this accession is resistant against salt stress. Foliar treatment of CTS boosted antioxidant enzyme activities, enhanced secondary metabolites (leaf proline and total phenolics), including endogenous melatonin level. These amendments in the internal cell environment enabled plants to tolerate threshold of salinity. CTS supplementation also lowered the level of H_2O_2, hence reducing lipid peroxidation. CTS reduced electrolyte leakage through improving RMP. These synergistic effects of CTS supplementation enhanced growth and ultimately yield of both *P. sativum* accessions in control as well as stressed environment. It is concluded that foliar treatment of CTS mitigated the deleterious effects of salt stress and modulated growth and yield attributes of *P. sativum* plants under salinity stress. Therefore, it is recommended that CTS mediated abiotic stress amelioration should be exploited in other crops, as well.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112509.

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