**A comparative study on the growth and yield of some pea cultivars under salt and heavy metal stress**

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**Abstract**

A greenhouse experiment was conducted to evaluate the effect of salt and heavy metals on morphological and biochemical parameters of pea (*Pisum sativum* L), using eight cultivars with four replicates. Results of analysis of variance, stress tolerance index and regression analysis showed significant differences among the cultivars based on different parameters studied under both control and stress conditions. In general, the salinity and heavy metal stress significantly affected the growth, yield and biochemical parameters at higher concentrations with pronounced effects on cvs. Lina Pak, Pea-450 and Pea-267. The applied stress treatments at lower concentrations increased the growth, antioxidant activity and protein content in some cultivars, significantly in Climax and Rondo. A gradual decrease was observed in photosynthetic pigments and number of leaves in all the cultivars but less significantly in cvs. Climax and Rondo. These results indicate a differential behavior among pea cultivars. Climax and Rondo showed greater potential for tolerance against salinity and heavy metal stress. A better growth performance was manifested by these cultivars with increase in antioxidant activity and protein content and less reduction in plant dry biomass while the cultivar Lina Pak was found to be the most sensitive against both stresses.

**Key words:** *Pisum sativum* L., salt stress, heavy metals stress, morpho-physiological parameters, antioxidant activity.

**Abbreviations:** Cd, Cadmium; Cr, Chromium; Pb, Lead; ROS, Reactive oxygen species; DAP, Diammonium phosphate; ANOVA, Analysis of Variance; ppm, parts per million; FW, fresh weight; DW, dry weight, DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate; IC50, Half-maximal inhibitory concentration; STI, Stress Tolerance Index; LSD, Least significant difference; NaCl, Sodium chloride.

**Introduction**

Pea (*Pisum sativum* L.) is an important leguminous plant belonging to family Fabaceae. It is a protein-rich legume crop cultivated worldwide as food and forage. It is considered as a potential source of proteins, carbohydrates, fibers, and digestible nutrients (Miljus-Djukic *et al.* 2013; Quafi *et al.* 2016). Environmental stresses like salinity and heavy metals have been reported to have a significant impact on growth and productivity of pea (Naeem *et al.* 2008).

Soil salinity is one of the major problems limiting agricultural production worldwide. All over the world, about 6% of the total agricultural land is salt-affected due to salinity (397 million hectares) and is not suitable for growing crops (Bhattarai *et al.* 2020). The presence of high salt concentration in the root zone causes higher osmotic pressure in soil solution than in plant cells, reducing the ability of plants to uptake water and essential minerals like potassium and calcium. Osmotic stress initially reduces leaf growth and eventually causes a reduction in shoot development and reproductive growth, primarily due to the water deficit in plant tissues (Munns & Tester, 2008).

Many heavy metals are required by plants at lower concentrations as trace elements which are essential in normal growth and development of plants. However, exposure to heavy metals at higher concentrations can have deleterious effects on plants including inhibiting seed germination and development, reducing plant biomass (stem, leaves, roots), inhibiting root growth, and effecting biochemical processes such as protein synthesis, photosynthesis, and different metabolic pathways (Tovar-Sanchez *et al.* 2019).

Plants grown in soil containing excessive levels of Cd show reduction in photosynthesis, nutrients, and water uptake, which results in chlorosis, retardation in growth, browning of root tips and eventually death of the plants (Clemens, 2001). Toxic effects of Cr on exposure to high levels include reduction in plant growth resulting in low yield. Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition. (Shanker *et al.* 2005). High level of Pb also causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition. Exposure of plants to toxic level of Pb adversely effects morphology, growth, and photosynthetic processes of plants (Nas & Ali, 2018).

It has been reported that the antioxidant activities of plants are due to the presence of phenolic compounds including tannins, flavonoids, phenolic diterpenes and phenolic acids (Lee *et al.* 2004). The synthesis of these polyphenolic compounds and their accumulation in plants has been attributed to abiotic or biotic stresses such as heavy metal or salt stress. These compounds protect the plants by scavenging reactive oxygen species (ROS) which are produced due to disruptions in different metabolic pathways induced by environmental stresses (Bettaieb *et al.* 2011). In addition to phenolic compounds, the protein content of plants can also be affected by environmental stress. These proteins may provide a storage form of nitrogen to be utilized when the stress is over.

Although plants have evolved strategies to cope with heavy metal toxicity such as reduced uptake into the cell, binding with phytochelatins, production of osmolytes and antioxidants, overexpression of genes and altered expression of enzymes (Sharma *et al.* 2011), but there is a need to identify stress tolerant plants for growing under such stressed conditions. Different morphological, biochemical, and molecular approaches have been applied for the identification of the most tolerant cultivars (Khalil *et al.* 2020).

In Pakistan, soil salinity is a serious problem, and more than six million hectares of land is affected by salt (Qureshi, 2009). Similarly, in many agricultural areas, the problem of metals pollution is increasing in the absence of any adequate preventative measures. Therefore, the development of crop cultivars which are salt tolerant is being considered as an alternative and effective strategy for producing sustainable crops. The objective of the present work is to identify the most tolerant and sensitive cultivars of pea to salt and heavy metals stress by using morphological and physiological characteristics and to correlate them with stress combating compounds produced in plants.

**Materials and Methods**

**Experimental Set up and Plant Material**

Seeds of eight pea cultivars (Climax, Janass, Lina Pak, Meteor, Rondo, Pea-09, Pea-267 and Pea-450) were obtained from Ayub Agriculture Research Institute, Faisalabad. The seeds were planted in earthen pots containing sandy loam soil and fertilized with diammonium phosphate (DAP). The pots were irrigated with tap water for 20 days and then given different stress treatments in greenhouse. The experimental design was Randomized Complete Block Design with four replicates per treatment. Statistical analysis was carried out using Analysis of Variance (ANOVA) using SPSS 19 Statistical software to determine the significance of differences among the treatments and cultivars and the means were compared at 5% probability level.

**Application of Salt and Heavy Metal Treatment**

Three weeks old seedlings were given NaCl (0, 20, 40, 80 and 100mM) solutions through irrigation. At the same time, they were treated with PbCl2 (50ppm,100ppm), CdCl2 (50ppm,100ppm), and CrCl2 (50ppm,100ppm) through irrigation. Each group had ten pea seedlings of each cultivar with one group selected as a control from each cultivar.

**Morphological Observations**

Morphological parameters including, height of plants, number of shoots, nodes, pods, and leaves, internodal length and leaf area were recorded. All plants were carefully harvested along with roots at the end of growing season and root length was recorded of all treated plants. Fresh weight (FW) and dry weight (DW) of shoots, roots and pods were taken after harvesting. Dry weight of each plant was recorded after keeping in an oven at 70oC for 3 days. Chlorophyll content of each plant (control and treated) was checked with the help of SPAD 502 meter (Minolta, Japan).

**DPPH Scavenging Assay**

Radical scavenging activity DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) of methanol leaf extract was estimated according to method of Gyamﬁ *et al.* (1999). Three ml (from different dilution 5-50ug/ml) of sample extracts were added to 1.0 mL of DPPH (0.1mM prepared in methanol solution), incubated for 30mins in dark and the absorbance was taken at 520nm. The standard curve was prepared using Ascorbic acid. The mixture of water and methanol was used as control. The following formula was used for calculations.

DPPH scavenging ability = [(Abs control – Abs sample)/ (Abs control)] × 100

IC50 value: IC 50 = (50-b)/a. where a= intercept value and b= slope value

**Determination of Total Protein Content**

Seed extract was prepared in 0.1M phosphate buffer. Total protein content was analyzed according to Bradford (1976) using BSA as a standard. Amount of total protein was measured spectrophotometrically at 595nm.

**Statistical Analysis**

Mean value of all parameters including growth, yield, chlorophyll, protein content and antioxidant activity was compared by LSD using Tuckey’s t-Test (Li, 1964). For understanding relationship among different parameters and cultivars MS excel regression analysis was used. For evaluating tolerance in cultivars STI (Stress Tolerance Index) was determined following Fernandez (1992) using the formula:

STI = (Yp\*Ys)/Yp2,

where Yp and Ys is the yield of cultivars in control and stressed conditions, respectively. Yp2 is the mean of all cultivars in control treatment.

**Results**

The effects of salt and heavy metals stress on morphological (shoot length, number of shoots, number of nodes, length of internodes, number and area of leaves, days to flowering, number and weight of pods, length of root, fresh and dry weights of shoots and roots) and biochemical (chlorophyll and soluble protein content and antioxidant activity) parameters of eight pea cultivars is shown in Figs. 1-7.

Most of the growth parameters showed a decrease under both type of stress conditions in most of the cultivars as compared to control, while some cultivars showed better growth under low levels of stress.

**Morphological characteristics**

In the salt treated plants, the length of shoots increased at low NaCl concentration and significantly decreased at high concentration of salt (100mM). Significant increase in the height of the plant was observed in cvs. Pea-267 and Rondo and maximum reduction was shown by cvs. Pea-450 and Climax (Fig. 1a, 1d). The average number of nodes per shoot generally showed a decreasing tendency as compared to control plants (Supplementary data). Under heavy metal stress, most of the cultivars showed better response with respect to height of plants at low concentrations of Pb and Cr stress than Cd except cv. Climax. Among all the cultivars, Rondo, Climax and Lina Pak showed better growth response while maximum reduction in above given parameters was shown by Pea-09 and Pea-450 (Figs 1).

**Fig: 1.** Variations in the plant height of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05).

**Number and area of leaf**

In salt treated plants, minimum reduction in the average number and area of leaves was observed in cvs. Meteor and Pea-267, while Pea-450 and Climax were most effected at high salt concentrations as compared to other cultivars (Fig 2a-b). In general, the average number of leaves in all the metal treated plants showed a decrease in all the treatments except for Meteor in which a slight increase in number of leaves was observed at 50ppm of Cr (Fig. 2a). Measurement of average leaf area of the treated plants showed a decrease in four cultivars (Rondo, Pea-09, Pea-267 and Pea-450), while an increase was observed only at 50ppm Cr in cvs. Climax, Lina Pak and Meteor (Fig. 2b). Among the heavy metals tried, Cd treatment showed a significant adverse effect on all the above parameters as compared to others.

**Fig 2**. Variations in the vegetative growth performance of eight cultivars of pea in response to different concentrations of NaCl and heavy metals, (a) number of leaves and (b) area of leaves of eight pea cultivars.

Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

**Flowering**

Among all the pea cultivars under control conditions, Lina Pak and Meteor were early flowering while Pea-450 and Pea-267 were late flowering cultivars. Generally salt and heavy metals stress induced early flowering in cvs. Climax, Janass and Pea-267, while a delay in flowering was observed in cvs. Meteor, Rondo, Pea-09 and Lina Pak as compared to control (Fig. 3a). Among the metal treated plants, Cr stress significantly delayed the flowering in all cultivars except Climax. Under Pb and Cd stress, an early flowering was observed only in cvs. Janass and Meteor.

**Fig 3**. Variations in days of flowering of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

**Effect on number and size of pods**

Generally, the average number and weight of pods increased at 20mM NaCl concentration and decreased at high concentration and under heavy metals stress and as compared to control. A significant increase in pod weight was recorded in Pea-09 (11.3gm) in salt stress and maximum decrease was observed in Pea-450 and Lina Pak (Figs. 4a and 4b). Among all the tested heavy metals, Cr significantly decreased the number of pods in all the pea cultivars.

**Fig 4**. Variations in the yield of eight cultivars of pea in response to different concentrations of NaCl and heavy metals, (a) number of pods and (b) weight of pods. Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

**Root length**

The lower salt concentration of 20mM induced an increase in root length in all the cvs. except Lina Pak as compared to control. In cv. Meteor, further increase in the length of roots was observed at 40mM salt concentration, while a decrease in root lengths was recorded at all the higher concentrations in all the cultivars (Fig 4). Root length of heavy metals treated plants showed an increase under Cr stress in most of the cultivars while Pb (50ppm) stress increased the root length of some cultivars (Climax, Janass, Meteor and Pea-267). In case of Cd stress, only cv. Janass showed an increase in root length. Minimum reduction in root length was recorded in cv. Rondo in all the metals as compared to control while maximum reduction was observed in Lina Pak and Pea-09 (Fig.5).

**Fig 5.** Variations in the length of roots of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

**Shoot and root biomass.**

In case of shoot biomass, the maximum reduction was observed in cvs. Pea-450 and Pea-09, while the minimum reduction in biomass was recorded for cvs. Meteor and Janass (Fig.6a and 6b). While an increase in both FW and DW of roots was seen in four cultivars including Janass, Rondo, Pea-267 and Pea-450 as compared to control. Minimum reduction in biomass of roots was observed in cvs. Meteor, Pea-267 and Janass, while maximum reduction was shown by cvs. Pea-09 and Rondo under salt stresses (Figs. 7a and 7b).

**Fig 6.** Variations in the F.W and D.W of shoots of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

The data recorded for FW and DW of shoots under heavy metals treatment showed a significant decrease in cv. Pea-450, Pea-09 and Janass. Significant reduction in DW of shoots was shown by cvs. Pea-450 and Janass.

**Fig 7.** Variations in the F.W and D.W of roots of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05).

Generally, Cr did not significantly decrease the FW and DW of shoots of all cultivars except Rondo as compared to other heavy metals (Figs. 6a, 6b). Effect of heavy metal stress on FW and DW of roots of all the cultivars were also recorded. An increase in FW was observed at 50ppm Pb, 50ppm Cd in cv. Rondo and at 50ppm Cr in cv. Meteor while maximum reduction was observed in Pea-09 (Fig. 7a). An increase in the DW of roots under Cr stress was observed in cvs. Climax, Janass, Meteor, while in case of cv. Rondo, the DW was increased in low concentrations in all the heavy metal treatment. Maximum reduction in DW was recorded in Pea-09 and Pea-450 cultivars as compared to others (Fig. 7b).

**Effect on chlorophyll content**

Salt stress seems to have a negative influence on the chlorophyll content of the leaves and a sharp decline was observed in all the cultivars at all the concentrations. Salt stress significantly decreased the chlorophyll content in cvs. Lina Pak and Meteor while minimum reduction was observed in cvs. Pea-09 and Pea-267 as compared to other cultivars (Fig. 8). Among the control plants, maximum chlorophyll content was recorded for cvs. Meteor, Rondo and Pea-09. A general trend of decrease in chlorophyll content after heavy metal treatment was observed in all the pea cultivars with significant reduction under Cd stress. Among all the cultivars, Rondo and Janass showed minimum reduction, while Lina Pak and Pea-09 were significantly affected by heavy metal stress (Fig. 8).

**Fig 8.** Variations in the chlorophyll content of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of three replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

**Effect on antioxidant activity**

The results of DPPH activity of the leaf extracts of eight pea cultivars under different salt concentrations is shown in Fig. 9. A general increase in antioxidant activity was observed at lower concentration (20mM) of NaCl in all the cultivars tested except Pea-450, and the values decreased with an increase in concentration. Among all the cultivars, cvs. Rondo (IC50 1.4) followed by Meteor and Pea-09 showed maximum activity at 40mM salt concentration. Maximum decrease at 100mM Salt concentration was observed in Pea-267(IC50 10.7) and Pea-450 (IC50 10.2). High salinities negatively affected the growth and biochemical attributes of pea cultivars. Among the salt treated plants of pea, cvs. Pea-09 and Rondo showed maximum potential for salt tolerance, whereas cv. Pea-450 showed a significant reduction in growth and biochemical parameters. Among the cultivars, maximum antioxidant activity in control treatments were shown by Janass and Rondo. Under heavy metal stress at lower concentrations an increase was observed in cvs. Rondo (50ppm Cr, Cd and Pb), Meteor (50ppm Cd and Cr), Janass and Climax (50ppm Pb) while a significant reduction was observed in cvs. Lina Pak and Pea-450 (Fig. 9).

**Fig 9.** Variations in the antioxidant activity of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of three replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

**Effect on total soluble proteins**

Total soluble protein content estimated by Bradford method ranged from 1.0mg/gm - 8.0mg/gm of Ascorbic acid equivalent to pea pod extract. Salt stress treatment increased the protein content in most of the pea cultivars except Climax, Pea-267 and Pea-450 at lower salt concentration (20mM) as compared to control. A significant increase was observed in cvs. Pea-09 and Rondo with an increase in salt concentration as given in Fig. 10. Effect of heavy metal stress on total soluble proteins from seeds of all the pea cultivars is given in Fig. 7c. Maximum protein content was recorded in control of cv. Lina Pak which significantly decreased because of heavy metal treatment. A negative effect of treatment with Cd and Pb on the amount of seed proteins was recorded in almost all the cultivars. Under Cr stress a significant increase in protein content was recorded for cv. Climax, while a significant increase was observed in cv. Rondo at both the concentrations of Pb (Fig. 10).

**Fig 10.** Variations in the antioxidant activity of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of three replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05)

**Regression Analysis**

Results of regression analysis (R2) showed that the parameters including number of nodes and shoots, DW of roots, weight of pods, their protein content and antioxidant activity showed highly significant changes as compared to other parameters tested (Table 1). Among the different cultivars, Climax showed the maximum value of R2 in almost all the parameters as compared to other cultivars, while maximum variations in growth parameters were shown by Pea-267 and Janass.

**Stress Tolerance Index (STI)**

Stress tolerance index given by Fernandez, (1992) was used to determine the stress tolerant and stress sensitive cultivars of Pea that have the maximum productivity under control and stress conditions (Table 2) (Supplementary data). According to the data given in the Table 1 (Supplementary data), Pea cultivars showed a high diversity of STI to salt and heavy metal stress. The cvs. Climax and Pea-267 in control while cvs. Climax and Rondo had maximum yield (number of pods) value, while cvs. Lina Pak and Janass showed minimum productivity in both conditions showing their sensitivity towards both the stress treatments.

**DISCUSSION**

The current study was aimed at investigating the effects of salt and heavy metals stress on growth, yield, and antioxidant activity of different pea cultivars, in order to characterize stress tolerant and stress sensitive cultivars among the cultivated peas being grown in Pakistan.

Plants can adapt to short-term fluctuations by showing increase or decrease in different morphological characters. Short term environmental changes and stress conditions can change the growth pattern like height, internode length, leaf area and shape and shoot-root ratio (Amist & Bano, 2019). In this study, an increase in shoot length was observed at low salt and heavy metal concentration in cvs. Rondo and Pea-267 and then a gradual decrease was observed at higher concentrations used. Similar results were reported by Amira & Qados, (2011) on *Vicia faba.* These changes may be attributed to the reduction in osmotic potential of soil and in turgor pressure within cells, resulting in ionic and nutritional imbalance which hampers plant growth and development (Ahmad *et al.* 2019). These stresses induce morpho-physiological disorders in plants resulting in decreased crop productivity (Shahid *et al.* 2015).

In this work, the number and area of leaves were negatively affected by salinity and heavy metals stress, however a slight increase in leaf area was observed in some cultivars at 20mM NaCl concentration. The decrease in leaf number may be due to the accumulation of NaCl in the cell walls and cytoplasm of the older leaves. Similarly, the reduction in leaf area could be explained by the negative effect of stress on photosynthesis that leads to the reduction of leaf area and ultimately plant growth (Netondo *et al.* 2004). Wang *et al.* (2014) also reported differential response of three *Salix integra* cultivars to heavy metal stress which may be attributed to the differences in the genotypes of these cultivars.

Significant variations in days to flowering were observed among the cultivars under both stress conditions. Cho *et al.* (2017) observed that under stress conditions, plants either produce flowers earlier or delay due to slow metabolism rate. A low salt concentration induced earlier flowering was recorded in some cultivars (Climax, Janass and Pea-450) in the present study. A significant increase in days to flowering under salt and heavy metal stress was observed in cv. Lina Pak. Shekari *et al.* (2019) reported the negative effect of heavy metals in cucumber while Zapryanova & Atanassova, (2009) reported earlier blooming in some ornamental plants under NaCl stress.

Similarly, the number and weight of pods increased at low concentration of salt (20mM) in cvs. Pea-09, Climax, Janass and Rondo and significantly decreased under high salt stress (100mM NaCl). Reduction in grain yield due to salt stress has also been reported for some other crop species as well (Sohrabi *et al.* 2008), which has been explained as the result of lowering of water potential which induces ionic stress and results in a secondary oxidative stress.

Heavy metals stress mostly reduced the number and weight of pods in most of the treated plants. A significant negative influence on all the yield components in turnip and lettuce plants irrigated with water containing heavy metals was reported by Hassanein *et al.* (2013). The negative effect of heavy metals on growth and crop yield may be due to decrease in water uptake by roots which results in decrease in the uptake of nutrients, thus inhibiting metabolic processes required for normal plant growth.

The root length at 20mM salt concentration increased in all the cultivars except Lina Pak and gradually decreased with increase in salt concentration. A significant reduction in FW and DW was observed in all cultivars which may be due to decrease in the number of roots as compared to root length. Accumulation of salts in the root zone decreases leaf water potential preventing the absorption of nutrients and water by plants. The water status of plant gets decreased due to physiological drought experienced by plants when subjected to salinity stress (Munns & Tester, 2008).

In case of heavy metals, Cr significantly increased the length and FW and DW of roots as compared to Pb and Cd. Establishment of roots helps the plants to cope with stress, but high evapotranspiration rate induces an osmotic stress and root dehydration. This results in decrease in growth and productivity of plants under stress due to decrease in the uptake of water leading to symptoms of drought (Paul *et al.* 2019). The FW and DW of shoots of pea plants significantly decreased under salt stress but some cultivars (Rondo and Pea-09) showed an increase in FW and DW at lower NaCl concentrations. The FW and DW depend on the number of nodes and internodal length and accumulation of salt ions in leaves. Among all the treatments, Cd was found to be most toxic and significantly reduced the yield and plant biomass of almost all the cultivars. Only cvs. Climax and Janass were moderately tolerant to Cd stress at lower levels. The decreased root and shoot FW and DW are due to the reduced water absorption, which in turn causes a reduction in the amount of water in plant tissue (Sharma *et al.* 2004). According to our results, a decrease in shoot to root ratio has been observed under salt stress while in case of heavy metals it increased at lower concentration. The decrease in the ratio is a potential indicator of salt stress. This may be due to decrease in water uptake rather than salt specific effect and also due to the accumulation of toxic ions in the aerial parts (Acosta-Motos *et al.* 2017).

The growth of plants depends on the photosynthetic activity and determination of chlorophyll level checks plant tolerance to stress conditions (Xu *et al.* 2008). In the present work, the salt stress mostly affected the chlorophyll content of pea cultivars as compared to other parameters which gradually decreased in all cultivars and more significantly in cv. Lina Pak. The decrease in chlorophyll levels in salt-stressed plants has been considered a typical symptom of [oxidative stress](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/oxidative-stress) and is often attributed to the inhibition of chlorophyll synthesis, together with the activation of its degradation by the enzyme [chlorophyllase](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/chlorophyllase%22%20%5Co%20%22Learn%20more%20about%20Chlorophyllase%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) ([Santos, 2004](https://www.sciencedirect.com/science/article/pii/S0254629915326739%22%20%5Cl%20%22bb0190)). In case of heavy metal stress, a significant decrease in chlorophyll content was observed in all cultivars especially under Cd stress. This decline is most probably due to the inhibition of the reductive steps in the biosynthetic pathways of photosynthetic pigments due to the high redox potential of many heavy metals (Chandra & Kang, 2016). Similar decrease in chlorophyll content under heavy metal stress in *Helianthus annuus* (Zengin & Munzuroglu, 2006) and almond (Elloumi *et al.* 2007) has been reported.

In the present study, protein content was increased in most of the pea cultivars at lower concentration of salt which may be due to a higher tolerance level of these cultivars to these NaCl concentrations. The decrease in proteins under higher salt concentrations in most of the cultivars could be due to the effect of salt on protein synthesis (Omar *et al.* 1993). Plants develop a survival mechanism by accumulating proteins that protect the plant cells from the effects of stress (Wang *et al.* 2003). An increase in protein concentrations in cvs. Rondo and Pea-09 in all the concentrations showed their increased tolerance level towards salinity as compared to other cultivars.

In case of heavy metals stress, the total protein content showed an increase in both the concentrations of Cr in cv. Climax, Pb in case of cv. Rondo and a significant decrease was observed in cvs. Lina Pak and Pea-450, while in the remaining cultivars a decrease in protein content was observed at higher concentration of heavy metals. Proteins are basic component of cells and are easily influenced by stress conditions. The reduction in protein content is associated with high protease activity induced by stress. The metals react with the thiol group of proteins band replace it with metal and form metalloproteins (Pena *et al.* 2006). The increase in protein content in cvs. Rondo and Climax may be attributed to the production of stress proteins under stress conditions. Our results show that among the heavy metals, the protein content was significantly affected by Cd stress followed by Pb and Cr.

Our studies revealed that antioxidant activity increased at lower concentration of salt (20mM) and upon further increase it decreased in most of the pea cultivars. Similar increase in antioxidant activity due to NaCl treatment has been reported by Lechno *et al.* (1997). Increase in antioxidant activity may be due to the free radical scavenging activities of bioactive compounds such as phenols, flavonoids and tannins synthesized by plants to protect their vital metabolic functions. In general, the plants prevent stress damage by increasing the levels of antioxidants, thereby increasing resistance to various stresses.

In the present work, an increase in antioxidant activity was observed at lower concentrations of heavy metals in cv. Rondo than most of the pea cultivars, while a significant decrease in antioxidant activity was shown by Pea-450 followed by Lina Pak. It has been reported that free radical formation and ROS (reactive oxygen species) is considered as the initial response to environmental stress and these are harmful to cellular components. To repair this damage, plants have developed different antioxidant systems as a defense strategy to cope up with the stress conditions (Nadarajah, 2020). Our results show that Cr significantly decreased antioxidant activity in most cultivars followed by Cd and Pb. The use of standard multiple regression analysis could be useful to determine the relationship between growth and biochemical parameters of control and stress treated genotypes (Tadayyon *et al.* 2011). Salt tolerance model developed by Maas & Hoffman, (1977) was commonly used to express salt tolerance of the crops. According to the model, plants tolerate or withstand soil salinity until a threshold value is reached and then a linear yield reduction occurs for per unit increase in soil salinity as has been indicated in the present work.

**Conclusion**

Salt and heavy metal stress caused significant effect on growth, yield and biochemical parameters of eight pea cultivars. According to our results, stress tolerance index and regression analysis, cvs. Climax and Rondo are salt and heavy metal tolerant cultivars while cv. Lina Pak is the most sensitive one. The maximum reduction in growth and yield was observed in cvs. Lina Pak, Pea-267 and Pea-450, while cvs. Climax and Rondo showed remarkable resistance to salt and heavy metal stress.

**Acknowledgements**

The present research work was funded by University of the Punjab, Lahore, Pakistan.

**Conflict of interest**

The authors declare that they do not have any conflict of interest.

**Author contributions**

This article is a part of the PhD dissertation of F.I. H.A. and F.B are the supervisor and co-supervisor, respectively. The experiments were performed, and data was analyzed, and the first draft of the manuscript was written by F.I. H.A. and F.B. revised the manuscript. All the authors read and finalized the manuscript.

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**Supplementary Figure and Tables.**

**Fig:**  Variations in the number of nodes of eight cultivars of pea in response to different concentrations of NaCl and heavy metals. Each value is the mean of four replicates and vertical bars represent the standard error (SE) of mean. (*P*≤0.05).

**Table 1.** Simple linear regression (R2) of different parameters of pea cultivars under stress condition.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | Climax | Janass | Lina Pak | Meteor | Rondo | Pea-09 | Pea-267 | Pea-450 |
| Length of shoots | 0.88 | 0.99 | 0.93 | 0.95 | 0.98 | 0.97 | 0.95 | 0.98 |
| Number of shoots | 0.93 | 0.72 | 0.61 | 0.63 | 0.65 | 0.8 | 0.6 | 0.63 |
| Number of nodes | 0.68 | 0.67 | 0.57 | 0.65 | 0.89 | 0.88 | 0.94 | 0.91 |
| Length of internodes | 0.95 | 0.95 | 0.95 | 0.96 | 0.86 | 0.95 | 0.92 | 0.98 |
| Number of leaves | 0.97 | 0.93 | 0.96 | 0.96 | 0.95 | 0.98 | 0.96 | 0.99 |
| Area of leaves | 0.98 | 0.97 | 0.97 | 0.99 | 0.96 | 0.96 | 0.96 | 0.95 |
| Days to flowering | 0.95 | 0.94 | 0.98 | 0.97 | 0.6 | 0.98 | 0.44 | 0.94 |
| Number of pods | 0.96 | 0.94 | 0.85 | 0.9 | 0.93 | 0.84 | 0.99 | 0.92 |
| Weight of pods | 0.96 | 0.93 | 0.88 | 0.93 | 0.93 | 0.89 | 0.84 | 0.89 |
| Length of roots | 0.96 | 0.96 | 0.97 | 0.96 | 0.92 | 0.98 | 0.98 | 0.96 |
| F.W of shoots | 0.89 | 0.94 | 0.96 | 0.96 | 0.96 | 0.96 | 0.92 | 0.98 |
| D.W of shoots | 0.97 | 0.96 | 0.94 | 0.95 | 0.96 | 0.95 | 0.93 | 0.96 |
| F.W of roots | 0.91 | 0.91 | 0.96 | 0.92 | 0.87 | 0.9 | 0.93 | 0.9 |
| D.W of roots | 0.81 | 0.95 | 0.95 | 0.9 | 0.85 | 0.78 | 0.97 | 0.95 |
| Chlorophyll content | 0.97 | 0.91 | 0.95 | 0.98 | 0.97 | 0.97 | 0.97 | 0.96 |
| Protein content | 0.8 | 0.95 | 0.79 | 0.9 | 0.95 | 0.96 | 0.9 | 0.93 |
| Antioxidant (IC50) | 0.84 | 0.59 | 0.89 | 0.96 | 0.98 | 0.96 | 0.67 | 0.76 |

**Table 2**. Stress Tolerance Index (STI) of pea cultivars under stress conditions.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatments | Climax | Janass | Lina Pak | Meteor | Rondo | Pea-09 | Pea-267 | Pea-450 |
| Control (Yp) | 14 | 6.6 | 6 | 10.6 | 11 | 8.6 | 11.6 | 10.6 |
| NaCl 20mM | 2.44 | 0.58 | 0.24 | 1.3 | 1.57 | 0.59 | 1.3 | 1.08 |
| 40mM | 1.9 | 0.33 | 0.22 | 0.7 | 1.38 | 0.49 | 1.14 | 0.6 |
| 80mM | 0.76 | 0.13 | 0.18 | 0.25 | 0.74 | 0.44 | 0.63 | 0.43 |
| 100mM | 0.29 | 0.1 | 0.14 | 0.17 | 0.4 | 0.28 | 0.39 | 0.25 |
| Pb 50mg/l | 1.52 | 0.37 | 0.36 | 0.39 | 1.64 | 0.49 | 0.9 | 0.68 |
| Pb 100mg/l | 0.8 | 0.22 | 0.22 | 0.14 | 0.85 | 0.2 | 0.511 | 0.32 |
| Cd 50mg/l | 2.3 | 0.37 | 0.18 | 0.54 | 0.67 | 0.22 | 0.66 | 0.32 |
| Cd 100mg/l | 1 | 0.13 | 0.1 | 0.28 | 0.56 | 0.11 | 0.23 | 0.14 |
| Cr 50mg/l | 1.43 | 0.42 | 0.18 | 1.04 | 0.56 | 0.49 | 1 | 0.8 |
| Cr 100mg/l | 0.94 | 0.29 | 0.1 | 0.6 | 0.29 | 0.2 | 0.78 | 0.43 |
| Mean yield of pea cultivars = 97.5 |