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Screening of pea genotypes against salinity at seedling stage

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Abstract

Abstract

Different 15-genotype of Pea (*Pisum sativum* L.) were grown in germination trays containing fine sand as a growth medium and exposed to five different salts (NaCl, $MgSO_4$, $CaCl_2$, Na_2SO_4) treatment, i.e., 0, 2.5, 5.0, 7.0 and 10 dS m^{-1}. The germination and emergence percentage, root /shoot length, shoot/root dry weight, leaf area, and number of leaves per plant and chlorophyll content of all genotypes showed a significant decrease as salts levels increased. On the basis of percentage increase or decrease in the above-mentioned traits, the investigated genotypes were considered as salt-tolerant, intermediate tolerant and salt-sensitive groups. Therefore, Samarina zard, Climax, Sadique-1 and sprinter were found to be salt tolerant. While 6173, F-16, Jurass, Olympia and Green cross showed an intermediate salt-tolerant response. Whereas Ambasidar, Green arrow, Meteor and Assar were observed as highly salt-sensitive genotypes. Tolerant genotypes (Samarina zard, Climax, Sadique-1 and Sprinter) were successful in maintaining the maximum dry matter, low Cl^- and Na^+, while high P, Mg, Ca and K^+ under saline conditions.

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Introduction

Pea (*Pisum sativum* L.), locally known as "Matar" belongs to the family Fabaceae, commonly used as fresh pea, edible-podded type and dry pulses. Pea considers an excellent human food; it contains carbohydrates, protein (Hussain *et al.*, 2006), minerals, fats, vitamins (A, B and C) in a reasonable amount (Choudhary, 1990) and antioxidants and water-soluble fiber (Noreen *et al.,* 2009). In Pakistan, Pea is an important crop that plays an important role in the farmer's economy. It is the most common crop and enjoys a great commercial demand due to its nutritional value. It is cultivated during winter in plains and during summer in highlands (Habib and Zamin, 2003).

Traditionally Pea seeds are used as nutrients, refrigerants, laxatives, astringent, and appetizers and also used in treating acne, diabetes, phlegm, intestinal inflammation and wrinkled skin. Antioxidant, antimicrobial land hypoglycemic activities of seeds also have been reported (Saima and Azra 2011). Furthermore, the pericarp of pea pods showed potential Antihyperglycemic activity (Taha *et al.*, 2011). Fruits and seeds contain starch, albuminoids, alkaloids, galactolipids, trigonelline, piplartine and essential oils. Petiole and tendril yielded kaempferol-3-triglucoside, quercetin-3- triglucoside, and their p-coumaric esters (Rastogi and Mehrotra, 1990). It is usually used in the cure of heart diseases, as a blood purifier and diabetes (Zilani *et al.,* 2017).

Worldwide food safety is seriously dependent on crops and their supplies, which require significant increases for investigative the gap among production and demand (Majeed and Muhammad, 2019). The requirement of successful crop production has been much more emergent in the previous years due to the increasing population, which will exceed 9.7 billion by 2050. Undoubtedly, increases in the population will exert pressure on crops and food resources (Soliman *et al.*, 2018). At the same time, global warming, as well as several abiotic and biotic strains, delays the growth and yields of agricultural crops (Soliman *et al.*, 2018). Among abiotic stresses, salinity is recognized as one of the leading restricting causes affecting the growth and productivity of agricultural crops, mainly in arid and semiarid regions (Elkeilsh *et al.*, 2019). Under osmotic stress conditions, plants are forced to escape from the salt accumulated area and make roots to be adapted to the saline condition. Under the long period of salinity stress, water availability to the root zone is decreased; consequently, resulting in low absorption of nutrient elements by roots. Reduction in nutrient uptake and metabolism and protein synthesis under salt stress conditions has been reported by several investigators (Dubey *et al.*, 2014; Lotfi *et al.*, 2015).

Abiotic stress issues of the environment, mainly soil salinity and drought, are the main reason for the decline of agriculture yields. Increasing human population and a decrease in land existing for cultivation are two threats to agricultural sustainability (Shahbaz and Ashraf, 2013). Soil salinity is one of the most common global problems faced by farmers, which harmfully affect particularly 20% of the lands and causes a reduction in the total yield of crops (Qadir *et al.*, 2014; Negrão *et al.*, 2017).

Approximately salinity affected 800 million hac of land and 32 million hac of cultivated land. (Wichelns and Qadir, 2015). Several investigations have suggested that soil salinity stress has a marked influence on plant morphology and Biochemical responses of plants like reduction in fresh and dry weight, stunted growth and altered enzymatic activities at a cellular level (Chartzoulakis and Klapaki, 2000). Plants' response to salinity can be defined in two main stages: first, the shoot ion-independence reaction occurs, with minutes to days, and is supposed to be related to sodium ion sensing and signaling (Roy *et al.*, 2014).

Salt sensitive plants are, when grown in low or moderate saline soils, unable to show proper growth and development. In contrast, salt-tolerant plants are able to grow and reproduce in high saline soils (Munns and Tester, 2008). The germination delays due to salinity, resulting in decreased plant growth and total crop yield (Azzedine *et al.,* 2011; Basiri *et al.*, 2013).

Saline soils prevent water uptake by the plants due to osmotic stress produced by an excess of salt ions in soil which decreases the turgor pressure inside the cell and causes wilting. Another prominent effect of salinity is the gathering of toxic excise ions in the root zone, which produces discrepancy of ions and results in ion toxicity, reduction in the availability of useful ions and hence causes malnutrition in plants (Munns and Tester, 2008).

Materials and methods

Experimental detail

Seeds of 15 different pea genotypes were collected from Ayyub Agriculture Research Institute, Faisalabad. Seeds were sown in germination trays placed in a growth chamber at the Department of Horticulture, College of Agriculture, University of Sargodha. Seeds of pea were sown in germination trays. After emergence, numbers of seedlings were thinned to four by removing all weak and unhealthy seedlings. The seeds were watered according to the need of the plant by observing the moisture of sand. The salinity levels i.e., 0, 2.5, 5.0, 7.0 and 10.0 dS m^{-1} ($NaCl$, $MgSO_4$, $CaCl_2$, Na_2SO_4) Salts were applied at 20 days after germination. The desired salinity levels were created by dissolving the specific amounts of Salts in distilled water. The experimental unit was arranged in a CRD with four replications. Data for the following parameters were recorded.

Morphological parameters

Germination percentage: After disinfecting the Pea seeds with 10% sodium hypochlorite, the germination test seeds of 15 genotypes of pea were accomplished in Petri dishes. Each petri dish had twenty-five Pea seeds in soluble Whatman filter paper, soaked with relevant desired saline solutions (0, 2.5, 5.0, 7.0,10 dSm^{-1} $NaCl$, $MgSO_4$, $CaCl_2$, Na_2SO_4Salts) and distilled water used for control. The treatments were replicated and placed in the growth chamber at 20 to 22 ^{0}C. Germination was calculated for five days. The germination percentage was calculated as under.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Emergence percentage: Seeds of all 15 pea genotypes were sown in germination trays. Half strength Hogland solution was used as a nutrient medium. Desired salinity levels (0, 2 .5, 5.0, 7.0 and 10 dSm-1Salts) were created before the sowing seeds. The number of seeds that emerged (4.0cm) from the sand was calculated by using the formula.

$$\text{Emergence percentage} = \frac{\text{Number of seeds emerged}}{\text{Total number of seeds sown}} \times 100$$

Seedling shoot and root length (cm): Shoot and root length per plant were taken by using a measuring tape in cm and the average was calculated (Sarwar *et al.*, 2016). Seedling root, shoot dry weight (g) per plant on the completion of experiment plants were taken out of the pots and dry weight of root, the shoot was taken by drying the plants at 65^{0}C for 48 hours in the oven (Memmert-110, Schawabach). After that, weight is taken in (g) by using an electric balance.

Numbers of leaves per plant per seedling: Data regarding number of leaves were taken manually. Every visible leaf on the plant was counted, including the tips of the new leaves just beginning to emerge (Wood and Roper, 2000).

Leaf area (cm2): Leaf Area was measured with the help of leaf area meter (LI-3100; LI-COR, Inc., Lincoln, Nebr.). For this purpose, third leaves from the tip of branches were used and the average was worked out according to Michael *et al.* (2000).

Chlorophyll contents (mg/g fresh wt.): Chlorophyll contents were calculated by using a portable device (Model; SPAD-502; Konica Minolta, Japan) was used (Khan *et al.*, 2003).

Results and discussion

Effect of salts stress on germination and emergence

Pea genotypes significantly responded to increasing salinity levels. Although salt stress decreased the germination and emergence percentage in all treated genotypes (Fig. 1&2), the maximum reduction in germination was recorded in seeds submitted to high salinity levels, i.e., 7.0 and 10 dS m^{-1} Salts. The genotypes, Samrena zard, Meteor, Climax, Sadique-1 and 6173, performed well by maintaining the highest germination percentage under saline conditions, while Rohna, Assar, Olympia, Green arrow and Ambasidar gave the poorest response in this regard.

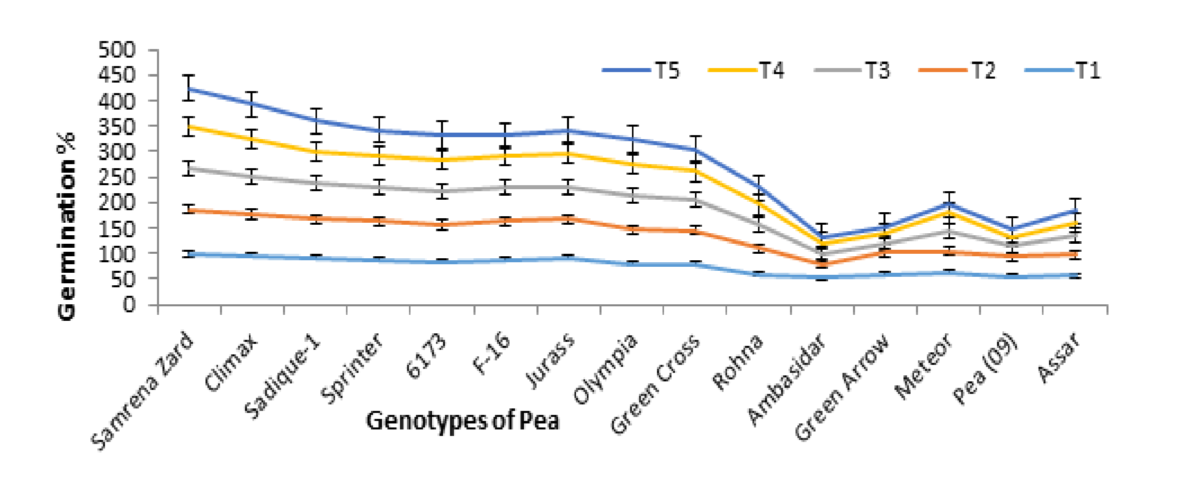

Fig. 1. Percentage reduction in the germination of pea genotypes under different levels of salinity. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at ($P \leq 0.05$).

The least mean % reduction in germination and emergence percentage was recorded for Samrena zard (17.86%) and (20.99%), respectively. A maximum decrease was noticed at T_5 (10dSm^{-1}) in all the treated pea genotypes, but Ambasidar shows the maximum decrease in germination and emergence percentage (62.70%) and (49.82%), respectively. These results agree with the results of Dhkil *et al.* (2014), who concluded that overall increased NaCl, $MgSO4$, $CaCl_2$, Na_2SO_4 levels led to the reductions in final germination and emergence percentage, but these reductions were higher for non-primed compared to primed seeds. Shahid *et al.* (2012) concluded that salt stress significantly decreased the germination percentage, emergence percentage. Similarly, it was found that increasing amounts of potassium and NaCl mostly affected the cucumber emergence and seedling growth adversely (Türkmen *et al.*, 2000).

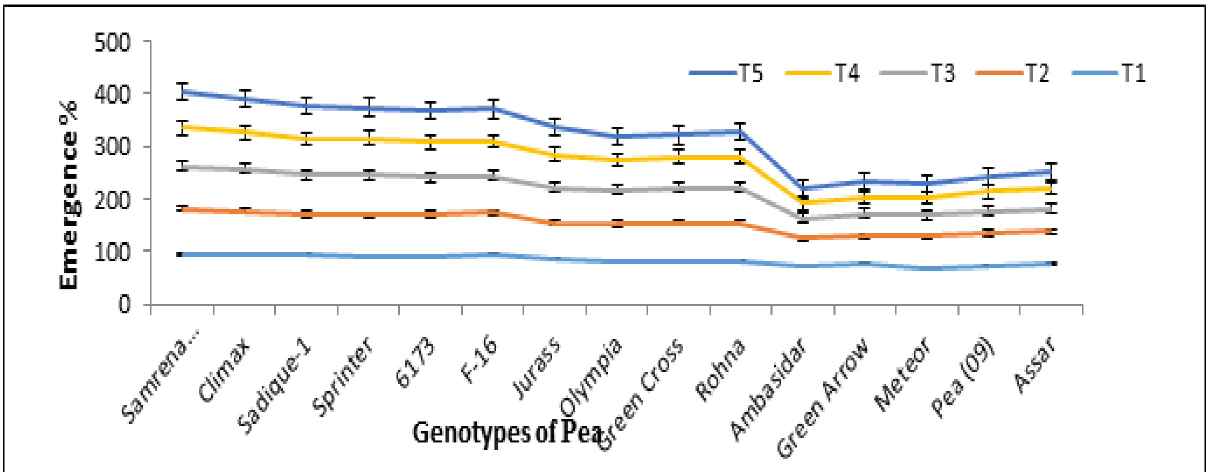

Fig. 2. Percentage reduction in Emergence of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at ($P \leq 0.05$).

The sensitivity of numerous plant species differs at seed germination, early seedling growth, and advanced growth stages with respect to salt stress (Mokhtar *et al.*, 2015). Our result accordance with the reports of Petrovic *et al.* (2016), which reveals that pea has been presented to be more tolerant to salt than water stress during germination and early embryo growth. Investigated cultivars showed greater susceptibility to both abiotic stresses when it comes to growth parameters compared to seed germination. Similarly, as Huang and Redmann (1995) reported that salt-induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity. According to our results, germination percentage decreased as the level of salinity increased so that our results correlate with the findings of (Mauromicale & Licandro, 2002, Gulzar *et al.*, 2001). Our findings agree with Abdel-Farid *et al.* (2020), who reported that salinity stress slightly delayed the seeds germination rate and significantly reduced the percentage of germination as well as shoot length under the highest salt concentration (200 mM) in cucumber.

In tomato, a prominent delay in seeds germination rate, the germination percentage and seedlings growth (shoot and root lengths) were significantly influenced under all concentrations of NaCl.

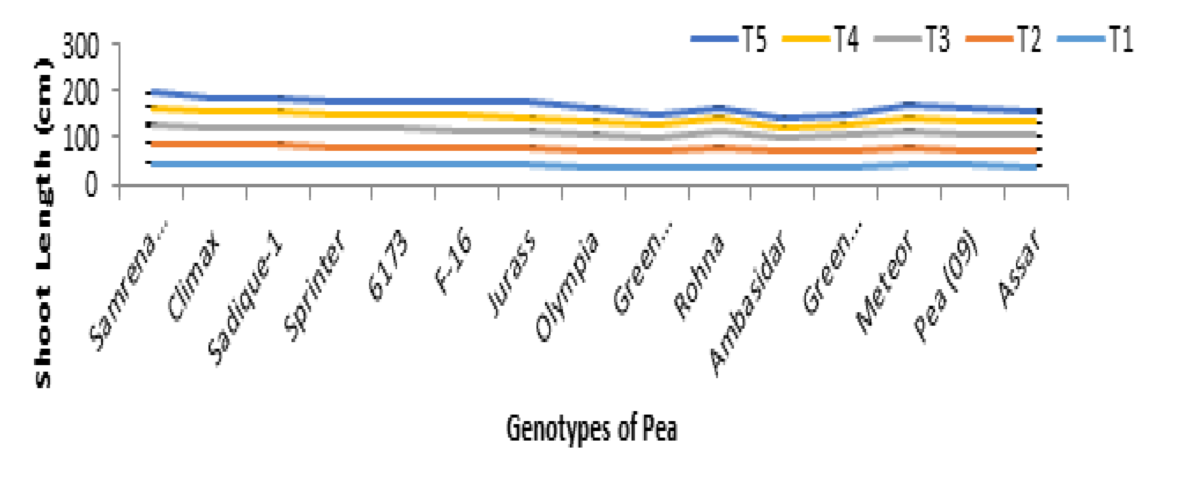

Fig. 3. Percentage reduction in shoot length of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at ($P \leq 0.05$).

Effect of salts stress on the seedling shoot and root length (cm)

Salt stress significantly reduced the plant shoot and root length in all treated genotypes (Fig. 3 & 4). But the genotypes, Samrena zard, Meteor, Climax, Sadique and 6173, showed maximum salt tolerance potential in terms of having the least % reduction in plant shoot length at all imposed salinity levels (2.5, 5.0, 7.0 and 10 dS m^{-1} Salts). However, the genotypes Rohna, Assar, Olympia, Green arrow and Ambasidar proved to be highly salt-sensitive due to the highest % decrease in plant shoot length at 2.5, 5.0, 7.0 and 10 dS m^{-1} Salts with respect to control (Fig 1). Similarly, the plants subjected to salinity stress exhibited a significant decrease in plant root length (Fig 1.), but maximum reduction was caused by 7.0 and 10 dS m^{-1} Salt's stress. The genotypes, Samarina zard (19.16 %), Meteor (23.87%), Climax (20.87%), Sadique (23.50%) and 6173 (22.24%) had the least mean % decrease in plant root length over the control, whereas Rohna (33.43%), Assar (27.91%), Olympia (24.36%), Green arrow (42.28%) and Ambasidar (42.44%) found to be the most severely affected genotypes in this regard.

The genotypes with the least % reduction in plant shoot and root length were categorized as salt-tolerant genotypes, while those showing the greatest % decrease were categorized as salt sensitive.

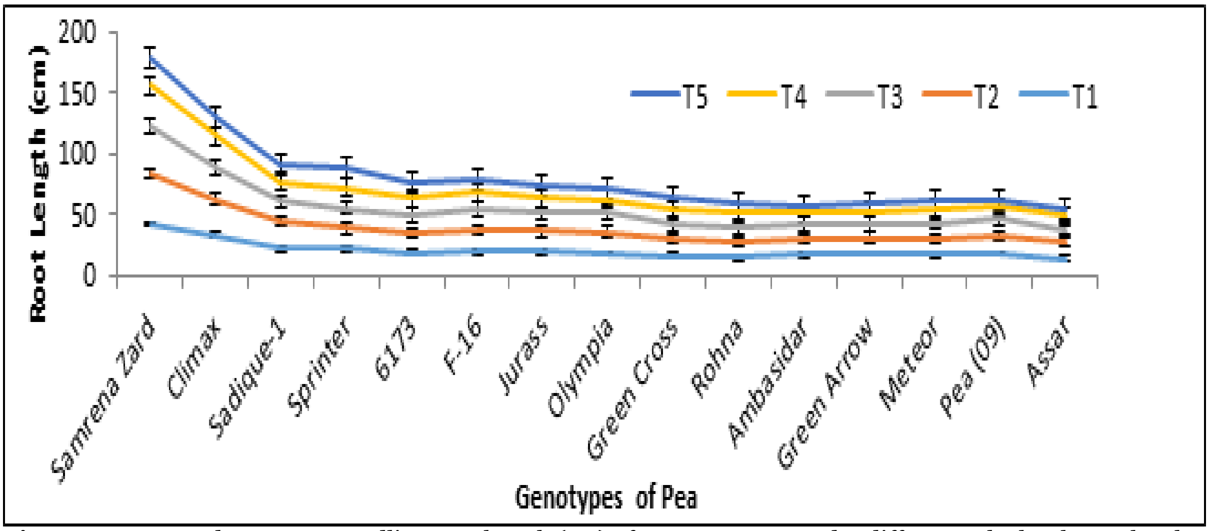

Fig. 4. Percentage decrease on seedling root length (cm) of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at (P≤0.05).

In accordance with the above findings of Demir and Arif, (2003) observed that the root growth was more adversely affected as compared to shoot growth by salinity. Our results correlate with the results of Jeannette *et al.* (2002) reported that the total fresh weight of root and shoot of cultivated accessions was reduced with increased salt stress. Our results show that the shoot length of the pea genotypes decreased at high and moderate salinity levels, but salts tolerant shows maximum shoot and root length. It is reported that moderate and high salinities negatively affect the shoot and root length of BS (Rahneshan *et al.*, 2018).

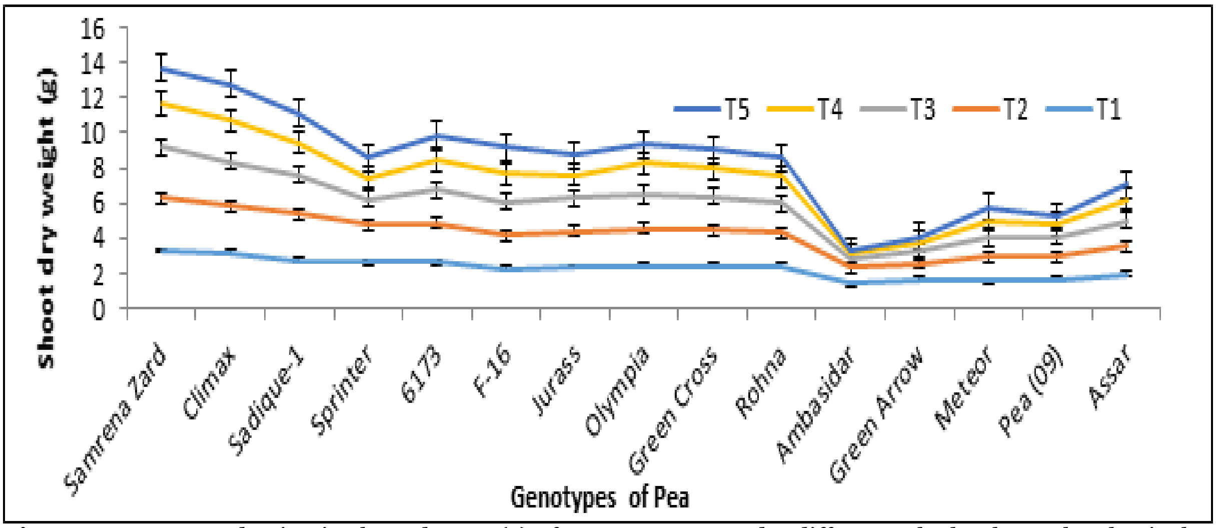

Fig. 5. Percentage reduction in shoot dry wt. (g) of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at (P≤0.05).

Effect of salts stress on seedling shoot dry and root dry weight (g)

Maximum reduction was noticed at T_5 (10 dSm^{-1}) in all the treated pea genotypes (Fig. 5 & 6). On the basis of overall percentage reduction at all the applied salts levels, the genotype (Samrena zard) showed the best performance by depicting minimum percentage reduction in shoot dry and root dry weight (19.15%) and (21%). The results show that maximum percentage (66.33%) and (61.105%) decrease was observed in Amabsidar categorized as salt sensitive. The current study also supported by the findings of (Shahid *et al.*, 2014). Salinity imposes prominent changes in morphology, physiology and biochemical attributes of plant metabolism. Similarly, studies were conducted by Jamil *et al.* (2007), who investigated hot pepper cultivars under saline conditions and concluded that salinity caused a significant decrease in leaves' fresh as well as dry weight. Salinity affected final cell size as well as the rate of cell production and thereby resulting in reduced shoot and dry root weight. The results are in agreement with the findings of (Alam *et al.*, 2004; Mahmood *et al.*, 2009). In accordance with our findings that higher biomass reduction in high NaCl stressed soils (200 mM) Ahmad and John (2005) investigated that salinity influence on the pea plants such as physio biochemical parameters in plants Sodium chloride caused a reduction in dry weight of root and shoot and fresh weight of root and shoot of pea plants. Potassium ions decreased and chlorophyll contents also decreased. The nitrate reductase activity decreases and proline and sugar content increase in leaves.

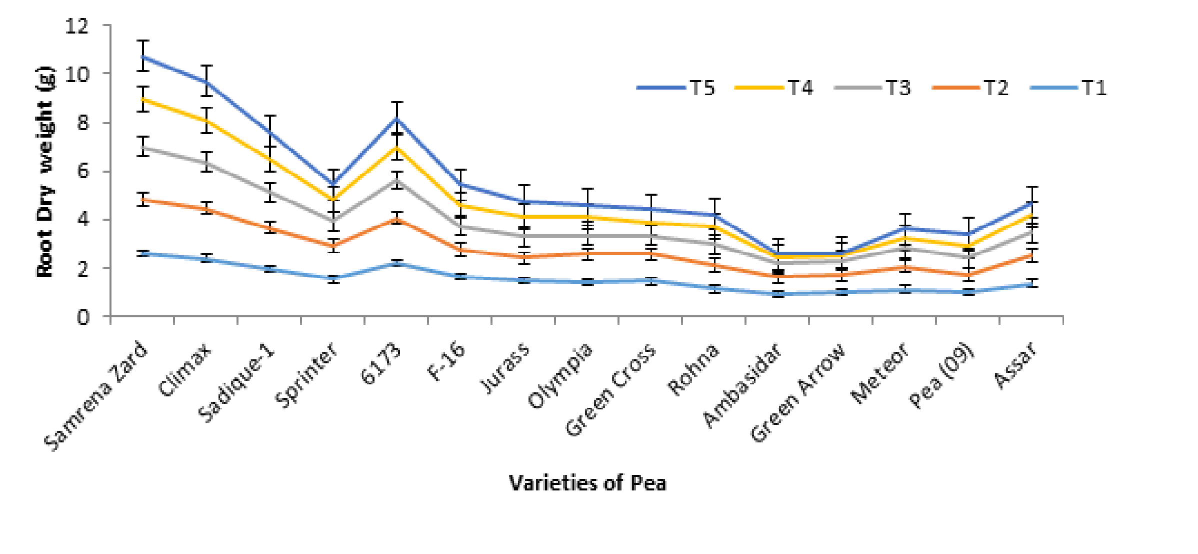

Fig. 6. Percentage reduction in Root dry wt. (g) of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at ($P \leq 0.05$).

Effect of salts stress on leaf area, number of leaves and chlorophyll

Under salinity stress, data presented in Fig.7 shows the percentage reduction in leaf area, number of leaves and chlorophyll of different treated genotypes, when compared with their respective control (Fig. 7, 8 & 9). There was a gradual increase in percentage reduction in leaf area, with the increasing salts level. Maximum reduction was noticed at T_5 (10dSm^{-1}) in all the treated pea genotypes. The genotype (Samrena zard) showed the best performance by depicting minimum percentage decrease (24.025%), (12.675%) and (9.83%) respectively. Overall percentage reduction in leaf area of pea plants shows that Ambasidar has maximum leaf area, number of leaves and chlorophyll reduction (50.922%), (46.142%) and (49.83%). Our findings in accordance with El-Sabagh *et al.* (2015) the reduction of the shoot and dry root mass might be due to salt stress reduced photosynthesis per unit leaf area which turned into a limited supply of carbohydrate needed for shoot growth. Our results correlate with the results of Rahneshan *et al.* (2018) concluded that salinity significantly had no effect on the leaf area of BZ, but the treatment of plants with NaCl, MgSO4, $CaCl_2$, Na_2SO_4 at the rate of 100 and 150 mM induced a significant decrease in leaf area of cultivar BS. Similarly, as in addition, salinity affected final cell size as well as the rate of cell manufacture and thus causing a decrease in shoot and dry root mass.

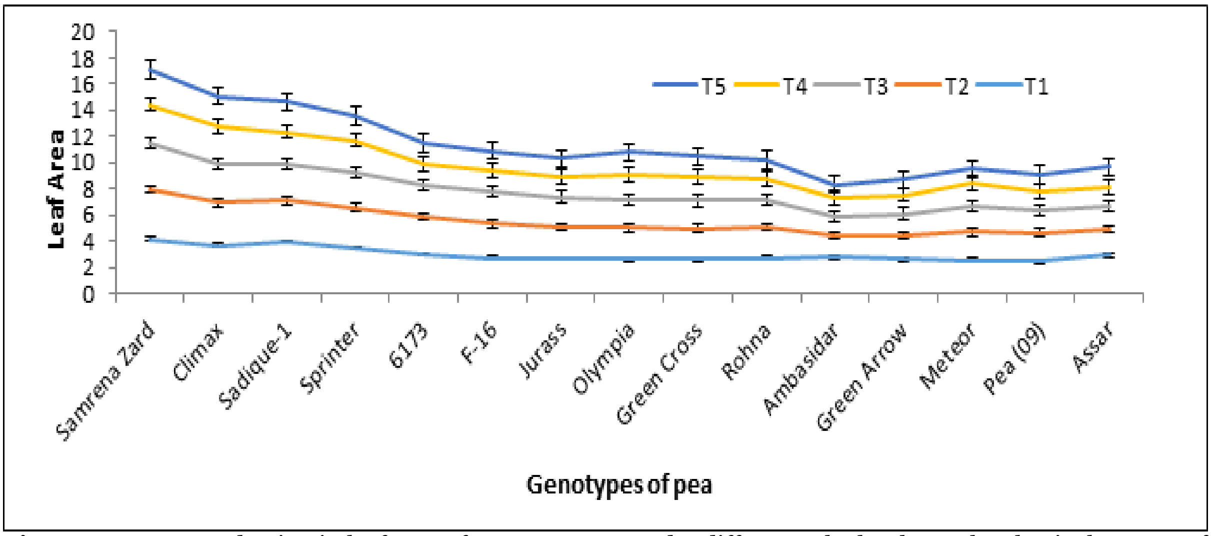

Fig. 7. Percentage reduction in leaf area of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at ($P \leq 0.05$).

The results are in agreement with the findings of (Alam *et al.* 2004; Mahmood *et al.*, 2009).

According to Rahneshan *et al.* (2018), their results showed a negative relationship between salinity stress and vegetative growth parameters such as RGR, leaf number, leaf area, and shoot length. However, the responses of cultivars were different. The above results showed a negative relationship between salinity stress and vegetative growth parameters such as the number of leaves of the pea plant, similar kind of results were found by Karimi *et al.* (2014), who concluded that during stress, leaf number, leaf area and shoot length of pistachio rootstocks was decreased.

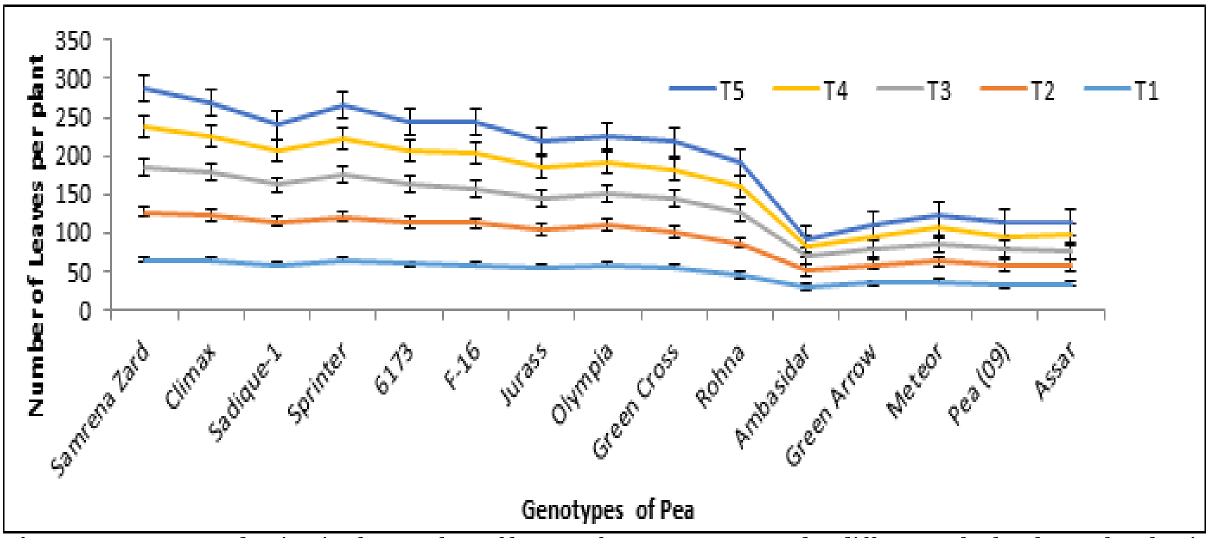

Fig. 8. Percentage reduction in the number of leaves of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at ($P \leq 0.05$).

The above results were in accordance with the findings of Kaya *et al.* (2003) reported that spinach chlorophyll content was reduced by 60 mM NaCl solution. Salinity reduces the chlorophyll content in salt susceptible plants and increases in salt-tolerant plants. Salinity reduced growth in radish (*Raphanus sativus* L.) at high salinity level could be attributed to a reduction in leaf area expansion and hence to a lower light interception (Marcelis & Hooijdonk, 1999). Salt tolerant plants show increased or unchanged chlorophyll levels under salinity conditions, whereas chlorophyll contents decreased in salt-sensitive plants (Ashraf and Harris, 2013). These results support our findings chlorophyll contents decreased in salt-sensitive genotypes of a pea.

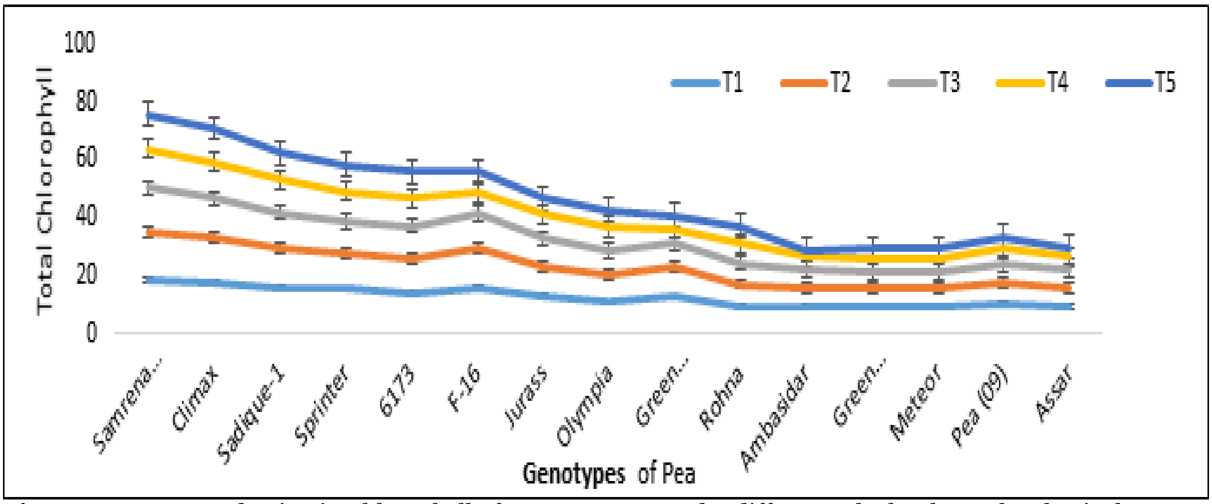

Fig. 9. Percentage reduction in Chlorophyll of Pea genotypes under different salts levels. Each value is the mean of four replications and the error bar shows the standard error (SE) of the mean. According to the HSD Tukey's test, treatments with different letters are significantly different at ($P \leq 0.05$).

Conclusion

Various genotypes of pea (*Pisum sativum* L.) differentially responded to salts stress results showed that salinity affects the traits of pea such as emergence and germination percentage, seedling shoot and root height, shoot and root dry weight, number of leaves, leaf area and chlorophyll decreased as salts stress levels increase as compare to control. The interaction between salts level and the genotypes was significant. On the basis of this current study, the genotypes such as Samrena zard, Climax, Sadique-1 and 6173 showed maximum germination. The genotypes (Sprinter, F-16, Green Cross, Jurass and Pea-09) showed intermediate performance. In conclusion from the current study that the genotypes (Rohna, Assar, Olympia, Green arrow and Ambasidar) consider as highly salts genotypes of a pea.

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