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EVALUATION OF PEA GENOTYPES FOR SALT STRESS TOLERANCE

E. RASHID[1*], Z. HUSSAIN[2], M.A. NAWAZ[1], and S. RASHID[1]

[1]Department of Horticulture, College of Agriculture, University of Sargodha, Sargodha, Pakistan
[1]Department of Horticulture, Ghazi University, Dera Ghazi Khan, Pakistan
*Corresponding author's email: erumrashid91@gmail.com
Email addresses of co-authors: zhussain@gudgk.edu.pk,
azher49@gmail.com, shahlarashid2007@gmail.com

SUMMARY

Pea (*Pisum sativum* L.), a highly nutritious vegetable, is extremely sensitive to salt stress conditions. A pot study evaluated four pea genotypes (Samrina Zard, Climax, Ambassador, and Green Arrow) by exposing them to control, 2.5, 5.0, and 7.0 dS m^{-1} by applying NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts. The pots under a completely randomized design (CRD) layout had four replications. Immense genetic variations occurred among the pea genotypes under salt stress. Samrina Zard showed better physiological (transpiration and photosynthesis rates, stomatal conductance, water use efficiency, and chlorophyll) and morphological traits (shoot/root length, shoot/root dry weight, number of leaves per plant, and leaf area). Genotype Samrina Zard significantly maintained the highest percentage of shoot length (14.54%), root length (28.28%), shoot dry weight (19.58%), root dry weight (36.36%), number of leaves (27.24%), and leaf area (21.59%) at a higher level of salinity 7 dS m^{-1} compared with the control and all other treatments. In contrast, the Ambassador genotype was categorically salt-sensitive based on the least percentage increase in shoot length (22.42%), dry weight of shoot (67.57%), dry weight of root (59.59%), number of leaves (47.69%) and leaf area (23.72%). However, salinity reduced the physiological attributes in both genotypes. Regardless of salt treatments, Samrina Zard performed better than Ambassador regarding photosynthesis (48.07%), transpiration rate (18.76%), stomatal conductance (45.42%), water use efficiency (55.88%), and chlorophyll contents (29.44%). According to study findings, Samrina Zard performed best against salinity stress.

Keywords: Pea (*Pisum sativum* L.), genotypes, salt stress conditions, genetic variation, morphological and physiological traits, plant growth, leaf area, photosynthesis, stomatal conductance

Key findings: This study focused on screening pea genotypes against salinity. The genotype Samrina Zard performed better in saline-sodic soil. Genotype Samrina Zard revealed best suited to arid and semi-arid regions with insufficient freshwater resources.

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INTRODUCTION

The world population rapidly increases day by day. Although cultivated land gradually declines by 1% to 2% yearly, it threatens sustainable production from inadequate land resources to address food and nutrient demand. Currently, salt-affected land covers approximately 1,125 million ha, while human-induced activities account for 76 million ha (Hossain, 2019; Mishra *et al.*, 2023). Dahal *et al.* (2019) stated that various abiotic stress factors hinder crop production to fulfill food demand. Food loss due to several environmental factors is a primary concern, sustaining the food supply to the growing population (Naik *et al.,* 2011). Salt affects almost 2,000 ha of land daily (Gerhardt *et al.,* 2017).

The salinity may result in membrane damage, changed levels of growth regulators, nutrient imbalances, enzyme inhibition, metabolic dysfunction, and photosynthesis, which cause plant death (Hussain *et al.,* 2018). Globally, biotic stress factors are chief threats to crop production. Plants have to face variations in salinity, which leads to morphological, metabolic, and physiological defects in plants. Salinity causes water starvation at a cellular level, oxidative stress, nutrient deficiencies, and ion toxicity, leading to various problems, such as, growth retardation, molecular damage, and, eventually, plant death. Each year, agricultural produce worth USD 10 billion is lost worldwide due to salt-damaged lands (Xu and Mou, 2016).

Stress disturbs plant metabolism, which leads to reduced growth and other developmental defects. According to an estimate, only 10% of the world's arable land is stress-free. Environmental stresses significantly create a gap between yield and maximum performance (Fathi and Tar, 2016). Salts in the soil sphere affect seed germination, seedling growth, and crop establishment, changing the plant's physiological properties reducing marketable weight, and resulting in low crop yield (Ashraf, 2009). Vegetables are high in phytochemicals and nutrients essential for several metabolic processes in the human body (Noreen and Ashraf, 2009; Singh *et al*., 2015).

Pea crop is one of the most prominent vegetable moisture. It is the world's fourth-largest grain crop. In Pakistan, it is the second sizable legume crop after chickpeas (Graham and Vance, 2000). Peas are usually eaten as greens or as raw vegetables. Green peas belong to a group of plants known as "nitrogen-fixing" crops, which makes them an environmentally friendly food (Duke, 1981). Pea seed is high in crude protein, fiber, tannin, and minerals (Wang *et al*., 2010; Kotlarz *et al*., 2011; Ciurescu *et al*., 2018). Pea production has progressed in Europe for the past 15 years due to self-sufficiency in protein-rich feedstock. Since the digestible energy content is similar to that of soybeans, the white, yellow, green oval and dark dried peas are high-protein feeds for livestock in developing countries.

Green peas are high in anti-inflammatory and antioxidant nutrients that are good for well-being and contain various nutrients. Green peas contain the antioxidant epicatechin and catechin, which belongs to the flavonoid family. Peas include α- and β-carotenes and are high in antioxidant vitamins, such as, E and C, and zinc. Another anti-inflammatory nutrient, omega-3 fatty acid, is also present in peas (Singh *et al*., 2017; Semba *et al.,* 2021). Consequently, this study produced valuable findings regarding morphological and physiological traits of salt-resistant and salt-sensitive pea genotypes.

MATERIALS AND METHODS

Experimental detail

Four selected pea genotypes (Samrina Zard, Climax, Ambassador, and Green Arrow) underwent four levels of salinity application and maintenance, *i.e.,* 0 (control), 2.5, 5.0, and 7.0 dS m^{-1} using NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts. Pea seeds sowing used plastic pots. Changing the number of plants per container to four ensued after the emergence of the first true leaf (15 days after germination), irrigating when required. After the 20th day of sowing, plants received a halfstrength (0.5×) Hoagland nutrient solution. After one month, exposing plants to salt stress transpired, gradually raising salt concentrations by 2.5 dS m^{-1} every two days until reaching the required dilution to prevent osmotic shock.

Morphological parameters

The average length measurement of the shoots and roots of pea plants used a measuring tape (Sajid *et al.,* 2016). Upon completion of the experiment, uprooted plants continued drying in an oven at 65 °C for 48 h (Memmert-110, Schawabach) to determine the dry weight of the shoots. An electric balance aided to measure weight (g). Manual data collection proceeded to determine the number of leaves per plant. Counting every leaf visible on the plant, even the tips of the new leaves poking out, ensued (Shahid *et al.,* 2013).

At the third-leaf stage, leaf area was measured using a leaf area meter (LI-3100; LI-COR, Inc., Lincoln, Nebr.) and the average was determined in accordance with Ahmed *et al.* (2017). A portable chlorophyll/SPAD meter (Model: SPAD-502; Konica Minolta, Japan) was used to record chlorophyll content (Khan *et al*., 2002).

Gas exchange characteristics

Three young, healthy leaves selected from each plant (two plants in each replication per treatment) attained their stomatal conductance, net transpiration rate, and photosynthesis rate measured in sufficient light at 9:00 AM. Placing selected leaves in an Infra-Red Gas Analyzer (IRGA) followed the method by Qureshi *et al*. (2022). The ratio between photosynthesis and water caliber was the basis for calculating water use efficiency (WUE) with the following formula:

$$WUE = \frac{\text{photosynthetic rate}}{\text{transpiration rate}}$$

Statistical analysis

Statistical analysis used a completely randomized design (CRD) with four replications. The assessment of the significance of changes between the genotypes and salt treatments employed the least significant difference (LSD) at $P < 0.05$.

RESULTS

Morphological parameters

The effect of concentrations of various salts (NaCl, $MgSO_4$, $CaCl_2$, and Na_2SO_4) on the shoot and root lengths of pea genotypes showed a significant ($P < 0.05$) decrease with rising salt levels (Table 1). Based on the findings that all applied salt treatments (2.5, 5.0, and 7.0 dS m^{-1}) decreased morphological growth, the genotype Samrina Zard performed best by giving the least percent reduction in shoot length, with genotype Ambassador (2.5, 5.0, and 7.0 dS m^{-1}) provided the highest percent decrease compared to the control (Figure 1).

At the maximum salinity level of 7.0 dS m^{-1}, Samrina Zard (14.54%) and Climax (17.94%) performed better than Ambassador (22.42%) and Green Arrow (20.73%), which showed a significant percentage reduction in plant root length (Table 1). The root length significantly ($P < 0.05$) shortened with salinity; however, this result was robust in the salt-sensitive genotypes Ambassador and Green Arrow (Figure 2). Compared with Ambassador (58.82%) and Green Arrow (44.26%), Samrina Zard (40.15%) and Climax (28.28%) gave the best performances when the mean percentage of root length declined substantially at level 7.0 dS m^{-1} (Figure 2).

The dry weight of the root and shoot of pea genotypes significantly ($P < 0.05$) decreased with a rise in salt levels. Under the highest salt treatment of 7.0 dS m^{-1}, the maximum reduction in dry shoot weight occurred (Table 1, Figures 3 and 4). Samrina

Table 1. Analysis of variance of the effect of salts on shoot and root lengths, shoot and root dry weights, number of leaves, leaf area, and chlorophyll content of pea genotypes.

Source of variation	d.f.	Mean squares						
		SL	RL	SDW	RDW	Number of leaves	Leaf area	Chlorophyll contents
Treatments	3	681.515*	95.5719*	0.72817**	0.05573*	1844.31*	15.4651**	36.1302**
Genotypes	3	850.788*	91.2577**	0.63622**	0.08205*	4201.52**	24.7119*	68.3970**
Treatments vs. Genotypes	9	36.750**	10.3801**	0.06875*	0.00996**	205.13*	1.2342**	2.0552**
Error	48	8.508	3.7470	0.06032	0.04027	37.17	0.2002	0.2511
Total	63							

P* < .05;*P* < .01;****P* < .001, Ns: non-significant, SL: Shoot length, RL: Root length, SDW: Shoot dry weight, RDW: Root dry weight.

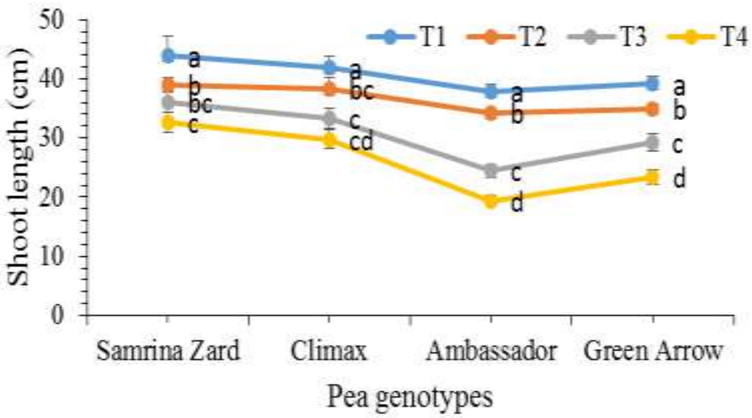

Figure 1. Effect of salt stress on shoot length of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

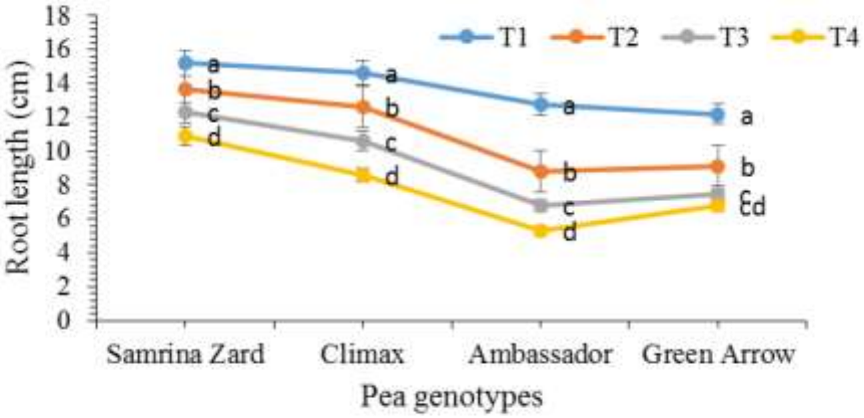

Figure 2. Effect of salt stress on root length of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

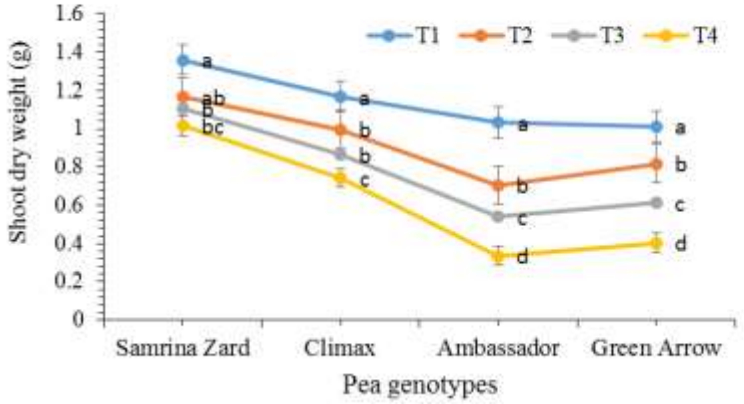

Figure 3. Effect of salt stress on shoot dry weight of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

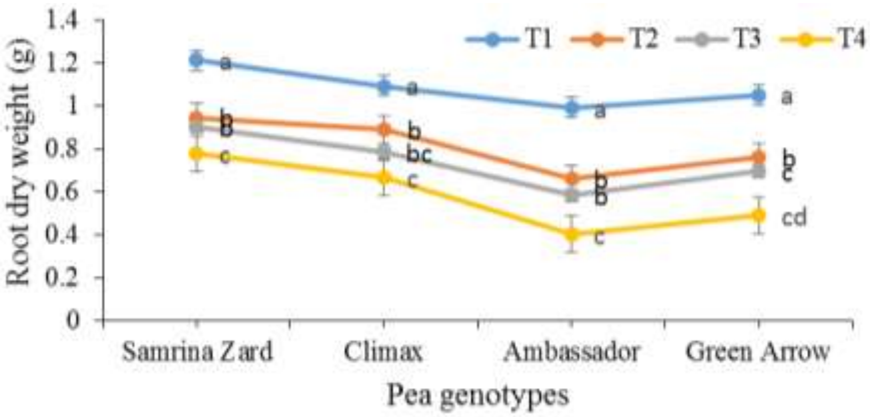

Figure 4. Effect of salt stress on root dry weight of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

Zard (19.58%) and Climax (25.17%) provided the best functions when compared with Ambassador (67.57%) and Green Arrow (57.21%). A recorded maximum root dry weight appeared in control plants. Samrina Zard (39.44%) and Climax (36.36%) had the highest root dry weight at 7.0 dS m^{-1}, performing better than Ambassador (59.59%) and Green Arrow (53.27%) (Figure 3).

The maximum leaf area resulted in the control and Samrina Zard (salt-tolerant genotype). A similar trend emerged for leaf area and the number of leaves. Samrina Zard (27.24%) and Climax (27.77%) had the best results among the salt treatments when compared with Ambassador (47.69%) and Green Arrow (44.19%), which had the maximum number of leaves per plant at 7.0 dS m^{-1} (Figure 5). Samrina Zard (21.59%) and Climax (22.78%) demonstrated superior performance versus Ambassador (23.72%) and Green Arrow (24.59%) in all genotypes treated with the highest salinity level (Figure 5). The Ambassador’s (salt-sensitive genotype) leaf

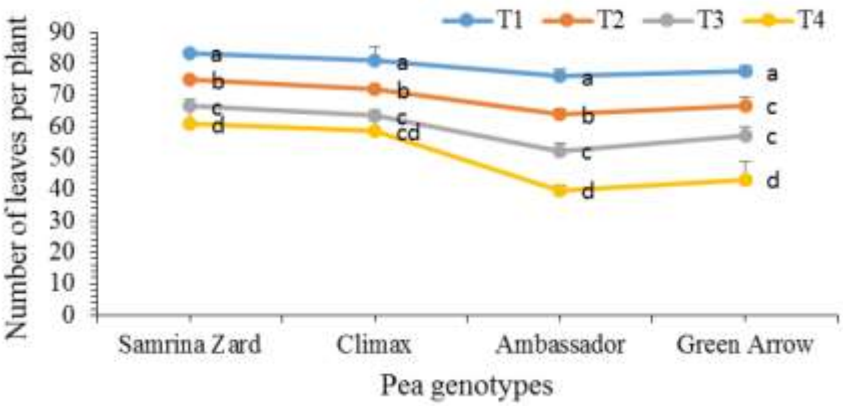

Figure 5. Effect of salt stress on number of leaves per plant in different pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

area decreased as the salt concentration increased (Figure 6). Among treatments, there was a significant decrease in the leaf area of the Ambassador (23.72%) and Green Arrow genotypes (24.59%). The best performance came from Samrina Zard (21.59%) and Climax (22.78%) (Figure 6).

Chlorophyll content

In this study, the results based on ANOVA showed that salt stress reduced the chlorophyll contents of salt-tolerant and salt-sensitive genotypes ($P < 0.05$) (Table 1). However, Samrina Zard retained better chlorophyll contents (40.98%) when subjected to 7.0 dS m^{-1} salts stress compared with Ambassador (40.98%) (Figure 7).

Photosynthetic rate, stomatal conductance, and transpiration rate

Changes occurred in gas exchange attributes of pea genotypes, recorded at 2.5, 5.0, and 7.0 dS m^{-1}. The salt stress meaningfully ($P < 0.05$) influenced the photosynthesis rate, stomatal conductance, and transpiration rate of all pea genotypes (Table 2). The comparison of means shows that a maximum decline was noticeable under the highest salt treatment, 7.0 dS m^{-1}. Among all genotypes, the photosynthetic rate of Samrina Zard (48.07%) performed well compared with Ambassador (61.36%) when exposed to salt stress at 7.0 dS m^{-1} (Figure 8).

Cultivar Samrina Zard (18.76%) demonstrated better retention of transpiration rate than Ambassador (33.95%) when subjected to salt stress at 7.0 dS m^{-1}. However, maximum stomatal conductance recording was in the control plants (Figure 9). According to research findings, Samrina Zard (45.42%) retained better stomatal conductance compared with Ambassador (52.17%) during salt stress at 7.0 dS m^{-1} (Figure 10).

Water use efficiency

The water use efficiency of pea genotypes significantly ($P < 0.05$) increased with an upsurge in salt concentrations (Table 2). Among all the genotypes where water use efficiency reportedly had influences at 7.0 dS m^{-1}, Samrina Zard (55.88%) exhibited better water use efficiency than Ambassador (27.27%). In conclusion, Samrina Zard performed better among all treatments by demonstrating increased water use efficiency. In contrast, Ambassador showed the lowest change in water use efficiency compared with the control when exposed to salt stress at 7.0 dS m^{-1} (Figure 11).

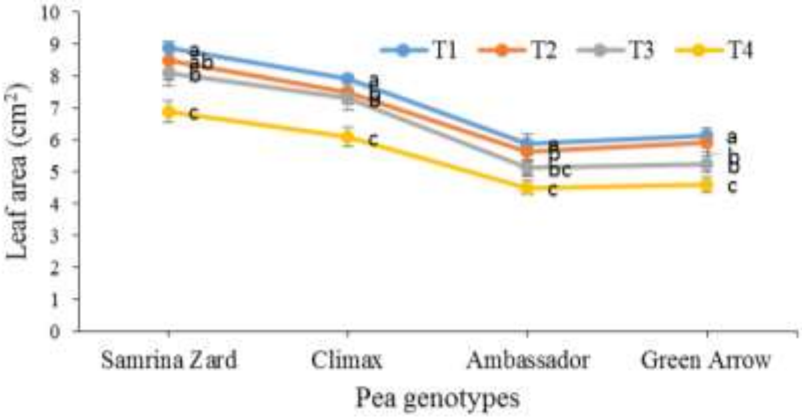

Figure 6. Effect of salt stress on leaf area of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

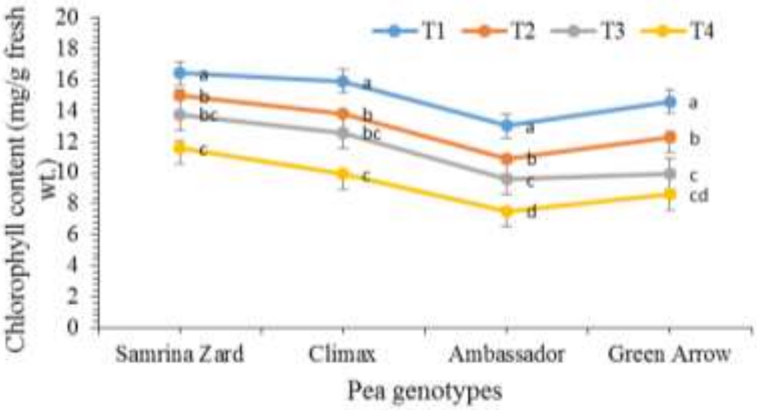

Figure 7. Effect of salt stress on chlorophyll content in leaves of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

Table 2. Analysis of variance of the effect of salts on photosynthesis rate, stomatal conductance, transpiration rate, and WUE of pea genotypes.

Source of variation	Mean squares				
	d.f.	Photosynthesis rate	Stomatal conductance	Transpiration rate	WUE
Treatments	3	1.6443 **	2.9831 **	2.36833**	1.90766 **
Genotypes	3	13.1706**	12.0981**	9.88875**	3.32891**
Treatments vs. Genotypes	9	0.0622**	0.0393**	0.11847*	0.15155**
Error	48	0.0590	0.0159	0.01542	0.00943
Total	63				

ns, non-significant; *$P < .05$; **$P < .01$; ***$P < .001$.

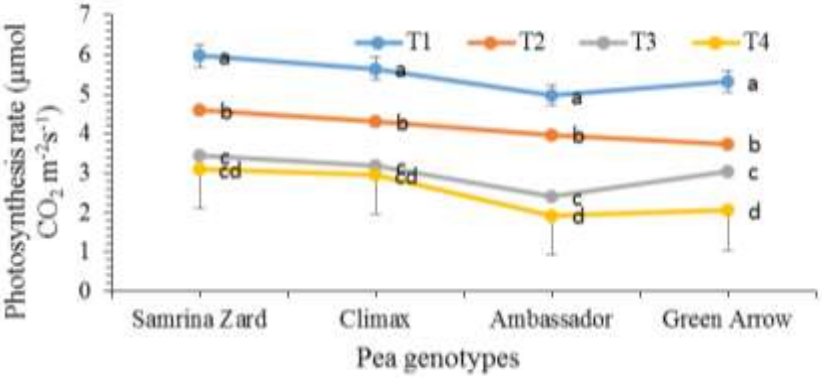

Figure 8. Effect of salt stress on photosynthesis rate of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

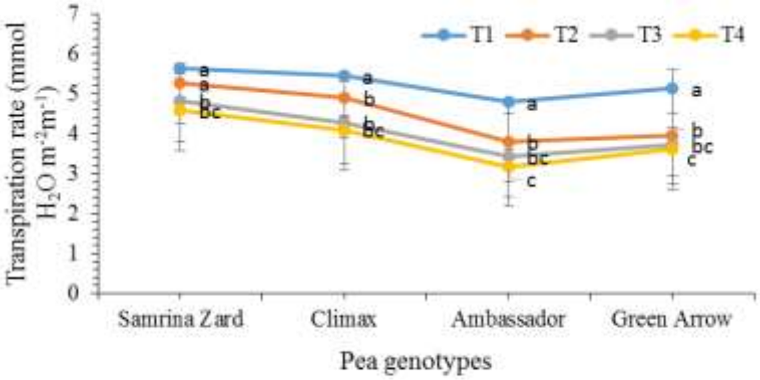

Figure 9. Effect of salt stress on transpiration rate of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

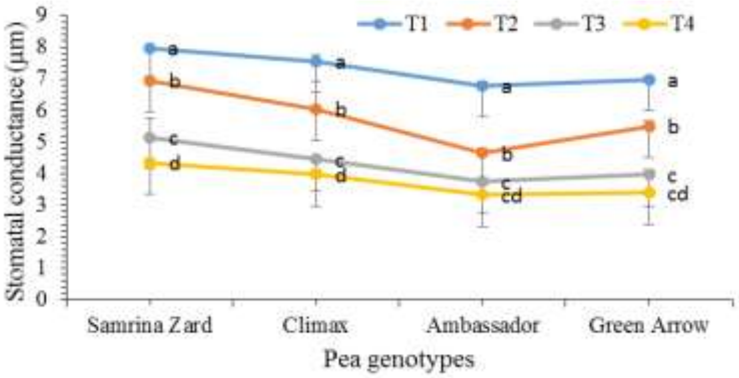

Figure 10. Effect of salt stress on stomatal conductance of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

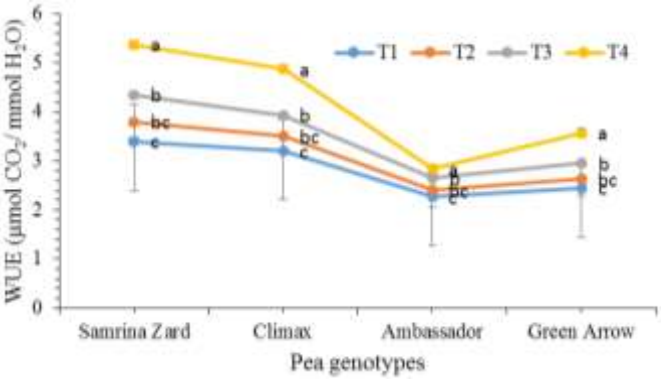

Figure 11. Effect of salt stress on water use efficiency (WUE) of pea genotypes. Treatment means followed by a different letter are significantly different ($P \leq 0.05$). Vertical bars indicate average ± standard error within means. Abbreviations – T1, control; T2, 2.5 dS m^{-1}; T3, 5.0 dS m^{-1}; T4, 7.0 dS m^{-1} NaCl, Na_2SO_4, $MgSO_4$, and $CaCl_2$ salts.

DISCUSSION

This study showed that salt stresses significantly affected plant growth (plant height, dry weight, number of leaves per plant, and leaf area) and physiological attributes (photosynthesis rate, stomatal conductance, water use efficiency, and transpiration rate). Salt stress considerably impacts germination and early seedling growth. Hence, these become useful as salt-stress markers. Based on these crucial indicators, classifying genotypes can be as tolerant or sensitive to salt stress. High seed germination and seedling development helped the plant to withstand salt stress, indirectly contributing to improved growth and productivity (Shahid *et al.,* 2012a). According to several sources, salt-tolerant characteristics vary depending on the developmental stage; one may have severe impacts, while another may tolerate salts. Salt tolerance is a genetically regulated phenomenon. Thus, the species gene pool must have a significant genetic variation to improve salt-tolerant attributes (Wibowo and Armaniar, 2019).

Salinity developed water and ionic imbalance in plants because of toxic ions. Plants under stress conditions showed stunted growth, making the leaves darker (Rani *et al*., 2019). Increased salt stress had a strong negative link with growth characteristics, including fresh and dry weight of the shoot and dry weight of the root, plant shoot, and root lengths. Based on the significant changes observed in the earlier-mentioned growth qualities between non-saline and saline regimes in all pea genotypes, they can serve as an effective method for screening under challenged conditions. Similarly, a positive association between the lengths of the root and shoot and shoot and root biomass demonstrated that these growth characteristics could be reliable and helpful markers for assessing salt tolerance in pea genotypes. Contrasting to salt-sensitive genotypes (Ambassador and Green Arrow), tolerant genotypes (Samrina Zard and Climax) showed a slight loss in dry shoot weight, root dry weight, shoot length, and root length, possibly due to their ability to sustain cell turgor under salty conditions. These outcomes corroborated the findings of Shahid *et al*. (2012b; 2013) and Sajid *et al*. (2016). The results regarding leaf area are consistent with those of Nizam *et al.* (2017), who showed that the inhibition of cell elongation caused by a higher concentration of Na^+ ion causes delayed plant growth and leaf development.

The research supports the findings of Baghel *et al*. (2019), who found that the maximum stress level (100 mM NaCl) reduced leaf area. Outcomes from the presented study agree with those of Nizam *et al.* (2017), who found that a higher concentration of Na ion inhibited cell elongation, resulting in slower plant growth and leaf development. Such developmental defects may relate to membrane disruption and inhibition of cell division and expansion (Deivanai *et al.,* 2011). The maximum amount of chlorophyll came from Samrina Zard. Previous studies have shown that saline conditions reduced the shoot/root dry weight, number of leaves, chlorophyll content, leaf area, and yield of pea genotypes. The relevant investigation supports the conclusion reported by Ishrat *et al.* (2022). The presented results on chlorophyll correlate with the findings of Shahid *et al*. (2012).

The results established the amount of chlorophyll decreased as salt increased, and the foremost inhibiting effect occurred at high salt stress. Study findings followed the conclusion of Jha (2019), who found that chlorophyll and carotenoid contents in maize shoots significantly reduced after applying salt stress. In the existing study, salt treatments strongly decreased photosynthesis and transpiration rate justifying the findings of Kang *et al.* (2019) that salinity changes photosynthetic parameters, as well as water and osmotic potential (Zahra *et al.,* 2020), and transpiration rate (Shahid *et al.,* 2022b). The above results regarding stomatal conductance are similar to those of Abbas *et al.* (2015) and Mustafa *et al.* (2014), who reported that salinity has proven to impact the leaf water potential, stomatal conductance, and transpiration rate of chili plants.

These results further align with Gupta and Huang (2014), who concluded that the individual and synergistic effects of osmotic, ionic, and nutritional imbalances are considerable possible strategies to mitigate salt stress. Salt stress also drastically affects the water usage efficiency of Ajowan plants (Ramezani *et al*., 2012). The latest findings of water use efficiency also correlate with Shahid *et al.* (2022). Water use efficiency has positive correlations with biomass and seed yield. However, similar results showed from the work by Khataar (2018), who reported that increased salt stress caused a decrease in the water use efficiency of wheat and bean plants.

CONCLUSIONS

The growth and development traits, i.e., shoot/root lengths, shoot/root dry weights, the number of leaves per plant, and leaf area, are essential screening parameters for salt tolerance in pea genotypes. On the other hand, photosynthetic activity, stomatal conductance, and transpiration rate had adverse effects from salinity stress. Furthermore, the notable study demonstrates that genotype Samrina Zard was more salt-resistant than the Ambassador was.

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