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ORIGINAL ARTICLE / ORIGINALBEITRAG

Growth and Biochemical Responses of Grass Pea (*Lathyrus sativus* L.) Genotypes Under Salt (NaCl) Stress Generated by Irrigation Water, and Changes in Soil pH and EC

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Abstract

The effect of salt stress (40, 80, 120, 160 mM, 4, 8, 12, 16 dS/m) created with irrigation water on germination seedling growth and biochemical content in fife grass pea genotypes consisting of five registered variety and two landraces was examined. The experiment was done in pots. The effect of salt stress (SS), genotype and their interactions were significant on the traits examined. Depending on the traits, negative effects began to occur in 80 or 120 mM SS, but the high detrimental effects of 160 mM on germination and growth was clearly recorded in all genotypes. This showed that 120 mM SS could be a critical level for grass pea in general. The variety Gap Mavisi was more tolerant to SS and this was associated with their stronger soot and root development Also shoot growth was more sensitive to SS than root growth in grass pea genotypes. Crude protein and ODAP content of shoots in the grass pea genotypes significantly increased in parallel with the SS and averaged the highest (31.86% and 8.26 mg g^{-1}, respectively) at 160 mM. In addition, as a result of soil analysis, it was determined that the applied salt doses were also effective on the pH and EC of the soil, and that the pH decreased in parallel with the SS, while the EC increased. In addition, as a result of soil analysis, it was determined that the applied salt doses were also effective on the pH and EC of the soil, and that the pH decreased in parallel with the SS, while the EC increased. It is thought that this changes in soil may affect the tolerance of grass pea genotypes to SS, therefore, integrating salinity studies with pH regulating agents may be produce more effective results agricultural aspects.

Keywords Salt stress · Grass pea · Protein · ODAP · pH

Introduction

Soil salinity is an ever-present threat to crop production worldwide, particularly in arid and semi-arid regions (El-nasharty et al. 2017). Arslan et al. (2018) reported that salinity is the one most important abiotic stress, which have affected almost every aspect of plant growth and development, including seed germination, vegetative growth, and reproductive development. Salinity is evident on 397 M ha agriculture land (Setia et al. 2013) and fifty percent of total cultivated and irrigated land is affected by salinity on global level (Gengmao et al. 2015). Climate change and improper agricultural practices make salinity a greater threat to agriculture day by day. Irrigation with saline water, low precipitation, and high evapotranspiration are main reasons caused salinization at a rate of 10% annually to agricultural lands meaning that more than 50% of arable land would be salinized by 2050 (Jamil et al. 2011; Ullah et al. 2021).

The tolerance and response mechanisms to salinity vary depending on the plant species (Gupta and Huang 2014). Due to their ability to fix biological nitrogen, high protein contents, and essential mineral contents, legumes have become a fascinating group of plants for many aspects including to improve fertility in saline soil (Piwowarczyk et al. 2016). There is an immense need to improve salt tolerant legume varieties to meet growing demand and for

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sustainable crop production worldwide (Farooq et al. 2017; Nadeem et al. 2019).

Grass pea (*Lathyrus sativus*), is a nutritious annual legume plant, resistance to abiotic stress such as drought, heat, cold, moderate salinity and periodic flooding that other legumes do not yield sufficiently high (Lambein et al. 2019). This makes the grass pea both a future crop of sustainable agriculture and an interesting object of basic research. Among *Lathyrus* species, grass pea is the most economically important and widely cultivated crop for human consumption and fodder for livestock feeding (Campbell 1997). With emphasis on worldwide demand for high-value feed and adaptation to climate change, grass pea has gained importance in recent years (Kumar et al. 2020).

Grass pea is valued for its high nutritional value and tolerance of abiotic stress and infertile soil (Hanbury et al. 2000). However grass pea contains a toxic component namely β-N-oxalyl-L-α,β-diamino propionic acid (ODAP) that may cause paralysis in humans and livestock if consumed for a long time in excessive amounts (Campbell 1997; Barpete et al. 2021). The ODAP content varies widely in grass pea depending on the genetic and growing environments (Das et al. 2021) ranging from 0.02 to 2.59% among the existing germplasm (Basaran et al. 2013; Kumar et al. 2013). It has been reported that stress conditions such as drought, salinity, excess iron, cadmium and low zinc in the soil can stimulate ODAP production in grass pea (Liu et al. 2017; Tokarz et al. 2021).

The aim of this study was to evaluate the response of different Turkish origin grass pea genotypes, including varieties and landraces, to the salinity stress generated with irrigation water at the early growth stage with references to germination, growth and biochemical aspects.

Material and Methods

The experimental materials comprised five Turkish origin grass pea (*Lathyrus sativus* L) genotypes, three of landraces (1603, 2006, 4403) and two of registered variety (Iptas and Gap mavisi). Salt solutions were prepared as four doses (40, 80, 120, 160 mM, 4, 8, 12, 16 dS/m) by diluting NaCl in distillate water and, distillate water was control.

Field soil taken from 0–30 cm with pH 8.26 and 271 µS/s was used in this study as growing media. After dried and passed through a 4-mm sieve, 4 kg soil was filed into plastic pots (30 × 40 × 7 cm) to 4 cm in height. 25 seeds of each genotype were sown pots as 2.5 cm depth and irrigated salt solutions up to field capacity (1200 ml/pot). Then pots were allowed to germinate and grow at climate room condition with 12:12 h light/dark photoperiod, 75% humidity and 25 °C constant temperature.

Germination (shoot emerging from soil) was determined at 8th days after sowing and, shoots rising at least 2 cm from the soil were counted as germinated. Seedling characters (shoot and root length, shoot and root dry weight) and biochemical traits (crude protein and ODAP) were determined in 21 days after sowing. For dry weight, total root and soot samples were dried at 60 °C in oven until the constant weight separately for each pot. After cooling and weighing the samples ground to pass through 1 mm screen. Biochemical characters (crude protein and ODAP content) of shoots were determined by using Near Reflectance Spectroscopy (NIRS, "Foss 6500") with software package program "IC-0904FE" (Basaran et al. 2011). Quantitative estimation of ODAP was done by the o-phthalaldehyde method according to Rao (1978).

The experiment was arranged in split plots design with three replications. Data was analyzed by SPSS 20.0 package program and the differences between the means were determined using Duncan's multiple comparison test. In addition, at the end of the experiment, the pH and EC (µs/S) values of the soil were determined for each treatment and how it changed according to the initial soil was revealed.

Results

The significant effect of salt stress "SS" ($p < 0.01$), genotype "G" ($p < 0.05$) and SSxG interaction ($p < 0.01$) was determined on the germination of grass pea (Table 1). Mean

Table 1 The effect of salt stress germination ratio (%) of grass pea genotypes

Genotype	Salt stress (mM)**					
	0	40	80	120	160	*Mean**
1603	98.33 a	90.00 a–d	100.00 a	93.33 abc	81.66 de	92.66 *A*
2006	98.33 a	98.33 a	100.00 a	91.66 a–d	83.33 cde	94.33 *A*
4403	98.33 a	95.00 ab	96.66 a	98.33 a	76.66 e	93.00 *A*
Iptaş	95.00 ab	90.00 a–d	91.66 a–d	85.00 b–e	58.33 f	84.00 *B*
Gap Mavisi	100 a	100 a	98.33 a	90 a–d	93.33 abc	96.33 *A*
Mean**	*98 A*	*94.66 A*	*97.3 A*	*91.66 A*	*78.66 B*	–

There is no difference between the treatments shown same small and capital letters
*: $p < 0.05$. **: $p < 0.01$

Table 2 The effect of salt stress on the shoot length (cm) of grass pea genotypes

Genotype	Salt stress mM**					
	0	40	80	120	160	*Mean*
1603	31.32 ab	29.15 b–e	26.20 efg	26.43 efg	23.92 ghı	27.40
2006	30.01 a–d	31.29 abc	25.37 fgh	24.86 fgh	21.37 i	26.58
4403	30.79 a–d	31.39 ab	30.50 a–d	29.20 b–e	23.10 hi	28.99
Iptaş	27.98 c–f	29.11 b–e	29.81 a–d	29.9 a–d	25.48 fgh	28.45
Gap Mavisi	32.87 a	31.38 ab	31.55 ab	27.61 def	24.98 fgh	29.67
Mean**	*30.59 A*	*30.46 A*	*28.69 B*	*27.60 B*	*23.77 C*	–

There is no difference between the treatments shown same small and capital letters
**: $p < 0.01$

germination ratio was similar at between 0 and 120 mM of SS, however significantly decreased at 160 mM. Over the salt doses, mean germination ratio was the lowest in landrace 4403 (84.00%) and, all other genotypes had the ratios that between 93.00 and 96.33% with no statistically differences. Looking at the SSxG interaction, it was seen that the effects of SS on germination were similar for all genotypes with the significant reduction at 160 mM. However, differently, the decrease in germination ratio begun at 120 mM of SS in Iptas variety and, it had the lowest ratio (58.33%) at 160 mM among the treatment.

Shoot length was significantly ($p < 0.01$) changed by SS, SSxG interaction (Table 2). Mean shoot length was similar between genotypes. However, it was decreased with increasing SS doses (≥ 80 mM) and was the highest (30.59 cm) at control and the lowest (23.77 cm) at 160 mM of SS. The GxSS interaction shows that the effect of SS on shoot length is generally negative. However, different responses of genotypes to SS were also observed, especially between 40–120 mM, and even shoot length in Iptas variety was higher than the control in this range. The adverse effect of SS at 160 mM was common in all genotypes, indicating that the doses ≥ 120 mM may be a critical for grass pea. However, the varieties were superior to landraces in shoot length at 160 mM.

SS, G and SSxG interaction were also significant ($p < 0.01$) on root length of grass pea (Table 3). Although relatively high values were detected at the 40 and 120 mM doses, root length averaged similar to the control at ≤ 120 mM, while significantly lower at 160 mM (12.95 cm). All genotypes exhibited greater or similar root length compared to the control at certain SS doses (< 160 mM), with the exception of landrace 4403 had a greater root length than its control (11.20 cm) even at 160 mM (14.33 cm).

Table 3 The effect of salt stress on the root length (cm) of grass pea genotypes

Salt stress mM**						
Genotype	0	40	80	120	160	*Mean***
1603	15.83 b–g	16.62 a–f	12.55 hi	13.97 fgh	9.52 j	13.69 *B*
2006	13.13 ghi	14.56 e–h	14.34 e–h	17.59 a–d	12.62 hi	14.44 *B*
4403	11.20 ij	14.78 e–h	14.04 fgh	15.38 d–g	14.33 e–h	13.94 *B*
Iptaş	18.16 abc	18.61 a	16.96 a–e	18.36 ab	15.06 d–h	17.43 *A*
Gap Mavisi	15.71 c–g	14.81 e–h	15.43 d–g	15.79 b–g	13.22 ghi	14.99 *B*
Mean**	*14.80 A*	*15.87 A*	*14.66 A*	*16.21 A*	*12.95 B*	–

There is no difference between the treatments shown same small and capital letters
**: $p < 0.01$

Table 4 The effect of salt stress on the shoot dry weight (g) of grass pea genotypes

Genotype	Salt stress mM**					
	0	40	80	120	160	*Mean***
1603	2.55 d–h	2.59 d–h	2.63 d–g	2.40 fgh	1.94 ij	2.42 *B*
2006	2.90 a–d	2.73 c–g	2.41 fgh	2.32 ghi	1.75 j	2.42 *B*
4403	2.48 e–h	2.54 d–h	2.56 d–h	2.20 hi	1.59 j	2.27 *B*
Iptaş	3.20 a	2.86 a–e	2.75 b–f	2.52 d–h	2.31 ghi	2.73 *A*
Gap Mavisi	3.07 abc	3.14 ab	3.11 ab	2.60 d–h	2.21 hi	2.82 *A*
Mean**	**2.84 A**	**2.77 A**	**2.69 A**	**2.41 B**	**1.96 C**	–

**: $p < 0.01$. There is no difference between the treatments shown same small and capital letters.

Table 5 The effect of salt stress on the root dry weight (g) of grass pea genotypes

Genotype	Salt stress mM**					
	0	40	80	120	160	*Mean***
1603	1.43 f–i	1.62 c–f	1.49 d–h	1.47 d–i	1.64 b–f	1.53 *B*
2006	1.21 h–k	1.17 ijk	1.04 k	1.11 jk	1.40 f–j	1.18 *C*
4403	1.51 d–h	1.11 jk	1.12 jk	1.53 d–g	1.65 b–f	1.39 *B*
Iptaş	1.84 abc	1.51 d–h	1.36 f–j	1.24 g–k	1.75 a–e	1.54 *B*
Gap Mavisi	1.52 d–h	1.97 a	1.44 e–i	1.77 a–d	1.94 ab	1.73 *A*
Mean**	*1.50 AB*	*1.47 AB*	*1.29 B*	*1.42 B*	*1.68 A*	–

There is no difference between the treatments shown same small and capital letters
**: $p < 0.01$

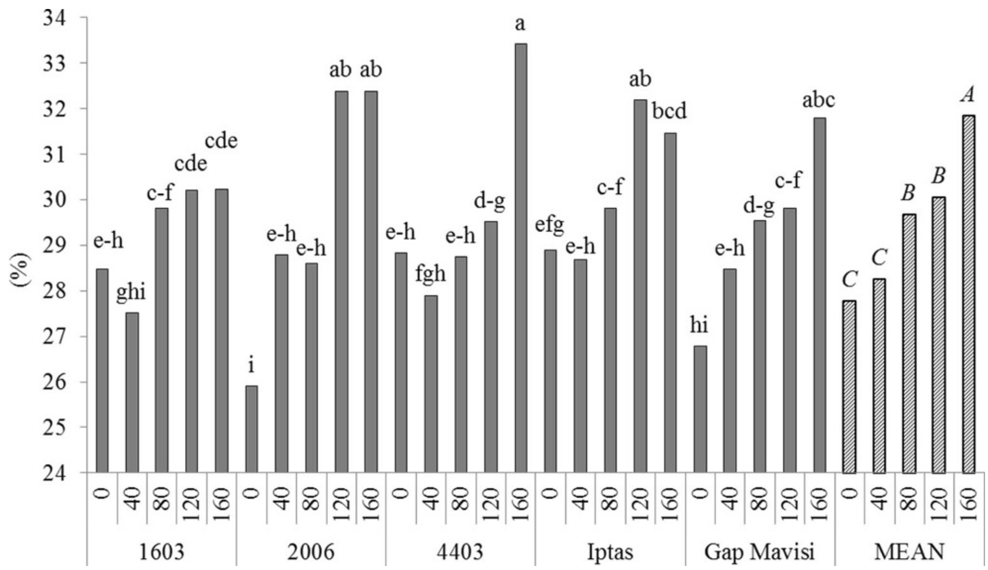

Fig. 1 The effect of salt stress (mM) on the shoot crude protein content in grass pea genotypes. (There is no difference between the treatments shown same small and capital letters on the bars ($p < 0.01$))

The lowest root length (9.52 cm) was detected in landrace 1603 at 160 mM of SS. Over the doses, Iptas variety was the highest genotype in root length (17.43 cm) and, others were in the same group. The Iptas variety was also superior at almost all doses in terms of root length, which may indicate its tolerance and stability to SS.

The effect of SS, G and SSxG interaction were significant ($p < 0.01$) on the shoot dry weight (Table 4). As an average, higher shoot dry weight was noted in the varieties compared to landraces. SS (>80 mM) decreased the mean shoot dry weight over the genotypes and caused the lowest value at 160 mM (1.96 g). The negative effect of SS (>80 mM) on shoot dry weight was observed in all the genotypes, and the lowest value was observed in landraces 2006 (1.75 g) and 4403 (1.59 g) at 160 mM.

Both treatments and their interactions were also significant ($p < 0.01$) on root dry weight (Table 5). Mean root dry weight decreased at 80 and 120 mM, but was in the same group as the control at 40 and 160 mm. Over the doses of SS, root dry weigh was averaged the highest in variety of Gap Mavisi (1.73 g) and the lowest in landrace 2006 (1.18 g). When the interaction was examined, it was determined that the genotypes give different responses to SS between 40 and 120 mM, but were positively affected at 160 mM, even had higher root dry weight than control. Differently, root dry weight of Iptas variety was similar at the control and 160 mM treatments.

In general, the effect of SS on crude protein of shoot was significant ($p < 0.01$) and positive (Fig. 1). Over the genotypes, crude protein content increased in parallel with the SS and was the highest (31.86%) at 160 mM doses. SSxG interaction showed that the positive effect of SS was valid for all species studied. In this regard, the crude protein content was noted the lowest in landrace 2006 at control dose (25.91%) while the highest in landrace 4403 at 160 mM (33.43%). However, the landrace 2006 (at 120 and 160 mM), variety of Iptas (at 120 mM) and Gap Mavisi (at 160 mM) were located at the highest group in crude protein content.

As with crude protein, SS had a significant ($p < 0.01$) effect on ODAP content of shoot and caused an increase (Fig. 2). Compared to the control, ODAP increased in parallel with the SS doses and reached the highest mean value at 160 mM (8.26 mg g^{-1}). Looking at the interaction, ODAP

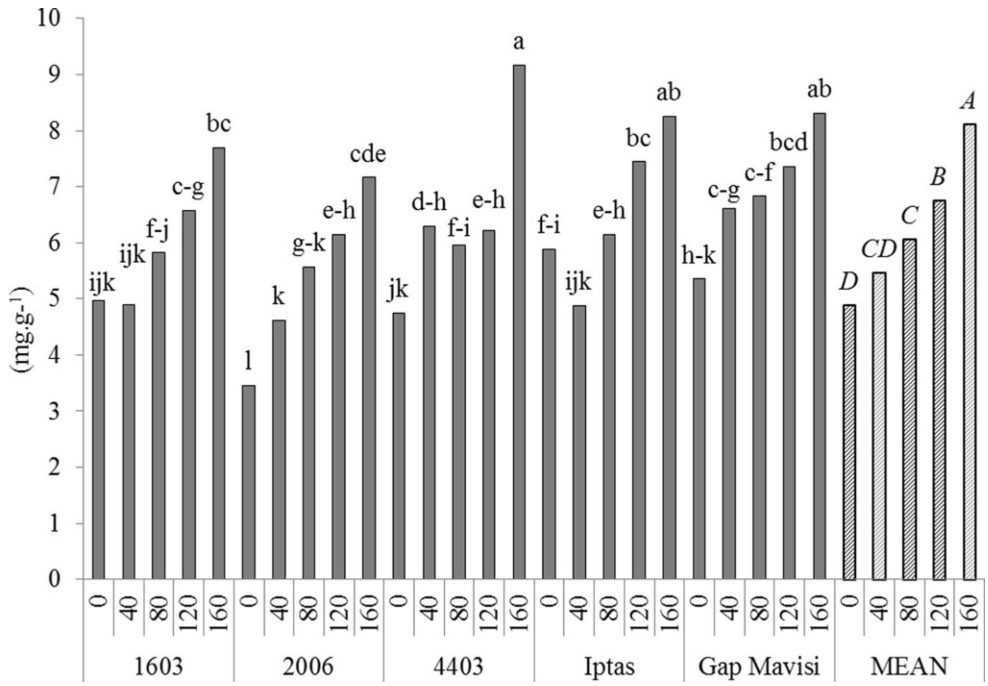

Fig. 2 The effect of salt stress (mM) on the shoot ODAP content in grass pea genotypes. (There is no difference between the treatments shown same small and capital letters on the bars ($p < 0.01$))

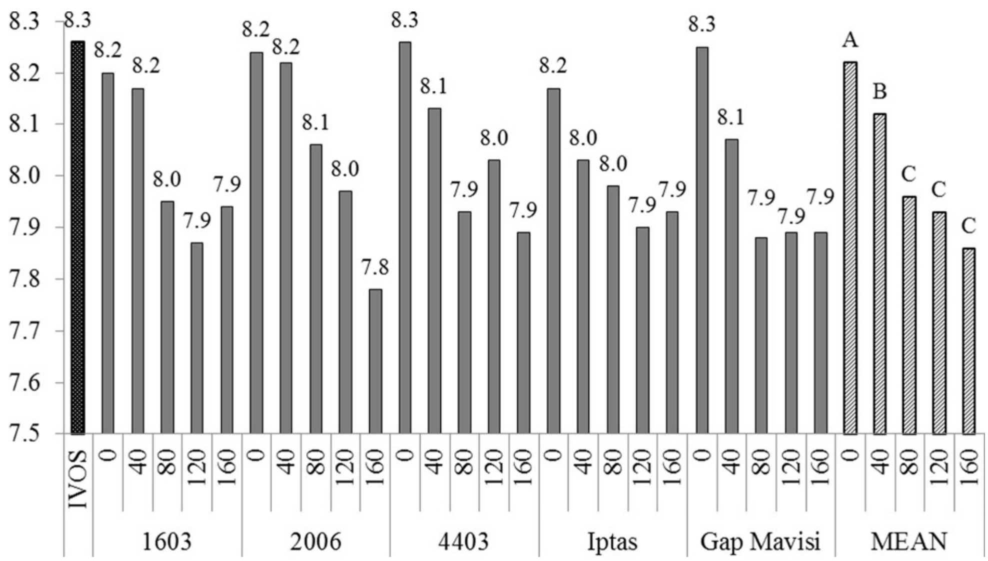

Fig. 3 pH values determined at the end of the experiment in soils applied different salt stress (mM). (*IVOS* the initial value of the soil used in the experiment)

was increased by SS in all genotypes except the value at 40 mM for Iptas variety and the highest was detected at 160 mM. The lowest ODAP content among to treatments was noted in landrace 2006 at the control (3.46 mg g^{-1}) while it was the highest in Landrace 4403 and Iptas and Gap Mavisi varieties at 160 mM of SS, respectively 9.17, 8.26 and 8.31 mg g^{-1}.

As a result of pH and EC analyzes performed after harvest, it was determined that SS application caused a significant change in both properties. Accordingly, SS decreased the pH (Fig. 3) and increased the EC of soil (Fig. 4) in parallel with the doses, as clearly seen in the mean of doses. When compared to initial value of the soil (pH: 8.3, EC: 271 µs/S), similar or slightly high values were observed in the control treatments, which indicates that the plant-based effect is very low and can be negligible for both pH and EC. Average results are also valid for genotype × SS interaction. In this context, for all genotypes, the pH value was higher in the control treatment and lower at 160 mM, while the EC was higher at 160 mM and lowest in the control treatment.

Discussion

Soil salinity is one of the most important constraints of today's agriculture in many parts of the world, and moreover,

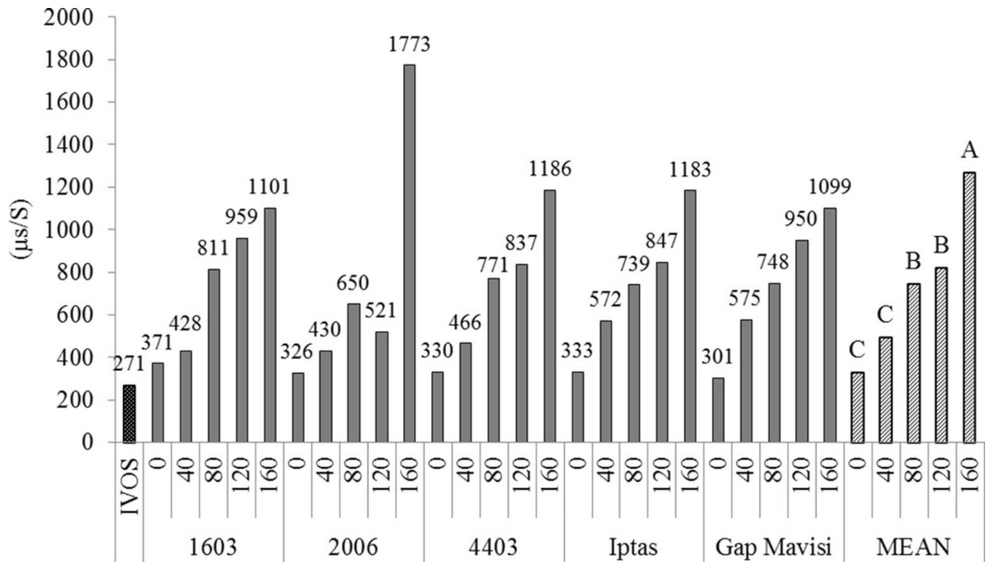

Fig. 4 EC values determined at the end of the experiment in soils applied different salt stress. (*IVOS* the initial value of the soil used in the experiment)

it is possible that global climate change will take this situation further. In this context, the detection of salinity-tolerant plants is an important step in terms of the future and sustainability of crop production. Because plants react differently to salt stress at the interspecies and even intraspecies level (Almansouri et al. 2001). Grass pea is known tolerance to moderate salt stress (Tokarz et al. 2020). Ahmed et al. (2014) reported that under laboratory conditions, salinity stress of 5 dS/m and 10 dS/m decreased germination percentage, root length, shoot length and total dry matter, and a salinity level of 15 dS/m was lethal for grass pea. The present study revealed that grass pea is a species that is moderately resistant to salt stress and gives different but clear responses at the morphological and biochemical level. In addition, SS (salt stress), G (genotype) and SSxG interaction were significant determinants of these responses. The high detrimental effect of 160 mM SS on germination and growth in all the genotypes was clearly recorded. Therefore, it can be said that 120 mM of SS is critical for grass pea and higher doses may have negative effects. Similarly, Piwowarczyk et al. (2016) reported that germination and seedling emergence of grass pea was not significantly affected by 50 and 100 mM of NaCl, however, SS at 200 mM decreased these parameters.

It is well known that germination is the most sensitive stage to SS The germination rates of the grass pea genotypes individually were similar to the control up to 120 mM (Table 1), but significantly lower at 160 mM. Considering the germination ratios at 160 mM, the most tolerant variety to SS was Gap Mavisi, while Iptas was the most sensitive.

The observed changes in the growth characteristics indicate the genotype effect is important to salt tolerance and, the varieties seem to be better than the landraces, especially with their performance at 160 mM. In addition, the differences between varieties and landraces in terms of growth characteristics can be seen in the control dose, with values in favor of varieties. This shows that strong habitus genotypes can be more tolerant to salinity with effective shoot and root development. In the current study, shoot growth was more affected by salt stress than root growth in grass pea genotypes. On average, the negative effect of salt stress occurred at 80 mM in shoot length (Table 2) and 160 mM in root length (Table 3). Bektas (2021) stated that salt stress started to affect shoot and root development in grass pea at doses of 100 to 150 mM, depending on the genotype. Tsegazeabe and Berhane (2012) reported that 4 dS/m SS caused a reduction in shoot length between 33.2% and 62.6% and root length between 43.79% and 52.91% in four Ethiopian grass pea landraces. In another plant (*Pisum sativum* L.), Onal Ascı and Zambi (2020), stated that salt doses up to 150 mM negatively affected root biomass, number of leaves, and above-ground biomass.

In terms of dry weight, the effects for SS shoot and root were different from each other. There was a significant decrease in shoot dry weight at 120 mM and especially at 160 mM of SS (Table 4). While the root dry weight decreased at 80 and 120 mM doses, it increased significantly at 160 mM and constituted first group with control and 40 mM. (Table 5). This result explains as that grass pea roots play an important role in combating salt stress by limiting growth and increasing the dry matter content. Naturally, the results of this strategy suppressed also shoot growth.

Crude protein (Fig. 1) and ODAP content (Fig. 2) of grass pea shoots significantly affected by SS and increased depending on salinity with the highest values at 120 and 160 mM, compared to control. Exceptionally, in landraces1603, 4403 and Iptas varieties, crude protein was recorded under control at 40 mM of SS. This situation was also observed in the ODAP content of 4403. Accordingly, the means and SS × G interaction clearly showed that SS led to significant increase in the crude protein content at ≥80 mM (Fig. 1), while in the ODAP content at ≥40 mM (Fig. 2). Compared to previous studies, the results of the change in ODAP are fully consistent, but the change in protein ratio is quite different. Although it has been stated in many studies that salinity stress decreased the protein ratio, our results showed an increase. In another study on the same genotypes, it was reported that alkaline stress also caused an increase in crude protein and ODAP content (Sen et al. 2021). Under salt stress, the proteome of plants exhibits a complex structure, and there are differences between sensitive and resistant genotypes and even between plant organs (Xiong et al. 2017; Razzaque et al. 2019). Proteins play an imminent role in plant stress response since they are directly involved in the acquisition of an enhanced stress tolerance (Kosová et al. 2013). Exposure of salt stress altered the protein profiles and promoted the accumulation of salt-specific proteins was depended on genotypes, the salt concentration and salt treatment duration in two blackgram varieties (Win and Oo 2017). Campos et al. (2012), after irrigation the J. curcas with a large range of NaCl concentrations for 50 days, detected a significant increases (32%) in the amino acids content of leaves especially in the heavily stressed (2.8 and 3.5 dS m^{-1}), however, the soluble protein content of leaves was differentially affected by the salt treatments, and decreased from 0.7 to 2.1dS m^{-1}, while 2.8 and 3.5 dS m^{-1} of salt produced no effects. Jiao et al. 2011 reported that variety of environmental stresses cause an increase in ODAP content. In addition, both environment and genotypes may play an important role in the biosynthesis of ODAP (Das et al. 2021). It is also stated that ODAP may be related to the stress resistance of grass pea and stress tolerance may decrease in genotypes with low ODAP content (Verma et al. 2022). In fact, when the decrease in shoot dry matter ratio is taken into account, the change in both ODAP and crude protein ratio corresponds to a much narrower range in terms of total values per plant. In this case, the increase in the ratio of ODAP and crude protein, besides may be being a defensive strategy, can be expressed as a results of lower dry matter in plants exposed to stress due to the restriction of growth.

In this study, how the applied salt doses affect the pH and EC of the soil was also investigated and interesting results were obtained. In the post-harvest analyzes, it was observed that the pH decreased in parallel with the dose of SS (Fig. 3), while the EC increased (Fig. 4). The pH and EC were also determined in the original soil, so it was possible to observe the change caused by the plant. As a matter of fact, based on data from control treatments, it can be said that the plant-based effect is negligible for pH, while EC was slightly increased by the plants. This finding is consistent with Campos et al. (2012) who concluded that the salt concentration in the irrigation water did not significantly ($p \leq 0.05$) change the soil pH, but increased the conductivity of the soil solution linearly. The increase in EC was similar in all genotypes. These results indicate that genotype is not effective in plant-induced change in soil pH and EC. Similarly, Al-Busaidi and Cookson (2003) found a negative relationship between pH and salinity in the calcareous soils varied from non-saline to excessively saline, and they argued that a possible reason for the negative relationship was that soils were influenced by increased solubility of Ca^{2+} ions in saline conditions under ambient atmospheric CO_2 concentrations that lead to a release of hydrogen ions. Kaiwen et al. (2020) reported that salinity (100 and 200 mM) was more effective than pH (7.0 and 9.0) on growth and physiological functions of alfalfa, however, at 200 mM salinity and 9 pH, there was higher Na uptake, and the root system was damaged. This may indicate that the low pH in the soil may help reduce the negative effects of salinity or help the plant acclimate to the salinity. Therefore, in the current study, the decrease in pH with increasing salinity may be a reason why some properties of grass pea are not affected even at the level of 120 mM.

Conclusion

Salt stress is one of the most important abiotic stress factors in today's agriculture. Determination of salt-tolerant species and varieties is of great importance for agricultural sustainability in this context. In this study, it was determined that, depending on the trait, grass pea is affected by salinity and is tolerant to moderate salt stress (<120 mM), and variety Gap Mavisi with good root and shoot development are better in this respect. At 160 mM salt dose, regression in growth and increasing in crude protein and ODAP content of all the genotypes were clearly observed. The effect of salt stress on shoot growth was relatively higher than on root growth in grass pea. Apart from this, it was determined that the pH and EC of the soil changed linearly depending on the applied salt doses. It is thought that especially the change in pH may have an effect on the response of plants to salinity. Therefore, observing or regulating the change in pH of the environment with the doses applied in salinity studies may provide useful results in terms of understanding the mechanism of plants' tolerance to salinity.

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Declarations

Conflict of interest K. Güleç Şen, U. Başaran, M. Çopur Doğrusöz, E. Gülümser and H. Mut declare that they have no competing interests.

Ethical standards Not applicable. **Consent to participate:** Not applicable. **Consent to publish:** Not applicable.

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