ROMANIAN AGRICULTURAL RESEARCH, NO. 40, 2023
First Online: January, 2023. DII 2067-5720 RAR 2022-81

EXOGENOUS APPLICATION OF ASCORBIC ACID TO INDUCE TOLERANCE AGAINST SALT STRESS IN COMMON BEAN PLANTS

Cigdem Aydogan, Zeynep Girici, Ece Turhan*

Department of Agricultural Biotechnology, Faculty of Agriculture, Eskisehir Osmangazi University, 26160, Eskisehir, Türkiye

*Corresponding author. E-mail: eturhan@ogu.edu.tr

ABSTRACT

Common beans have an important place in the world due to its high nutritional values in the human diet and with the largest cropping area among the legumes. Besides, they are named as quite sensitive to salt stress. Salinity is one of the utmost abiotic stress factors limiting agricultural production, which affects plant growth and development at different levels. Lately, exogenous applications of signalling and/or protective molecules to various parts of plants are used to combat salt stress before or at the time of stress. In this context, this research was conducted to assess the influence of foliar-applied ascorbic acid (AsA) on electrolyte leakage (EL), activity of antioxidative enzymes, total protein (TSP) content and protein profiles in the two common bean genotypes (salt-sensitive "Local Genotype" and salt-tolerant "Şeker Fasulye") at early growth stage under salinity (0, 50, 100, 150 mM NaCl). The genotypes were exposed to salt stress from fully developed true leaf at the third nodes emerged stage for two weeks, meanwhile 3 mM AsA was foliar-applied every three days. Salt stress increased EL in both genotypes and exogenous AsA application decreased EL value especially in "Local Genotype". Foliar-applied AsA generally reduced the adverse effects of NaCl on AsA content of both genotypes. Exogenous AsA application also increased the activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) in the salt-stressed common bean plants and did not play a role in the TSP content. However, it has been determined that SDS-PAGE protein profiles represent adaptive mechanisms for dealing with excess salt in common bean genotypes. The results suggested that foliar-applied AsA was effective in reducing the adverse effects of salinity especially in relatively salt sensitive common bean genotype.

Keywords: antioxidants, foliar application, *Phaseolus vulgaris*, protein, salt injury.

INTRODUCTION

Salinity, which causes more than 50% reduction in average crop yield, is one of the most limiting factors for agricultural production worldwide (Zhang et al., 2020). It is estimated that salinity affects 7% of the agricultural areas in the world, which accounts for about a third of irrigated agricultural land (Maršálová et al., 2016). Soil salinity, increases soil osmotic pressure, causes nutritional imbalance due to its interaction with plant nutrients and negatively affects plant growth through the specific ion effect or combination of these factors (Machado and Serralheiro, 2017). High salinity also triggers reactive oxygen species' (ROS) formation, whose excessive accumulation in plant cells causes oxidative damage to membrane lipids, proteins, nucleic acids, and carbohydrates (Suzuki et al., 2012).

The ROS, which are by-products of stress metabolism, can be toxic over a threshold level. Besides, it acts as signal transduction molecules during stress acclimation and regulate different pathways (Choudhury et al., 2017). The increment in ROS levels is often provisional and linked with the subsequent induction of defence mechanisms; such as generating antioxidative pattern (enzymatic and non-enzymatic antioxidants), accumulation of stress proteins and up regulation of stress-protective genes, which collectively ensure stress protection (Kerchev et al., 2020). Several enzymes such as superoxide dismutase (SOD: EC 1.15.1.1), catalase (CAT: EC 1.11.1.6), peroxidase (POX: EC 1.11.1.7), ascorbate peroxidase (APX: EC 1.11.1.11), glutathione reductase (GR: EC 1.6.4.2) are involved in the detoxification of ROS and are stimulated under salinity. In the non-enzymatic defence

Received 28 October 2022; accepted 18 January 2023.

system, antioxidant compounds like ascorbic acid (AsA), glutathione (GSH), phenolics, flavonoids, and tocopherols have a significant role in the removal of toxic oxygen compounds (Symes et al., 2018).

In recent years, some techniques have been proposed along with traditional breeding and biotechnological methods to reduce the negative effects of salinity on agricultural production. Seed or seedling priming, application of osmolytes, hydrogen peroxide (H_2O_2), and antioxidants including AsA, GSH and dehydroascorbate; which are implicated in signalling against stress (Romero-Romero and López-Delgado, 2009). Antioxidants can impromptu supply electrons to free radicals, alleviating the oxidative cellular environments induced by aerobic metabolism (Paciolla et al., 2019). Though recent scientific efforts have shown that the use of AsA has promising results in alleviating abiotic stresses such as salinity, chilling, and flooding (Jamil et al., 2015; Aliniaeifard et al., 2016; Ullah et al., 2017; Elkelish et al., 2020), the underlying mechanisms remain unclear (Al-Huqail et al., 2020). The favourable effects of AsA may be attributed to the increase in photosynthesis activity, as well as its role in various defence mechanisms against oxidative stress under salinity (Saeidi-Sar et al., 2013; Alnusaire et al., 2022).

The common bean contains full carbohydrates, vitamins, and various minerals, and plays an essential role in human diet (Hayat et al., 2014). However, it is quite sensitive to salinity at early growth stage, which poses a serious threat to its production (Assimakopoulou et al., 2015). Due to the growing demand to plant products and environmental pressures on agricultural ecosystems, it is becoming clear that grain legumes would play an important role in future cropping systems (Stagnari et al., 2017). Therefore, the elevation of salt tolerance is useful to expand the planting area of common bean on saline land. In addition, Taïbi et al. (2016) reported that future strategies for plant breeding studies in *Phaseolus vulgaris* aiming at developing new genotypes that are tolerant to salinity should focus on selecting plants with high antioxidant activity rather than plants that can accumulate more salt. There are some researches on the use of AsA by adding it to irrigation water or nutrient solution in order to eliminate the negative effects of salinity in bean plants (Dolatabadian and Jouneghani, 2009; Saeidi-Sar et al., 2013; Rady et al., 2018). However, it is reported that foliar application provides rapid absorption of AsA, and increases the salt tolerance of plants (Bybordi, 2012). In this respect, there is a question of whether foliar-applied AsA could activate particular defensive mechanisms, which may be associated with salt tolerance and whether AsA could be used as bio-fortifier in common bean plants? The present study was designed to answer these questions.

MATERIAL AND METHODS

Plant materials and experimental practices

In order to determine the effects of foliar-applied AsA to induce tolerance against salinity in common bean, "Şeker Fasulye" (salt-tolerant) and "Local Genotype" (salt-sensitive) genotypes were used (Gulmezoglu et al., 2016). The seeds were sown into pots (14 cm) filled with a mixture of peat:perlite:vermiculite (2:1:1). The experimental treatments were arranged in a randomized block design with three replicates. Each replicate consisted of 30 plants. Plants were grown in a climate cabinet (DAIHAN WGC-1000, South Korea), under 26°C/18°C (day/night) with approximately 60-70% relative humidity and a light density of 450 mol$\mu m^{-2}s^{-1}$ (Khadri et al., 2006).

Seeds were irrigated with doubly distilled water (ddH_2O) until fully developed true leaf emerged at the third nodes. Afterwards, seedlings were irrigated with ddH_2O containing 0 (control), 50, 100 and 150 mM NaCl. While half of the plants were sprayed with 3 mM AsA (Elwan et al., 2012; Ergin et al., 2014) every three days for 2 weeks (5 applications) in order to investigate whether AsA was effective on salt tolerance

of the genotypes, the other half were used as control plants. The amount of water to be given was determined by the pot tensiometer (Irrometer, Co. Riverside, Calif, USA). At the end of experiment, samples of each treatment were divided into two groups: One group was used to detect electrolyte leakage (EL), and the other group immediately was fixed in liquid nitrogen (N_2) and stored at -80°C until AsA, enzyme and protein analyses were performed.

Electrolyte leakage (EL)

Electrolyte leakage of leaf tissues was measured according to Arora et al. (1998) with some modifications: Leaf disks of 1.5 cm diameter from each treatment group were put into test tubes containing 15 mL of ddH_2O. The samples were then subjected to vacuum infiltrated at -0.15 MPa for 5 min and incubated at room temperature (24±1ºC) for 4 hours using an orbital shaker (Thermo Scientific MaxQ 4000, USA) at 250 rpm before the electrical conductivity of each solution (EC_1) was measured using a conductivity meter (Metler Toledo Seven Easy S30, USA) then immediately autoclaved (ALP CLG 32L, South Korea). Total conductivity (EC_2) was determined once more when the solution in test tubes cooled down to room temperature. Electrolyte leakage was calculated as follows: $EL(\%) = (EC_1/EC_2)$.

Ascorbic acid (AsA) content

Ascorbic acid content was determined according to Schoner and Krause (1990). Briefly, liquid N_2-frozen leaf tissues of 1.0 g were homogenized in 5 mL ice cold containing 4% (v/v) metaphosphoric acid, and centrifuged (Beckman Coulter Allegra 64R, USA) at 4000×g for 10 min at 4°C. Subsequently, 1 mL of the supernatant was sampled, mixed with 1 mL 50 mM Na citrate buffer, pH 2.6, and 1 mL 10 mM dichlorophenolindophenol and incubated for 1 min at 25ºC. Following incubation for 30 s, the AsA content in the mixture was determined at 524 nm using a UV/VIS spectrophotometer Perkin Elmer Lambda 25, USA) and compared with those of standard AsA solutions.

Extraction and determination of enzymatic antioxidant activity

To prepare enzyme extracts, 0.5 g of liquid N_2-frozen leaf tissues were ground in a pre-chilled mortar and pestle with 5 mL of the following ice-cold extraction solutions containing 1.0% polyvinylpolypyrrolidone (PVPP) (Moran et al., 1994): For CAT 100 mM potassium phosphate (K-phosphate) buffer, pH 7.0, containing 0.1 mM ethylenediamine-tetraacetic acid (EDTA), with addition 0.1% Triton, for APX and GR 50 mM K-phosphate buffer, pH 7.8, containing 50 mM AsA and 50 mM K-phosphate, pH 7.6, containing 0.1 mM EDTA, respectively. The homogenate was centrifuged at 15000×g for 20 min at 4°C. The supernatants were used to assay for the enzymatic activities and soluble protein analysis. All the enzyme analyses were carried out spectrophotometrically. The method of Rao et al. (1996) was employed for the assay of CAT by recording the decrease in absorption at 240 nm in a UV/VIS spectrophotometer as H_2O_2 (ε= 39.4 mM/cm) was consumed. The APX activity was recorded by the decrease in oxidized AsA (ε= 2.8 mM/cm) at 290 nm (Nakano and Asada, 1980). The GR activity was measured at 340 nm (ε= 6.22 mM/cm) by the method of Cakmak and Marschner (1992), equivalent to the oxidation of β-nicotinamide adenine dinucleotide phosphate. The soluble protein content of the crude enzyme extracts was determined according to Bradford (1976).

Total soluble protein (TSP) content and SDS-PAGE

The TSP content was determined by making some modifications in Shen et al. (2003)'s method. The TSP was extracted with a mortar and pestle from 250 mg of leaf samples at 4ºC with 1.0% PVPP and 1 mL of the extraction buffer containing 25 mM tris-base, 275 mM sucrose, 2mM

EDTA, 10 mM dithiothreitol (DTT), 0,5 mM phenylmethylsulfonyl fluoride (PMSF).

The extract was centrifuged (10000×g, 10 min, 4°C) and used for the Bradford assay (Bradford, 1976). The SDS-PAGE was performed with a PROTEAN tetra vertical electrophoresis unit (Bio-Rad, Hercules, USA) using "TGXTM FastCastTM premixed acrylamide solutions" kit (Bio-Rad, Hercules, USA). An equal amount of total protein (7.5 µg) was loaded for each sample and gels were stained with Coomassie Brilliant Blue G-250. Protein bands were examined in the imaging system (Vilber, Quantum ST4 Gel Imaging System, France). The SDS-PAGE molecular weight (MW) standard (Biorad, Low Range SDS-PAGE standard) was used during electrophoresis of the samples.

Statistical analysis

The experiment was set up using a randomized block design. Thirty biological and 3 technical replications were used for all assays. The data were subjected to analysis of variance (ANOVA) and the means were evaluated using Duncan test at $p<0.05$ using the SPSS software (version 22., Chicago, USA).

RESULTS AND DISCUSSION

Electrolyte leakage (EL)

The leaf EL values of both genotypes are shown in Figure 1A. Root zone salinity significantly increased the EL of both genotypes. Due to imposition of salinity, the genotypes showed similar increasing trends in EL attributes. The highest increase in EL values was recorded at 150 mM NaCl of 65.81% and 50.95% for "Local Genotype" and "Şeker Fasulye", respectively, as compared to control plants. In addition, the EL was higher in "Local Genotype" than in "Şeker Fasulye". Although these harmful effects seem alleviated by spraying AsA, statistical analysis revealed a significant effect of only NaCl treatment on EL (Table 1). On the other hand, plant growth also decreased with increasing salinity levels and foliar-applied AsA ameliorated inhibitory effects of salinity especially in "Local Genotype" (Figure 1B).

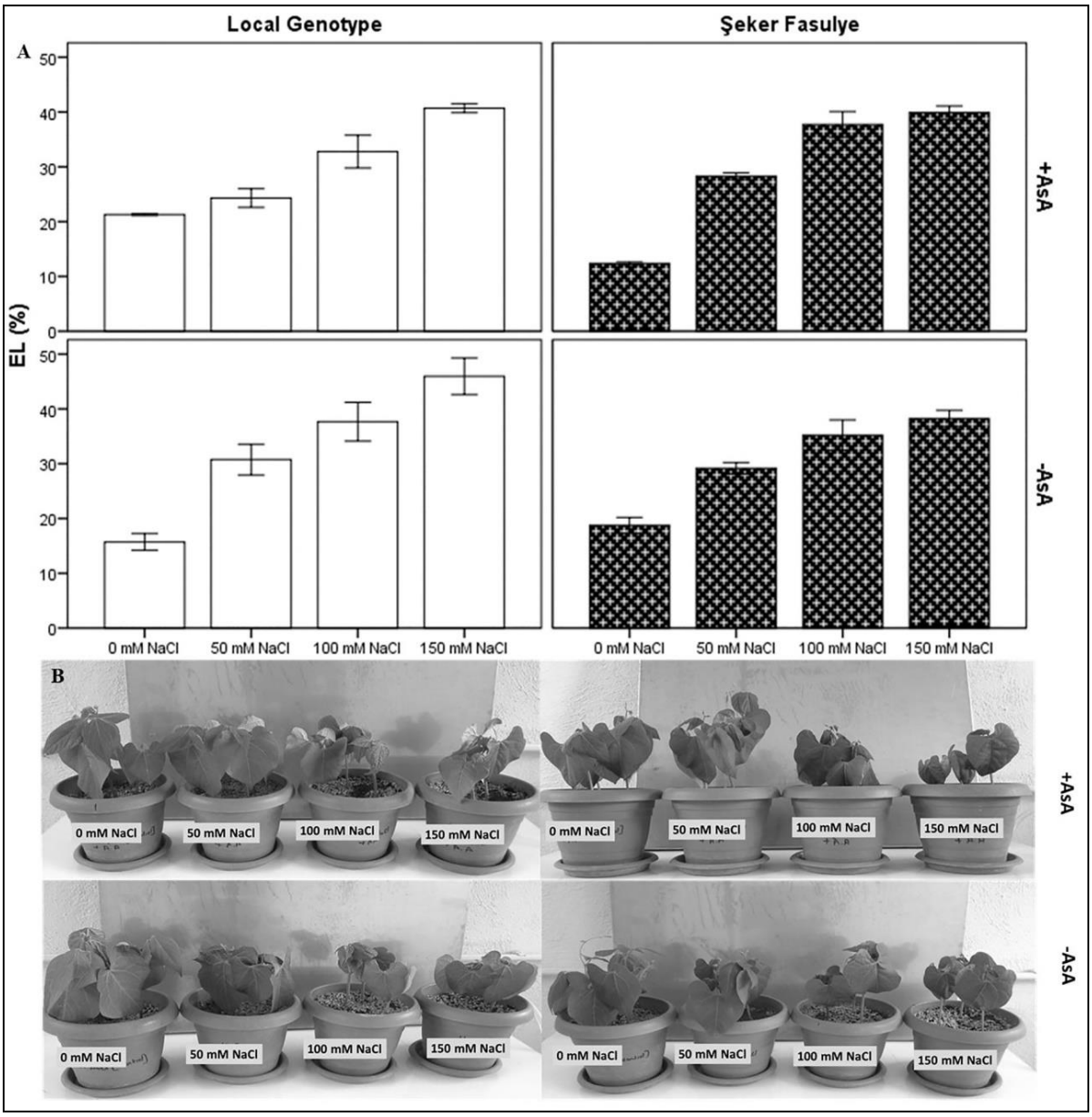

Figure 1. Electrolyte leakage in leaf tissues of two common bean genotypes as influenced by foliar-applied AsA under salt stress (Panel A), the effects of salinity and AsA applications on common bean plants (Panel B). Error bars represent ± SE of three replications.

Ascorbic acid (AsA) content

A significant alteration in the AsA content of both genotypes was recorded due to salinity treatment (Figure 2). 50 and 100 mM NaCl applications decreased the AsA content, while 150 mM NaCl increased the AsA content of "Local Genotype" without AsA application. On the other hand, salt induced increment of the leaf AsA content in the "Şeker Fasulye" at same condition. Exogenous application of AsA significantly reduced the adverse effects of salinity on AsA content of both genotypes except that of 50 mM NaCl (Figure 2). The highest increase in AsA content was recorded at 100 and 150 mM NaCl with 27.37% and 18.24% respectively, in "Şeker Fasulye" by foliar-applied AsA. The effect of genotype, AsA application, NaCl treatment and all their interaction on AsA content was statistically significant (Table 1).

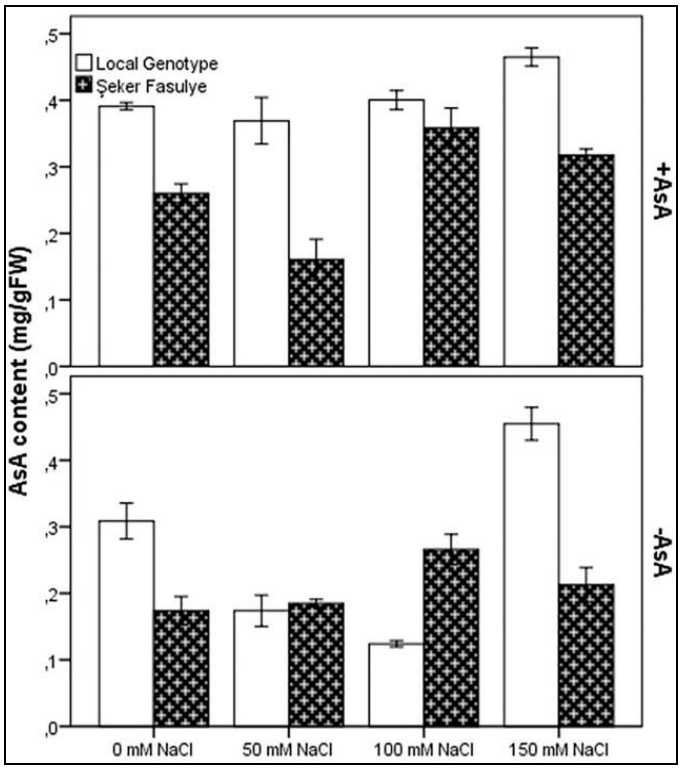

Figure 2. Ascorbic acid content in leaf tissues of two common bean genotypes as influenced by foliar-applied AsA under salt stress. Error bars represent ± SE of three replications.

Enzymatic antioxidants activities

Antioxidant enzymes like CAT, APX, and GR displayed different responses under salinity (Figure 3). Figure 3A shows the changes in the leaf CAT activity. Due to root zone salinity, significant increase in the CAT activity of leaves was recorded in both genotypes. Both genotypes showed similar increasing trends in CAT activity due to NaCl application except 150 mM NaCl. However, foliar-applied AsA was found effective in reducing the adverse effects of salinity on this attribute between 20.38% and 61.99% in both genotypes. More increases in the CAT activity due to the foliar-applied AsA was evident in the "Local Genotype" under salt stress. All the independent variables and their interaction was statistically significant except the genotype and interaction of genotype*NaCl treatment on CAT activity (Table 1).

The changes in the leaf APX activity are shown in Figure 3B. The APX activity of both genotypes decreased due to salinity except 50 mM NaCl for "Local Genotype". However, the exogenous application of AsA significantly increased the APX activity between 85.04% and 92.66% in "Local Genotype" but the opposite was true for "Şeker Fasulye" under salt stress. All the independent variable and their interaction was statistically significant except the genotype and genotype*AsA interaction on APX activity (Table 1).

The changes in the leaf GR activity are shown in Figure 3C. The GR activity of "Local Genotype" was increased while the activity decreased in "Şeker Fasulye" (except 50 mM NaCl) significantly due to salinity. Although foliar-applied AsA did not affect GR activity significantly, AsA application increased GR activity between 12.66% and 55.92% in both genotypes under salt stress as compared to control treatment. All the independent variables and their interactions were statistically significant except the AsA application on GR activity (Table 1).

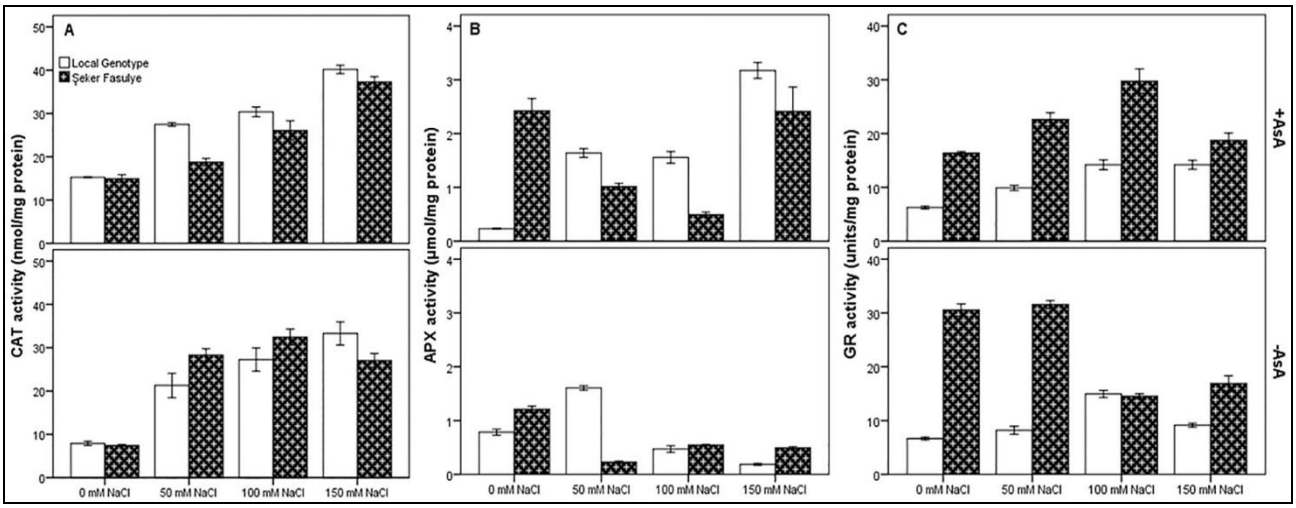

Figure 3. The activities of CAT (Panel A), APX (Panel B), and glutathione reductase (GR) (Panel C) in leaf tissues of two common bean genotypes as influenced by foliar-applied AsA under salt stress. Error bars represent ± SE of three replications.

Total soluble protein (TSP) content and SDS-PAGE

The changes in the leaf TSP content are shown in Figure 4A. While the TSP content increased significantly due to salinity in "Local Genotype", the opposite was true for "Şeker Fasulye". A non-significant effect of foliar-applied AsA was found on the TSP content of both genotypes. All the independent variable and their interaction on TSP content was statistically significant except the AsA application (Table 1).

Figure 4B shows the total protein profiles of both genotypes depending on NaCl and AsA applications. While many protein bands had been observed in SDS-PAGE with sizes ranging from 14.40 kDa to 127.46 kDa according to treatments, only polymorphic bands were marked on the gels. Ten polymorphic bands estimated as 127.46, 108.36, 81.97, 77.17, 72.65, 58.27, 54.56, 43.89, 22.86 and 20.88 kDa were noted in both genotypes. The 127 kDa protein band was observed only in 0 mM NaCl without foliar-applied AsA for "Local Genotype". The 108.36 kDa protein band disappeared in 100 and 150 mM NaCl in "Local Genotype" and in 150 mM NaCl in "Şeker Fasulye" without foliar-applied AsA. However, the size of 108.36 kDa protein band occurred in both genotypes except 150mM NaCl for "Şeker Fasulye" with foliar-applied AsA. The sizes of 81.97 and 77.17 kDa protein bands were hardly visible in 150 mM NaCl in "Local Genotype" and all the NaCl treatments in "Şeker Fasulye" without foliar-applied AsA. On the other hand, the 81.97 and 77.17 kDa protein bands were detected with foliar-applied AsA in 100 and 150 mM NaCl for "Local Genotype", in 50 and 100 mM NaCl for "Şeker Fasulye". Although the 72.65 kDa protein band disappeared at 150 mM NaCl without foliar-applied AsA, it occurred with foliar-applied AsA in both genotypes. A 58.27 kDa sized protein band in both genotypes disappeared by 150 mM NaCl without foliar-applied AsA and this band was detected in 150 mM NaCl with foliar-applied AsA for "Local Genotype". The sizes of 54.56 and 43.89 kDa protein bands also disappeared in 150 mM NaCl in two genotypes in both conditions. The 22.86 and 20.88 kDa protein bands disappeared at 150 mM NaCl in both genotypes without foliar-applied AsA. However, these protein bands appeared in both genotypes in 150 mM NaCl with foliar-applied AsA.

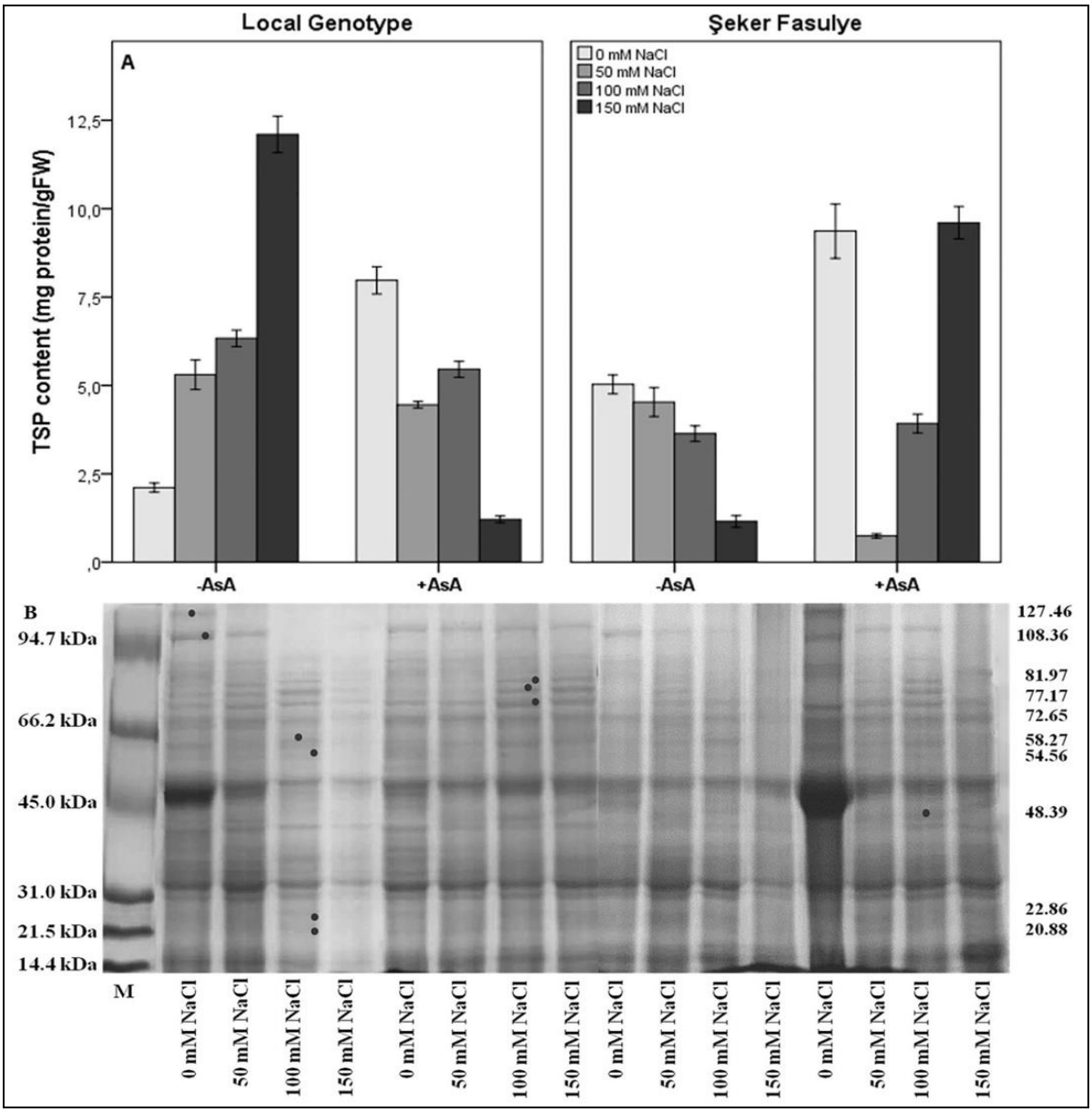

Figure 4. Total soluble protein content (Panel A) and protein profiles (Panel B) of leaf tissues in two common bean genotypes as influenced by foliar-applied AsA under salt stress. M: MW standard. Error bars represent ± SE of three replications.

Table 1. Results of analysis of variance (ANOVA) of genotype (G), NaCl, AsA and their interactions with EL, CAT, APX, GR activities, and TSP content in leaf tissues of common bean genotypes. Numbers represent F values relative to a significance level of 0.05.

Dependent variable	Independent variable						
	G	NaCl	AsA	GxNaCl	GxAsA	NaClxAsA	GxNaClxAsA
EL	0.784^{ns}	57.445*	1.733^{ns}	1.246^{ns}	0.546^{ns}	0.240^{ns}	2.542^{ns}
AsA	74.596*	27.134*	89.532*	22.232*	12.296*	6.417*	11.721*
CAT	2.746^{ns}	151.609*	15.594*	1.915^{ns}	11.609*	12.193*	7.451*
APX	2.170^{ns}	21.861*	175.682*	50.493*	0.323^{ns}	53.291*	25.906*
GR	610.685*	15.460*	0.010^{ns}	39.427*	8.636*	44.612*	45.650*
TSP	25.124*	41.238*	3.318^{ns}	35.263*	133.456*	90.087*	222.755*

(*) Significant within column at $p<0.05$, (ns) - nonsignificant.

Plasma membranes are the main region of ion-specific salt damage (Hasegawa et al., 2000). Therefore, EL is a common evaluation way for plasma membrane stability and an important criterion for selection of salt-tolerant plants (Hniličková et al., 2019; Aydogan and Turhan, 2020). In the current study, salinity ruptured plant tissues, leading to high EL in both genotypes. Similarly, it was reported that salinity induced important suppression in growth of *P. vulgaris* seedlings (Saeidi-Sar et al., 2013; Taïbi et al., 2016). It is suggested that the retardation in growth is due to inhibition of cell elongation (Abdelgawad et al., 2019). It has been determined that the EL value was lower in the "Şeker Fasulye"; which is more tolerant to salt, than in the "Local Genotype", while AsA application caused a reduction in EL of the "Local Genotype" under salt stress (Figure1). The results are in accordance with previous research in common bean (Rady et al., 2018), faba bean (Alzahrani et al., 2019) and mung bean (Nawaz et al., 2021), that also showed high EL under salt stress. In previous researches, the application of AsA reduced EL in the common bean and mung bean plant under salt stress (Rady et al., 2018; Nawaz et al., 2021). From the results, it might be suggested that AsA application regulates plant tolerance to reaction, and relieves the injurious effects of salinity on plant growth. This result is in agreement with previous results, which argue that AsA can effectively improve salt damages in tomato (Abdelgawad et al., 2019) and barley (Noreen et al., 2020). Furthermore, the results suggest that AsA is more effective on the "Local Genotype", which is relatively more sensitive to salinity, than on the "Şeker Fasulye".

Oxidative protection is an important component for determining the viability of a plant during stress. Ascorbic acid, one of the non-enzymatic antioxidants, is a scavenger of many ROS due to its electron donating ability. It is evident from the present study that foliar-applied AsA reduced the adverse effects of salinity on AsA content in both genotypes (Figure 2). Similar effects were also observed in several recent studies (Dolatabadian and Jouneghani, 2009; Ergin et al., 2014; Nawaz et al., 2021). The fast reduction of ROS by the AsA prevents the impairing of bio molecules before the activation of antioxidant enzymes (Paciolla et al., 2019).

Although ROS are produced in chloroplast, mitochondria and peroxysomes under optimal conditions, they may be generated by salinity that induces lipid peroxidation and destruction, which leads to membrane damage (Srour, 2015). Plants have generated an effective antioxidative pattern composed of enzymatic and non-enzymatic antioxidants to minimize and repair the ROS-initiated damage (Sharma et al., 2012). Catalase, found largely in peroxisomes, directly transforms H_2O_2 to H_2O and O_2 and it is more involved in detoxification of H_2O_2 not as a signalling molecule (Sofo et al., 2015). In the AsA–GSH cycle, APX reduces H_2O_2 to H_2O utilizing AsA as an electron donor (Sharma et al., 2012). Glutathione reductase is a possible enzyme of the AsA-reduced GSH cycle and acts a part in the defence system against ROS by maintaining the reduced position of GSH (Noctor et al., 2012).

It was evident from the results that, there was a notable increase in the CAT activity induced by salt applications (Figure 3A). The CAT activity of common bean (Dolatabadian and Jouneghani, 2009), and barley (Agami, 2014) increased due to salinity. Increments in the activity of CAT enzyme is considered as a response to the elevated H_2O_2 generation and may be effective in detoxifying salt stress induced H_2O_2. In addition, foliar-applied AsA increased the activity of CAT in both genotypes under salinity. Similar results were found in wheat (Athar et al., 2008), barley (Hassan et al., 2021) and common bean (Dolatabadian and Jouneghani, 2009; Rady et al., 2018) plants subjected to salinity with an AsA application. This implies an unequivocal role of AsA in scavenging H_2O_2 under salt stress. In addition, from the results, it can be suggested that AsA is more effective on the "Local Genotype", which is relatively more sensitive to salinity, than "Şeker Fasulye"on this attribute.

In the current work, the leaf APX activity was increased in seedlings exposed to salinity only when 50 mM NaCl was applied in "Local Genotype". Although foliar-applied AsA increased the activity of APX in all salt applications in the "Local Genotype", the APX activity was equal to those at the control plants only under 150 mM NaCl application and decreased in other applications with foliar-applied AsA in "Şeker Fasulye" (Figure 3B). Higher APX activity might improve the H_2O_2 scavenging system in chloroplasts and prevent the accumulation of H_2O_2 (Chaitanya et al., 2002). It means that foliar-applied AsA could be increased in genotype sensitivity to salinity especially salt sensitive common bean genotype. These results also suggest that, under mild saline stress, the alleviated levels of the antioxidant enzymes protect seedlings against the ROS (Younis et al., 2010). The association of APX activity with salt tolerance found by the current study is in good agreement with previous studies that have clarified the significant role of APX in increasing salt tolerance by AsA application (Kaya, 2017; Hassan et al., 2021; El-Beltagi et al., 2022).

According to the presented data, the GR activity showed different responses to salinity in both genotypes. Although not statistically significant, foliar-applied AsA generally increased GR activity under salt stress in both genotypes (Figure 3C). These results suggest that, higher GR activity may relate to higher salt tolerance of the common bean genotypes, which is consistent with previous finding of Taïbi et al. (2016). Besides, several previous reports have suggested that AsA application enhanced GR activities and this increased the induction of stress response due to the effect of AsA on antioxidant system (Wang et al., 2014; Kaya, 2017). This calls our attention to the fact that the AsA–GSH cycle may play an important role in salt tolerance in *P. vulgaris* (Taïbi et al., 2016).

A remarkable approach about salt stress adaptation is to study some mechanisms involving protein promotion and differentiated protein function (Sofy et al., 2017). Early changes caused by stress include reprogramming of signal transduction components, transcription factors and proteins associated with ROS metabolism under stressful conditions (Ashraf and Harris, 2004). The preservation of protein structures and functions under stress is very important for the survival of the cell (Wang et al., 2004). In the present study, the genotypes showed a different trend in case of TSP content in response to salinity. The TSP content of "Şeker Fasulye" at 150 mM NaCl reached control treatments' level with foliar-applied AsA (Figure 4A). Salinity increases the TSP content at low concentrations with the synthesis of new stress proteins, while at high stress levels the TSP content decreases with a decrease in photosynthesis (Abdoli Nejad and Shekafandeh, 2014). Under salinity, protein accumulation may be considered as a form of N storage that may utilized when stress ends and may play a role in osmotic regulation (Ashraf and Harris, 2004). The results showed that the TSP content was not an effective parameter in the salt tolerance of common bean, and foliar AsA application also did not play a role in this mechanism.

The changes in protein profiles of the genotypes under different salinity levels and treated with AsA are shown in Figure 4B. Salt and AsA applications stimulated an induction of some polypeptides and other polypeptides vanished. It has been determined that SDS-PAGE protein profiles represent adaptive mechanisms for dealing with excess salt in common bean. Similar results were obtained in faba bean and flax under salinity by AsA application (Younis et al., 2009; El-Bassiouny and Sadak, 2015).

Ten polymorphic bands, which may be members of heat shock protein (HSP) families; ranging between 14.40 kDa to 127.46 kDa, with roles to play in salt stress in plants were represented in the current work. The protein band with 108.36 kDa, may play the role of HSP100 proteins, which maintains the functional integrity of some key polypeptides by re-solubilizing protein aggregates via interactions with the small HSP (sHSP) chaperone system (Gupta et al., 2010). The protein band with 81.97 kDa, may function as HSP 90, which is mentioned as stress protein and as a key regulator of

normal growth and development (Sangster et al., 2007; El-Bassiouny and Sadak, 2015). The protein bands with 77.17 kDa and 72.65 kDa seems to be HSP 70 chaperones that function is prevention of protein aggregation (Park and Seo, 2015). The protein bands with 58.27 kDa and 54.56 kDa, may be members of the HSP 60 family which are significant in helping plastid proteins (Gupta et al., 2010). The protein bands with MW 43.89 kDa, of the HSP40 family are known to induce folding of proteins by HSP70 (Wang et al., 2004). Besides, the protein bands with 22.86 kDa and 20.88 kDa described the small HSP family where the MW ranges from 15 to 42 kDa, terminating unwanted protein-protein interactions and helping in refolding of denatured proteins (Gupta et al., 2010).

CONCLUSIONS

This study indicates that salt stress disrupts the growth of common bean plant by damaging cellular membranes. The results suggest that foliar-applied AsA, especially in the "Local Genotype", reduces EL through ROS scavenging, upregulation of the antioxidant system and stabilization of cellular membranes under salinity.

Besides the results indicates that foliar-applied AsA is an effective preservative for improving salt tolerance to activate the antioxidative system and salt responsive protein especially in the salt-sensitive common bean genotype in seedling stage. The findings also suggest that common bean genotypes develop different mechanisms in salt tolerance.

Although the results obtained are promising, before recommending the use of 3.0 mM AsA sprays in commercial formulations to enhance plant growth and the production of especially salt sensitive common bean genotypes under salt stress, further studies with more genotypes are required. Moreover, there is a need to decipher the protective mechanisms of AsA and the signalling cascades at the gene level in future studies.

ACKNOWLEDGEMENTS

The authors would like to thank Tchanou Tchakoute Rodolph for editing the English language of this research article.

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