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RESEARCH PAPERS

Effect of Salt Stress on Antioxidant Defense Systems, Lipid Peroxidation, and Chlorophyll Content in Green Bean[1]

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Abstract— Salt stress-induced changes in antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), total chlorophyll content, and lipid peroxidation measured as malondialdehyde (MDA) content, in leaves of a green bean genotype Gevas sirsk 57 (GS57) and cv. Fransiz 4F-89 differing in salt tolerance were investigated. Plants were subjected to three salt treatments (0, 50, and 100 mM NaCl) under controlled climatic conditions for 7 days. The salt-sensitive cv. 4F-89 exhibited a decrease in GR activity at all salt treatments, but the salt-tolerant genotype GS57 showed only a slight decrease in GR under 50 mM salt treatment and an increase under 100 mM salt treatment. CAT and APX activities increased with increasing salt stress in both varieties. CAT and APX activities were higher in the salt-tolerant GS57 than salt-sensitive cv. 4F-89. The two varieties showed an increase in MDA content with an increase in salinity, but the increase in sensitive cv. 4F-89 under salt stress was higher than that in salt-tolerant GS57 genotype. The increasing NaCl concentration caused a reduction in the chlorophyll content in cv. 4F-89 but not in GS57.

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Key words: Phaseolus vulgaris - antioxidant enzymes - salt stress - oxidative stress - lipid peroxidation - chlorophyll.

INTRODUCTION

Salinity in soil or water is of importance for agriculture because it limits a distribution of higher plants in certain natural habitats and induces a wide range of adverse metabolic responses in them. Although some crops are moderately tolerant to salinity, many crops are adversely affected by even low salt levels [1].

Salinity stress affects the metabolism of plant cells, leading to severe crop damage and a loss of productivity [2].

When plants are subjected to environmental stresses, reactive oxygen species (ROS) are generated in response to stress condition [3]. ROS can cause oxidative damage to many cellular components, including membrane lipids, proteins, nucleic acids, and chlorophyll [4]. The possible ROS involvement in the mechanism of damage by salt stress was already confirmed on cotton [5], eggplant genotypes [4], and melon [4], i.e., species differing in salt resistance.

To control the level of ROS and to protect the cells, plants possess a number of low-molecular-weight antioxidants (ascorbate, glutathione, phenolic compounds, tocopherols) and enzymes (SOD, CAT, APX, and GR) scavenging ROS and regenerating the active forms of antioxidants. Under normal conditions, ROS production and scavenging is well regulated. In this way, this enzyme system can eliminate the damaging effects of toxic oxygen species [8–10].

The role of antioxidant enzymes as the components of the main tolerance mechanisms developed in response to different environmental extremes has already been shown. Many studies showed a correlation between the resistance to environmental stresses and the efficiency of the antioxidant system [11].

ROS cause chlorophyll degradation and membrane lipid peroxidation, reducing membrane fluidity and selectivity. Chlorophyll loss, lipid peroxidation measured as MDA (malondialdehyde, a product of lipid peroxidation) content, and electrolyte leakage are considered to be indicators of oxidative damage [12, 13].

Common bean (*Phaseolus vulgaris*) is classified as a salt-sensitive plant [14]. It is a species widespread in Anatolia, but its responses to salinity stress are almost unknown. In a previous study [15], we have determined one salt-tolerant and one salt-sensitive local bean cultivars. In this study, we hypothesized that an increased activity of antioxidant enzymes (CAT, APX, and GR)

[1] This text was submitted by the authors in English.

Abbreviations: APX—ascorbate peroxidase; CAT—catalase; GR—glutathione reductase; MDA—malondialdehyde; ROS—reactive oxygen species.

contributes to the protection of bean plants against salt stress.

The aim of this work was to evaluate the effects of salt stress on the activity of antioxidant enzymes, lipid membrane peroxidation, and chlorophyll content in two green bean varieties in order to better understand their differences in salt stress tolerance.

MATERIALS AND METHODS

Plant material. A salt-tolerant (GS57) and salt-sensitive (Fransiz 4F-89) local Turkish green bean varieties, whose salt tolerance has been determined in our previous study [15], were used as an experimental plant material.

Plant growth and treatments. The seeds of green bean were germinated in plastic pots ($40 \times 25 \times 5$ cm) filled with Perlite in a growth chamber with a 16 photoperiod at 25°C and 70% humidity. After the emergence of the first true leaves, seedlings were irrigated with the Hoagland nutrient solution. Plants were grown in the pots until the emergence of the first two true leaves. Afterwards, they were transplanted to hydroponic culture. For hydroponic culture, plastic dishes ($40 \times 25 \times 5$ cm) filled with the Hoagland nutrient solution were used. The nutrient solution was renewed once a week.

Seedlings were grown under control conditions until emergence of the fourth true leaves, at which time salt stress treatment was initiated. In the salt treatment, the plants were exposed to salinity by adding NaCl to the growth medium in two steps at 12-h interval until the final concentrations of 50 and 100 mM NaCl were reached. Treatments were repeated four times, each replicate having 10 plants and arranged in a completely randomized design. Seven days after salt treatment, six plants of each genotype were harvested at random and the fresh weights of whole plants were determined. The leaf samples were kept at –80°C for further analyses.

Chlorophyll determination. Leaf segments (200 mg), either fresh or frozen at –40°C, were placed in 5 ml of 80% ethanol and heated in a water bath at 80°C for 20 min. Total chlorophyll was evaluated in the alcohol extracts from absorbance readings, using the appropriate extinction coefficient. Chlorophyll content (mg/g fr wt) was calculated as $1000 \times A_{654}/(39.8 \times$ sample fr wt) according to Tetley and Thimann [16].

Malondialdehyde content. Lipid peroxidation was measured as the amount of MDA determined by the thiobarbituric acid (TBA) reaction [17]. Frozen samples were homogenized with a prechilled mortar and pestle with two volumes of ice-cold 0.1% (w/v) TCA and centrifuged for 15 min at 15000 *g*. Assay mixture containing 1 ml of the supernatant and 2 ml of 0.5 % (w/v) TBA in 20% (w/v) TCA was heated at 95°C for 30 min and then rapidly cooled in an ice bath. After centrifugation (10 000 *g* for 10 min at 4°C), the supernatant absorbance was read at 532 nm, and the values corresponding to nonspecific absorption (600 nm) were subtracted. Lipid peroxidation products were measured as the content of TBA-reactive substances. The MDA content was calculated according to the molar extinction coefficient of 155/(mM cm).

Enzymes extraction and assay. Fresh leaf samples were submersed for 5 min in liquid nitrogen. The frozen leaves were kept at –80°C for further analyses. Enzymes were extracted from 0.5 g of leaf tissue using a mortar and pestle with 5 ml of extraction buffer containing 50 mM K-phosphate buffer, pH 7.6, and 0.1 mM Na_2-EDTA. The homogenate was centrifuged at 15000 *g* for 15 min, and the supernatant fraction was used to assay various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C.

CAT activity was determined by monitoring a disappearance of H_2O_2 according to the method of Cakmak and Marschner [18].

APX activity was determined according to Cakmak and Marschner [18] by measuring a decrease in the absorbance of the oxidized ascorbate at 290 nm. One unit of APX was defined as the amount of enzyme required to consume 1 μmol ascorbate/min.

GR activity was determined by measuring the enzyme-dependent oxidation of NADPH by following absorbance at 340 nm. One unit of GR activity was defined as the amount of enzyme that oxidized 1 μmol NADPH/min [18].

The experiment was designed as completely randomized plot with three replicates, each plot having 10 plants. Data were analyzed statistically, and the means of each treatment were analyzed by Duncan's Multiple Range Test using SAS software [19].

RESULTS

Plant Growth

Plant growth was assessed by measuring leaf, shoot, and root weights. NaCl treatments led to a gradual decrease in the plant fresh weight of both green bean varieties. At 100 mM NaCl treatment, reduction in fresh weight was more in both salt-sensitive and salt-tolerant green bean plants when compared with control and 50 mM NaCl treatments. The fresh weight of cv. Fransiz 4F-89 was lower than that of the GS57 genotype (Table 1).

Chlorophyll Content

The effects of increasing level of NaCl salinity on chlorophyll contents in plants of two varieties were determined after 7-day exposure to salinity. In cv. 4F-89, chlorophyll content decreased gradually with the increasing NaCl treatments. On the other hand, chlorophyll content in GS57 genotype was decreased by 50 mM NaCl but not changed significantly by 100 mM NaCl (Table 1).

Table 1. The effect of salt treatment on the content of chlorophyll, MDA, and fresh weights in two varieties of *P. vulgaris*

Variety	Chl, mg/g fr wt %			MDA, μmol/g fr wt %			Fr wt, g/plant		
	NaCl treatment, mM								
	0	50	100	0	50	100	0	50	100
GS57	0.20^{aA}	0.16^{aB}	0.19^{aA}	1.40^{aC}	3.01^{bB}	4.99^{bA}	10.70^{aA}	9.56^{aB}	7.04^{aC}
4F-89	0.17^{bA}	0.11^{bB}	0.09^{bC}	1.43^{aC}	5.48^{aB}	7.78^{aA}	9.53^{bA}	7.39^{bB}	4.59^{bC}

Note: Significant differences between means of salt concentrations are indicated by different capital letters+ADs- significant differences between means of varieties are indicated by different small letters.

Table 2. Antioxidant enzymes activities in leaves of salt-treated *P. vulgaris*, L. plants

Variety	CAT, μmol/(mg fr wt min)			APX, μmol/(mg fr wt min)+ADs-			GR μmol/(mg fr wt min)		
	NaCl treatments, mM+ADs-								
	0	50	100	0	50	100	0	50	100
GS57	8.91^{aC}	25.19^{aB}	37.36^{aA}	1.65^{aC}	6.38^{aB}	12.41^{aA}	14.97^{aB}	13.27^{aC}	20.23^{aA}
4F-89	7.59^{aC}	16.99^{bA}	10.35^{bB}	1.82^{aB}	5.06^{bA}	5.75^{bA}	12.87^{aA}	9.90^{bB}	8.43^{bC}

Note: Significant differences between means of salt concentrations are indicated by different capital letter+ADs- significant differences between means of varieties are indicated by different small letter.

Lipid Peroxidation

Lipid peroxidation in leaves of both green bean varieties, measured as MDA content, is given in Table 1. NaCl treatments led to a gradual increase in the levels of MDA in both green bean varieties. MDA accumulation in cv. 4F-89, however, was higher than in GS57, indicating a higher degree of lipid peroxidation due to salt stress.

Antioxidant Enzyme Activities

There were striking differences in antioxidant enzyme activities between the two green bean varieties with increasing NaCl concentration (Table 2). APX activity increased with increasing NaCl concentration in both cvs. 4F-89 and GS57. The rate of increase in APX activity was higher in GS57 genotype than that in cv. 4F-89 after 7 days of 50 and 100 mM NaCl treatments. A similar pattern was observed for CAT activity. GS57 genotype exhibited an increase in CAT activity with increasing salt treatments. However, in cv. 4F-89, CAT activity increased only in 50 mM NaCl treatment, but not in 100 mM salt treatment (Table 2).

GR activities of two green bean varieties under the effect of different NaCl concentrations are shown in Table 2. High NaCl concentration (100 mM) increased GR activity significantly, but low NaCl concentration (50 mM) caused reduced GR activity in the leaves of GS57 genotype as compared to control plants. On the other hand, cv. 4F-89 exhibited a significant decrease in GR activity with increasing NaCl treatment.

DISCUSSION

It has been reported that the typical symptom of salinity injury to the plant is growth retardation due to the inhibition of cell elongation [20]. In this study, when subjected to 50 and 100 mM NaCl, both GS57 genotype and 4F-89 Fransiz cultivar showed a reduction in plant growth. In term of fresh weight, the response of two green bean varieties to salt stress was found to be different. GS57 genotype showed better growth than cv. 4F-89 under salt stress conditions. Reduction of plant growth by salinity has been reported in several grain legumes, including *Phaseolus vulgaris* [21]

NaCl treatments led to a significant increase in the content of MDA in both green bean varieties. GS57 genotype showing better growth under salt stress had less MDA than cv. 4F-89, indicating that salt-tolerant genotype GS57 exhibited less active lipid peroxidation under both 50 mM and 100 mM salinity treatments than the salt-sensitive cv. 4F-89 (Table 1). In control groups, lipid peroxidation was similar in both varieties. This indicates that salt-tolerant genotype has a higher hereditary and induced capability of plant protection against oxidative damage caused by salt treatment. This protection might result from significantly higher constitutive activities of CAT, APX, and GR in the leaves of GS57 genotype. In fact, activities of these enzymes were found to be higher in GS57 genotype than in cv. 4F-89 at both 50 and 100 mM NaCl treatment. According to Scandalios [22], CAT and APX are the antioxidant enzymes most effective in preventing cell damage. A

lower lipid peroxidation resulting from elevated activities of antioxidants under salt stress was also reported in salt-tolerant wild tomato species [23], pea cultivars [24], eggplant [6], and in sugar beet [25, 26].

Overexpression of the APX gene in plants has been reported to improve protection against oxidative stress [27]. In plants, a number of enzymes regulate H_2O_2 intracellular levels; APX and CAT are considered to be the most important ones [28]. Our data showed that APX activity in both varieties was increased by salinity, and the activity of APX was higher in salt-tolerant GS57 genotype than in salt-sensitive cv. 4F-89 at both of 50 and 100 mM NaCl treatments. It is noteworthy that salt-induced APX activation in the GS57 genotype was accompanied by a greater increase in CAT activity. Therefore, it may be supposed that CAT and APX, both responsible for detoxification of H_2O_2, are probably equally important in the detoxification step in salt-tolerant genotype GS57. These results are consistent with observations of many researchers who reported that APX activity coordinated with CAT activity plays a central protective role during salt stress [2, 9, 11, 25, 29, 30]. These enzymes were also reported to be important in salt tolerance in mulberry [32], cotton [5, 9], and maize genotypes [33].

Increases in GR activity have been reported to play a role in tolerance to salt stress in pea [34] eggplant [6], maize [35], and rice [36]. Yasar et al. [7] also observed that NaCl decreased GR activity in salt-sensitive melon cultivar. Similarly, in this study, GR activity in the salt-sensitive cv. 4F-89 was decreased by NaCl treatment. On the other hand, in the salt-tolerant genotype GS57, GR activity increased significantly at 100 mM salt concentration, although it slightly decreased at 50 mM NaCl. Our results suggest that salt-sensitive variety exhibits a less active ascorbate-glutathione cycle and that the Halliwell-Asada enzymatic pathway may play a key role in salt tolerance in green bean plants.

A change in the leaf chlorophyll contents due to NaCl stress was similar to GR activities in both GS57 genotype and 4F-89 cultivar, probably because high antioxidant enzyme activities prevented degradation of leaf chlorophyll. Parallel decreases in chlorophyll content and enzyme activities due to NaCl salinity have been reported for cotton [37]. In the salt-sensitive cultivar (4F-89), the chlorophyll content decreased probably because there was a low activity of APX, CAT, and GR. No significant change in the chlorophyll content was found in 100 mM NaCl salt treatment plants of GS57 genotype. Meloni et al. [9] reported that in cotton cultivars the chlorophyll content in 50, 100 and 200 mM salt treatment decreased, but no significant changes in the salt-tolerant cultivar compared to control plants were found.

The results of this study showed that there were considerable differences between the salt-sensitive 4F-89 and salt-tolerant GS57 green bean varieties in their responses to salt stress. The significant differences in response to salt stress in green bean are closely related to differences in the activities of antioxidant enzymes. Finally, these results suggest that salt-tolerant green bean variety GS57 may have a better protection against oxidative damages by increasing the activity of antioxidant enzymes under salt stress.

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