Investigation of the relationship between the tolerance to drought stress levels and antioxidant enzyme activities in green bean (*Phaseolus Vulgaris* L.) genotypes

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The objective of this investigation was to establish the relationship between tolerances against drought stress and antioxidant activities of bean genotypes of the *Phaseolus vulgaris* L. species, namely Gevaş Bodur64 (GB64), Samsun100 (S100), Samsun95 (S95), 4F-89 Fransiz (4F-89), Gevaş Sırık57 (GS57), Gevaş Sırık26 (GS26), Samsun96 (S96), Sırık Barbunya (SB), Kırkgünlük (KG), and Oturak Barbunya (OB) collected from various regions of Anatolia. The seedlings of 10 different bean genotypes were cultivated in containers containing Hoagland's nutrient solution in a cultivation chamber, with climate conditions that were kept under control. For the application of drought stress, 10% polyethylene glycol 6000 (PEG-6000), corresponding to an osmotic potential of –0.40 MPa, was added to the nutrient solution. After the application, the fresh weight of green parts of the plants, the relative water content of leaves and the leaf antioxidative enzyme activities (superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were determined. The result showed that, the antioxidant enzyme activities were determined to be very effective on drought tolerance. Antioxidative enzyme systems that can survive under drought conditions were more active in the tolerant bean genotypes than in the susceptible genotypes. Under drought stress, the OB and GS57 genotypes were the most tolerant and the SB and 4F-89 genotypes were the most susceptible.

**Key words:** Beans, *Phaseolus vulgaris* L., drought, oxidative stress, enzyme activity, polyethylene glycol.

**INTRODUCTION**

In plants, the most important changes occur under drought stress, such as changes in plant growth performance, ion accumulation, and most importantly, the mobilization of antioxidative defense mechanisms (Bowler et al., 1992; Yasar, 2003; Turkan et al., 2005; Yasar et al., 2006, 2008a, b).

In plants, stomatal closing is a common response after exposure to drought stress, which causes a decline in the CO₂ uptake and an increase in the accumulation of nicotinamide adenine dinucleotide phosphate (NADPH). Hence, oxygen is the final electron acceptor in place of limited NADP, which results in the formation of superoxide (Zlatev et al., 2006). Through a variety of reactions, superoxide leads to the formation of hydrogen peroxide, hydroxyl radicals, and other reactive oxygen species (ROS), all of which can cause damage in various
ways (Hernandez et al., 1993; Sairam et al., 1998). The generation of ROS results in lipid peroxidation, protein degradation, and nucleic acid damages (Fazeli et al., 2007). Plants possess several antioxidant enzyme systems that protect their cells from the negative effects of ROS.

These include nonenzymatic antioxidants such as ascorbic acid, glutathione, and carotenoids, as well as antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) (Yasar, 2003; Yasar et al., 2006, 2008a, b; Choohkampaeng, 2011; Fu et al., 2012). Yasar (2003) have demonstrated that the antioxidant enzyme activities (SOD, CAT, APX, and GR) were higher in salt-tolerant eggplant cali than in sensitive eggplant cali. About 60% of the world’s common bean production occurs in areas prone to drought stress (White et al., 1994). Studies for selecting drought-tolerant cultivars have become more important during the latter half of the 20th century. The main selection criteria for tolerant plants are parameters of plant growth and production, which are time consuming to measure (Lizana et al., 2006). Therefore, utilizing stomatal conductance, lipid peroxidation, and antioxidant enzyme in the quest of drought-tolerant plants are preferred as fast and reproducible ways of screening.

Many reports suggest that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems (Yasar, 2003; Terzi and Kadioglu, 2006; Yasar et al., 2006, 2008a, b). The purpose of this study was to observe how the bean genotypes responded for the antioxidant enzyme activities after applying drought stress. The leaf fresh weight and leaf relative water content (RWC), and the SOD, CAT, and APX enzyme activities, as well as the increase of their tolerance levels to drought were investigated, enabling us to assess the effect of the antioxidant enzymes.

MATERIALS AND METHODS

A total of 10 bean (Phaseolus vulgaris L.) genotypes from various regions of Anatolia, Turkey, were employed in the present study. This included Gevaş-Bodur 64 (GB64), Kirkpınük (KG), and Oturak Barbunya (OB) of the bush types, and Sazova 1946 (Sazova), Samsun 95 (S95), Samsun 96 (S96), 4F Fransız (4F-89), Gevaş- Sırık 57 (GS57), Gevaş-Sırık 26 (GS26), and Sırık Barbunya (SB) of the climbing types (Table 1).

Growing the plants

Bean seeds were planted in pots made of foam, with a hole in the bottom of the pots, and filled with pumice. They were placed in a climatic room at a temperature of 25 ± 2°C and with a relative humidity of 50% under a light density of 500 μmol m⁻² s⁻¹. Later, they were left waiting at a light/dark photoperiod of 16:8. After their first real leaves appeared, the saplings were watered furtigated with Hoagland’s nutrient solution (Hoagland and Arnon, 1938). After the saplings had produced their second real leaves in pumice medium, they were transferred to water culture. Plastic dishes measuring 25 x 25 x 18 cm were filled with Hoagland’s nutrient solution and used for the water medium. The nutrient solutions were refreshed once a week and the dishes were repositioned to ensure that all of the plants benefited equally from the lightening conditions.

Inducing drought stress

The saplings were grown in the water medium for a week and then the drought stress application was started. The saplings had 3 to 4 real leaves at this stage. From each genotype, 15 plants were assigned for the test with 3 repetitions. A concentration of 10% polyethylene glycol 6000 (PEG-6000) corresponding to an osmotic potential of −0.40 MPa was added to the Hoagland’s nutrient solution for inducing drought stress in bean plants (Turkan et al., 2005; Kalefetoglu and Emekci, 2006).

Determination of leaf relative water content (RWC)

Percent leaf RWC was determined on 3 replicates of leaf tissues from each tray using the standard formula: RWC = ([fresh weight-dry weight]/[weight at full turgor-dry weight]) x 100. Water contents were gravimetrically determined by oven drying of leaves at 70°C for 48 h, and full turgor was determined from plants that had been watered and kept overnight in plastic bags as described by Farrant (2000).

Enzymes extraction and assay

Fresh leaf samples were submerged for 5 min in liquid nitrogen. The frozen leaves were kept at -80°C for further analyses. Enzymes were extracted from 0.5 g leaf tissue using a mortar and pestle with 5 ml extraction buffer containing 50mM potassium phosphate buffer pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15000 × g for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C. SOD was assayed according to Cakmak and Marschner (1992), by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. Measurements were carried out 40 models Analytic Jena Spectrophotometer. One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of the photochemical reduction of NBT. Catalase activity was determined by monitoring the disappearance of H₂O₂ according to the method of Cakmak and Marschner (1992). APX activity was determined by measuring ascorbate consumption by absorbance at 290 nm. One unit of APX was defined as the amount of enzyme required to consume 1 μmol ascorbate min⁻¹ (Cakmak and Marschner, 1992). All results were the means of three replicates, and each replicate consisted of ten plants data were analysed statistically and treatment means were separated by Duncan’s Multiple Range Test using SAS (1985) software.

RESULTS

This study determined the reaction of bean genotypes to drought stress using RWC and antioxidant enzyme activities in the leaves. Table 2 shows the variation among genotype for fresh weights and RWC in the
leaves of the control and the drought stressed treatments. In the 4F-89F, GS57 and GS26, significant differences between control and drought stressed treatments were not observed in terms of fresh weight, but the fresh weight of other genotypes was decreased significantly by 10% PEG-6000 application. Under stress conditions, the best plant growth was observed in the GB64 genotype, while the lowest development was observed in the KG genotype.

The relative leaf water potential of the control plants was higher than that of the drought treated plants (Table 2). The genotypes namely S95 and SB had the lowest relative water potential under drought stress, while GS57, GS26, and S100 had the highest value. The RWC of plants grown under drought stress showed a decrease for all of the genotypes compared to the control plants.

**The effect of drought stress on antioxidant enzyme activities in the bean genotypes**

In control treatment, there were significant differences in the SOD activities between genotypes, and S100, S96 and KG had higher SOD than other genotypes (Table 3). The lowest SOD activity was observed in GB64 in control treatment. While drought stress increased SOD activities of GB64, GS57 and OB, it decreased SOD activities of S100, S95, GS26, S96, SB, and KG. In the plants under drought stress, the highest SOD activity values were in the OB, GS57, and KG genotypes, while the lowest values were found in the SB, S95, and GB64 genotypes.

In the control treatment, generally, CAT activities of all genotypes were similar, except for GS57. CAT activity of these genotypes was quite higher than all other genotypes. Drought stress created by 10% PEG-6000 application increased CAT activities in all of the genotypes compared to the control plants (Table 3). The highest CAT activity was found in the genotypes OB and GS57 and the lowest was found in the genotypes 4F-89 and SB under drought stress condition.

GS57 had quite higher APX activity than all other genotypes used in this study in control treatment. Though not as much as GS57, genotype KG also had quite high APX activity relative to other genotypes. The APX activities of all genotypes were increased by drought stress, except for SB. The increases in APX activity caused drought stress were markedly higher in OB, S100, GS26 and S95 relative to other genotypes (Table 3).

**DISCUSSION**

Many researchers reported that in general, the effect of oxidative injury was seen in the leaves of the plants (Costa-França et al., 2000; Turkan et al., 2005). Bean genotypes under drought stress in this study showed different responses in terms of fresh weight. The fresh weights of the 3 genotypes exposed to drought stress were similar to the control, while fresh weight of the other genotypes was found to be decreased. Sairam et al. (1998) and Turkan et al. (2005) reported that PEG-treated plants showed a general reduction in plant fresh and dry weights, leaf area, leaf number, chlorophyll content and that the percent of RWC had been reduced in the drought-tolerant genotypes. In this study, significant differences between genotypes were not determined for RCW, and RCW was decreased by drought stress in all genotypes, and it was similar level for all cultivars under drought stress condition. Based on these result fresh weight and RCW can not be said as reliable parameter to determine the level of drought tolerance.

It was reported that a decrease in RWC in some barlet and wheat varieties was accompanied by the an increasing in the activity of SOD under drought stresses, and that this could be attributed to the fact that a reduction in RCW may result in loss of turgor, which leads to reduced CO₂ uptake, hence an increase in oxidative stress (Öztürk, 1996; Acar et al., 2001).

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**Table 1. List of green bean genotypes used in the study**: code, variety name, source and their growth type.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Code</th>
<th>Name</th>
<th>Location</th>
<th>Supplier</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GB64</td>
<td>Geväş Bodur 64</td>
<td>Van/Gevaş</td>
<td>Farmer</td>
<td>Bush type</td>
</tr>
<tr>
<td>2</td>
<td>S100</td>
<td>Samsun 100</td>
<td>Samsun/Kavak</td>
<td>Farmer</td>
<td>Climbing type</td>
</tr>
<tr>
<td>3</td>
<td>S95</td>
<td>Samsun 95</td>
<td>Samsun/Kavak</td>
<td>Farmer</td>
<td>Climbing type</td>
</tr>
<tr>
<td>4</td>
<td>4F-89F</td>
<td>4F-89 Fransız</td>
<td>Eskişehir</td>
<td>Anatolia Agric.Res.</td>
<td>Climbing type</td>
</tr>
<tr>
<td>5</td>
<td>GS57</td>
<td>Geväş-Sırık 57</td>
<td>Van/Gevaş</td>
<td>Farmer</td>
<td>Climbing type</td>
</tr>
<tr>
<td>6</td>
<td>GS26</td>
<td>Geväş-Sırık 26</td>
<td>Van/Gevaş</td>
<td>Farmer</td>
<td>Climbing type</td>
</tr>
<tr>
<td>7</td>
<td>S96</td>
<td>Samsun 96</td>
<td>Samsun/Kavak</td>
<td>Farmer</td>
<td>Climbing type</td>
</tr>
<tr>
<td>8</td>
<td>SB</td>
<td>Sırık Barbunya</td>
<td>Antalya/Korkuteli</td>
<td>Farmer</td>
<td>Climbing type</td>
</tr>
<tr>
<td>9</td>
<td>KG</td>
<td>Kırkgünüş</td>
<td>Eskişehir</td>
<td>Anatolia Agric.Res.</td>
<td>Bush type</td>
</tr>
<tr>
<td>10</td>
<td>OB</td>
<td>Oturak Barbunya</td>
<td>Antalya/Korkuteli</td>
<td>Farmer</td>
<td>Bush type</td>
</tr>
</tbody>
</table>
Contrary to literature given in the above, in the present study, it was clearly observed for some genotypes that while RCW was decreased by drought stress, SOD activity was also decreased. This could be due to that the genotypes have different defense mechanism to stress.

SOD catalyzes the dismutation of \( \text{O}_2 \) to \( \text{H}_2\text{O}_2 \) and \( \text{O}_3 \). It was reported from earlier studies of some plant species (Gossett et al., 1996; Yasar, 2003; Yasar et al., 2006, 2008a, b), that SOD activities was increased at different rate by drought stress depending on the structure of plant genetic. Acar et al. (2001) suggested that a correlation between plant resistant to drought stress and SOD activities. In this study, although it was determined that bean genotypes exposed to drought stress had different dismutating capacities, no relationship was found between drought tolerance and SOD activities of genotypes.

In plants, a number of enzymes are involved in regulate of \( \text{H}_2\text{O}_2 \) intracellular levels and APX and CAT are considered to be the most important ones (De André et al., 2006). According to Scandalios and Guan (2001), CAT and APX are the antioxidant enzymes most effective in preventing cell damage. The overexpression of the APX gene in plants has been reported to improve protection against oxidative stress (Wang et al., 1999). Our data showed that the APX and CAT activity was increased by drought in all bean genotypes used in this study. OB and GS57 appeared to have the highest APX increased by drought in all bean genotypes used in this study. OB and GS57 appeared to have the highest APX and CAT activities under drought stressed treatments. In the present study, it was clearly observed for some genotypes that while RCW was decreased by drought stress, SOD activity was also decreased. This could be due to that the genotypes have different defense mechanism to stress.

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drought-induced APX activation in the GS57 and OB genotypes was accompanied by a greater increase in CAT activity. Therefore, it may be supposed that CAT and APX, both responsible for the detoxification of H2O2, are probably equally important in the detoxification step in the drought-tolerant. These results are consistent with the observations of many researchers who reported that APX activity coordinated with CAT activity plays a central role during stress (Türkan et al., 2005; Yasar et al., 2008a; Kusvuran, 2010).

Consequently, in this study, although no relationship was determined between fresh weight and antioxidant enzymes activities, it was clearly observed that there was a significant difference between genotypes in terms of their hereditary and induced capability of antioxidant enzyme activation.

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REFERENCES


