

Effects of drought stress on physiological and biochemical changes in *Phaseolus vulgaris* L.

Sebnem Kusvuran¹ and H. Yildiz Dasgan²

Cankiri Karatekin University Kizilirmak Vocational High School,
18100, Cankiri, Turkey.

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ABSTRACT

The present study investigated different levels (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress) of drought stress on oxidative damages and variations in antioxidants in the two bean varieties Bn-16 (drought-sensitive), Bn-150 (drought-tolerant) to elucidate the antioxidative protective mechanism governing differential drought tolerance. The shoot fresh weight, shoot height, leaf number and area, RWC were reduced with different level of drought stress. However this reduction clearly occurred in Bn-16 (sensitive). Antioxidative enzyme activities, such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, had a greater increase in tolerant genotypes (Bn-150) than in sensitive ones (Bn-16). The level of lipid peroxidation was measured by estimating malondialdehyde content. Lipid peroxidation increased with increasing drought conditions in all genotypes, although Bn-150 was the least affected when compared with the other genotype. Total phenolic and flavonoid content increased in bean genotypes under S2 and S3 conditions. The highest total phenolic and flavonoid contents were attained in Bn-150 subjected to S3 treatment. These results indicated that an antioxidant defence system, osmolytes (such as proline), and secondary metabolites play important roles in common bean (*Phaseolus vulgaris* L.) during drought stress and recovery.

Key words: CAT, Common bean, Flavonoid, MDA, Proline, SOD.

INTRODUCTION

Plants respond and adapt to a variety of environmental stresses in order to survive. Among these stresses drought is one of the most adverse factors on plant growth and crop production. Drought stress induces various biochemical and physiological responses in plants (Shinozaki *et al.*, 1999).

Environmental stresses such as drought enhance the generation of reactive oxygen species (ROS). One oxidative stress product includes ROS, such as the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide, singlet oxygen (1O_2), and hydroxyl radicals (OH \cdot), which leads to an increase in the amounts of toxic oxygen compounds present in plant systems (Morsy *et al.*, 2007; Sharma *et al.*, 2012). Since ROS are associated with several forms of cellular damage, the identification of genes encoding for the enzymes involved in ROS detoxification has been a primary goal in plant stress research (Maggio *et al.*, 2003). Antioxidants and the enzyme systems involved in their synthesis and regeneration have been shown to help protect plants under environmental stress (Wang, 1995). Superoxide dismutase (SOD) are a group of enzymes that accelerate the conversion of $O_2^{\cdot-}$ to H_2O_2 (Hodges *et al.*, 1997). The H_2O_2 is then further scavenged by catalase (CAT) and ascorbate peroxidase (APX) into H_2O and O_2 (Anjum *et*

al., 2012). The oxidized ascorbate is then reduced by glutathione (GSH), generated from oxidized glutathione (GSSG) which is catalyzed by glutathione reductase (GR), at the expense of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Siringam *et al.*, 2011). The reports suggested that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their antioxidant systems.

The purpose of this study was to assess the physiological and biochemical responses, the mechanisms adapted by *Phaseolus vulgaris* L. to tolerate drought, and to assess whether a certain degree of drought stress could enhance the total flavonoid, phenolic and proline content of tolerant and sensitive bean genotypes.

MATERIALS AND METHODS

Two common bean (*Phaseolus vulgaris* L.) genotypes were used in this research: Bn-150 (drought-tolerant) and Bn-16 (drought-sensitive) (Dasgan *et al.*, 2010). Both seeds were obtained from the University of Cukurova, Department of Horticulture. Plants were grown in plastic pots (11 L) containing a peat: perlite (2:1) ration in a

*Corresponding author's e-mail: skusvuran@gmail.com

¹Cankiri Karatekin University Kizilirmak Vocational High School, 18100, Cankiri, Turkey.

²Cukurova University, Faculty of Agriculture, Department of Horticulture, 01330, Adana, Turkey.

greenhouse (temperature: $25\text{ }^{\circ}\text{C} \pm 2$ and relative humidity: $55\% \pm 5$). Starting from 38 d after sowing, three watering treatments were applied: one well-watered treatment [100% of field capacity (FC): S1] and two water-stressed treatments (50 and 0% of FC: S2 and S3, respectively). The plants were subject to drought stress for 14 days. Control plants were grown under non-stress conditions for the same period of time.

Responses of the genotypes to drought were evaluated using some plant physiological (shoot fresh weights, leaf number, leaf area, relative water content) and biochemical parameters such as proline; total phenolic content (TPC), flavonoids, and chlorophyll content; lipid peroxide content (MDA); superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase antioxidative enzyme activities.

The total phenolic content was determined using a Folin-Ciocalteu reagent. The phenolic content of leaves was expressed in milligrams. Gallic acid was used as a standard (Singleton *et al.*, 1999). Total flavonoid content was determined by colorimetric assay (Molina-Quijada *et al.*, 2010; Medina-Juárez *et al.*, 2012). The proline was measured following the methods of Bates *et al.* (1973). The absorbance of the upper phase was spectrophotometrically measured at 528 nm. The SOD was assayed according to Karanlik (2001), by monitoring the superoxide radical-induced nitro blue tetrazolium (NBT) reduction at 560 nm. The CAT activity was determined by monitoring the disappearance of HO. APX activity was determined by measuring the consumption of ascorbate from its absorbance at 290 nm. (Cakmak and Marschner, 1992). The GR activity was determined by measuring the enzyme-dependent oxidation of NADPH from its absorbance at 340 nm. The lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by the thiobarbituric acid (TBA) reaction (Heath and Packer, 1968).

RESULTS AND DISCUSSION

In this study, two common bean genotypes were subjected to three watering treatments for 14 days. A reduction in the fresh weight of common bean seedlings under drought conditions indicated an inhibition of growth.

Water deficiency reduced fresh weight of bean plants compared to the control (Table 1). The fresh weight was decreased by 40 and 60% under moderate stress (S2) in Bn-150 and Bn-16, respectively. However the decrease fresh weight reached 60% in Bn-150 and 84.6% in Bn-16 under S3 conditions compared with control groups. Drought stress had adverse effects, not only on seedling biomass, but also on other morphological parameters such as shoot length, leaf number while S2 and S3 drought conditions caused significant reductions ($P \leq 0.05$) in these descriptions. The shoot length of Bn-150 and Bn-16 dramatically decreased depending on different drought stress levels. The shoot length of the tolerant Bn-150 genotype under S2 and S3 drought conditions decreased by 4.9% and 14.8 %, respectively. However, that of the sensitive Bn-16 genotype under these stress conditions decreased by 47.7 % and 62.4 %, respectively. The decrease may have been due to decline in net assimilation, brought about by decreased leaf water potential. The effect of water stress on yield may be accentuated, since the rate of decline in photosynthesis may be more than that of respiration under water stress (Rao *et al.*, 2008). Also water stress reduces plant growth by reducing cell division and enlargement and causes a decline in transport to the root surface, which leads to a further decrease in plant growth (Pugnaire *et al.*, 1999). An early morphological response to drought stress is the avoidance mechanism through adjustment of plant growth rate such as a reduction in shoot height, basal diameter, and total fresh/dry mass in the two Bn-150 and Bn-16 species used in our experiment. Our study results are consistent with the previous studies (Lei *et al.*, 2006; Kusvuran *et al.*, 2011).

Plant leaf number and area, measured at the end of the stress period differed significantly between the two cultivars (Table 1). In addition, a significant decrease in the leaf number was observed in Bn-16 compared to the control in response to S3 treatment. With drought stress leaf area decreased by 25.7-37.3% in Bn-150, however, this decrease was determined to have been by 41.9-71.1% in Bn-16 under S2 and S3 treatments compared with the control groups, respectively. Emmam *et al.* (2010) reported leaf area of dry beans was reduced when the plants were exposed to drought stress during the vegetative growth stage. One of the factors

Table 1: Changes in the different morphological parameters of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

Genotype		Fresh Weight (g/plant)	Shoot Height (cm/plant)	Leaf Number (number/plant)	Leaf Area (cm ² /plant)	RWC (%)
Bn 150	S ₁	26.66 ±1.15 ^a	27.00 ±2.00 ^{ab}	17.00 ±2.00 ^b	438.65±30.30 ^b	88.33 ±3.21 ^a
	S ₂	16.00 ±1.00 ^b	25.66 ±0.58 ^{ab}	12.33 ±0.58 ^c	325.66±18.46 ^c	77.00 ±3.61 ^b
	S ₃	10.66 ±1.15 ^c	23.00±1.00 ^{bc}	11.00 ±1.00 ^{cd}	274.86±51.03 ^c	66.66 ±4.16 ^c
Bn 16	S ₁	30.33 ±0.58 ^a	36.33 ±2.31 ^a	23.00 ±2.00 ^a	563.23±56.08 ^a	89.33 ±2.08 ^a
	S ₂	11.66 ±1.53 ^{bc}	19.00 ±2.00 ^{bc}	13.33 ±2.52 ^c	327.08±19.40 ^c	61.66 ±9.45 ^c
	S ₃	4.66 ±0.58 ^d	13.66 ±1.15 ^c	8.66 ±1.53 ^d	162.28±16.69 ^d	45.00 ±3.61 ^d

*Results are means ±SD (n=3). The different superscript letters indicate statistically significant differences by a Duncan's multiple range test ($P \leq 0.05$)

associated with many critical aspects of plant growth and survival is specific leaf area which is the ratio of fresh foliage surface area to unit dry foliage mass or projected leaf area per dry mass. Specific leaf area is often positively correlated with seedling potential relative growth rate and leaf net assimilation rate and is reduced under drought conditions (Terzi *et al.*, 2010)

The relative water content (RWC) is a key marker for drought stress study (Table 1). The highest RWC values were obtained in control groups (88-89%). In common bean genotypes exposed to different levels of drought stress, RWC content decreased when compared to the controls. Under S2 and S3 stress conditions, the RWC decreased with the severity of drought stress. The decrease that was observed in the sensitive (Bn-16) genotypes on day 14 of the drought stress was by 49% at S3 compared with that of S1 (control). RWC measurement is a general method used to determine leaf water balance in plants during water deficient periods and estimates the percentage of water present in the leaf as a fraction of the total volumetric water that the leaf can hold at full turgor. When RWC can be maintained in cells and tissues, it allows continuation of the metabolic activity by osmotic adjustment and other traits of adaptation to drought (Slabbert and Krüger, 2014).

The results for chlorophyll for both genotypes under control and drought conditions are given in figure 1. The chlorophyll contents were significantly reduced by increasing the drought stress compared to bean plants grown in control conditions. After 14 days of exposure to the stress conditions, there was a decrease in the chlorophyll contents by 23 % and 60 % in the sensitive genotype; however, this decrease in the tolerant genotype was by 11 %–14 %, respectively. Photo-inhibition and the photo-destruction of pigments may have contributed to such alterations. The decrease in chlorophyll under drought stress is mainly the result of

damage to chloroplasts caused by active oxygen species (Mafakheri *et al.*, 2010).

Proline accumulation is an important physiological index for plant response to drought stress, as well as to other types of stress (Kaymakanova and Stoeva, 2008). The proline concentration in all of the bean genotypes increased after water stress (Fig. 2). After 14 days of water stress, the proline concentration of Bn-150 reached 16.26 and 24.77 $\mu\text{mol g}^{-1}$ FW in the S2 and S3 treatments. However, under the same conditions, proline concentration of the Bn-16 genotype was 18.39 and 19.95 $\mu\text{mol g}^{-1}$ FW, respectively. Drought increased proline content markedly in different drought sensitive and tolerant genotypes: greater proline accumulation in drought tolerant ones were observed, which correlates to drought adaptation. Plants accumulate various soluble substances in their cytoplasm and organelles to obtain osmotic equilibrium during stress exposure. These substances play an immediate role in the osmotic regulation in plants under stress by protecting the membrane integrity, except for providing a positive effect over enzymes. Many studies have proved a positive correlation between stress tolerance and the synthesis of organic substances like glycinebetaine and proline (Asraf and Foolad, 2007, Jia *et al.*, 2015). In this study, the proline content increased with different levels of water stresses. This increase was by 20 -93 % in the tolerant genotype (Bn-150), however this changed to 20-30% in the sensitive genotype (Bn-16) when compared to the control plants. These facts showed that proline is an effective organic substance, not only in functioning as an osmolyte, but also in cellular stabilization and regulation of OH. (Kusvuran *et al.* 2016).

The phenolic compounds in common bean genotypes were changed by water stress (Fig. 3). The total phenolic contents of Bn-150 significantly increased under moderate (S2) and several (S3) water stress conditions when

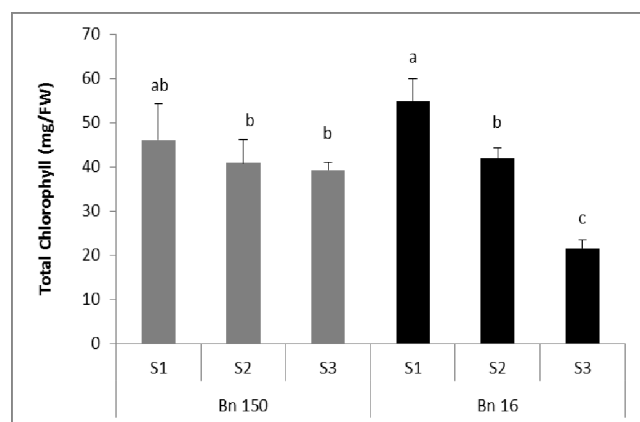


Fig 1: Changes in the total chlorophyll content in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

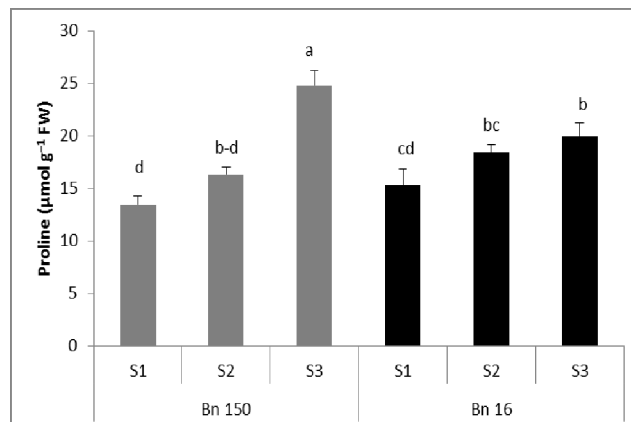


Fig 2: Changes in the proline content in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

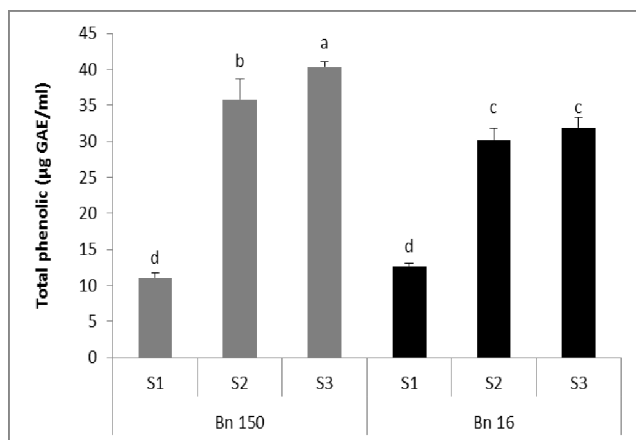


Fig 3: Changes in the total phenolic content in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

compared with the control seedling (223.59% and 265.18%, respectively). Similarly, total flavonoid content increased depending on water stress levels. In this study, total flavonoid content was determined to be 6.37 mgQE/100g (17% increase) and 9.40 mgQE/100g (73% increase) under S2 and S3 water stress conditions, respectively. On the contrary, in Bn-16 total flavonoid content decreased (4.9 mgQE/100g-19% decrease) under S3 treatment (Fig.4). In general, phenolic compounds in plants are produced through the phenylpropanoid pathway, and they can be induced by environmental stresses and elicitor (Yuan *et al.*, 2010). Mansori *et al* (2015) reported that polyphenols represent a large family of plant secondary metabolites and these may act as antioxidants to protect the plant against oxidative stress. Therefore, increase in total phenolic and flavonoid content by treatment of water stress in bean genotypes can be explained by enzyme activation.

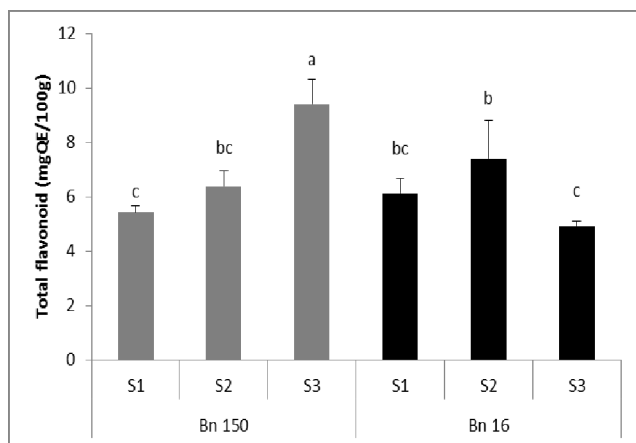


Fig 4: Changes in the total flavonoid content in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

MDA accumulation was determined in the leaves of the bean plant under different conditions (Fig. 5). The results showed that MDA increased significantly under water stress and reached highest levels (3.80 and 5.28 $\mu\text{mol g}^{-1}$ FW) under S3 treatment in Bn-150 and Bn-16, respectively. This change was more clearly observed due to the 794.91% increase in Bn-16 when compared to the control plants. Drought test causes free radical formation in plants. These free radicals lead to irreversible damage to lipids and proteins. Lipid peroxidation destroys the integrity of the cell membranes, and eventually, cell death occurs (Dolatabadian *et al.*, 2008). The lipid peroxidation increase is due to compounds such as superoxide radicals ($\text{O}_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) in chloroplasts. In this study, lipid peroxidation of both genotypes increased with drought stress. However, this reduction was significant in the sensitive genotype (Bn-16) in different drought levels compared to the tolerant genotype (Bn-150). In a previous study (Rosales *et al.*, 2012; Li *et al.*, 2013; Mansori *et al.*, 2015; Kusvuran, 2015), the investigators showed that the MDA levels increased, especially in the susceptible phenotypes, depending on drought stress, and this increase was related to ROS formation. These results may be imputed to varieties in their genotypic ability to scavenge ROS and/or to be protected against their oxidative properties.

Higher plants are sessile therefore are continuously exposed to different environmental stress factors, such as drought, salinity, heavy metals, nutritional disorders, radiation without any protection. Most of these stresses produce certain common effects on plants, like induced oxidative stress by overproduction of reactive oxygen species (ROS), besides their own specific effects. Thus, plants have developed their own specific response(s) against each of these stresses as well as cross-stress response(s). ROS are generated in plant cells by normal cellular metabolism or

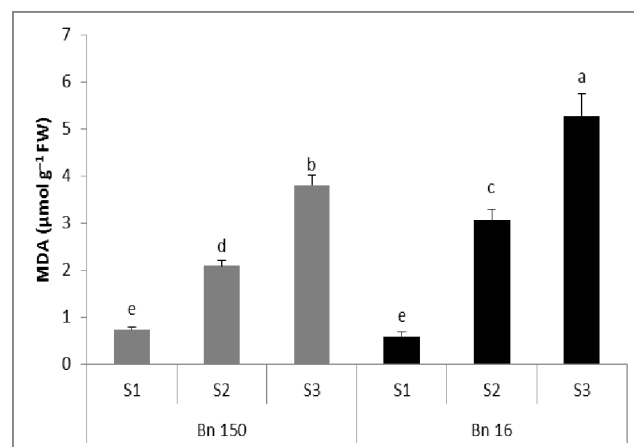


Fig 5: Changes in the MDA content in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

due to unfavorable environmental conditions such as drought, salinity, heavy metals, herbicides, nutrient deficiency, or radiation. Their productions are controlled by various enzymatic and non-enzymatic antioxidant defense systems. Enzymatic antioxidant defense systems, including CAT, APX, POX, SOD, MDHAR, DHAR and GR (Sen, 2012).

Water stress leads to oxidative damage in plants by inducing the production of active oxygen species. The activities of certain enzymes increase as a result of water stress (Dubey, 1999). The antioxidative enzyme (SOD, CAT, GR, and APX) activities were determined to investigate whether the scavenging enzymes have a possible effect on the stress-tolerance mechanisms in the common bean genotypes. The drought stress activated the antioxidant system in bean genotypes. In the S3 treatment, the SOD activity reached $238.22 \text{ U min}^{-1} \text{ mg}^{-1} \text{ FW}$ in Bn-150 (tolerant genotype); however, it only reached $187.74 \text{ U min}^{-1} \text{ mg}^{-1} \text{ FW}$ in the sensitive genotype (Fig. 6). The SOD activities increased with drought stress. This increase was higher in the tolerant genotypes (118.74%) compared to the sensitive genotypes (54.09%) under S3 treatment. Similar trends were observed for CAT activity which increased during the S2 treatment, reaching maximal levels during the S3 treatment (Fig. 7). However, CAT activity of the Bn-150 was significantly higher (227.51-713.13%) than Bn-16 (88.94- 199.82%) during both the S2 and S3 applications. Superoxide radicals that emerge as a result of stress in plant tissues are transformed into hydrogen peroxide (H_2O_2) by the SOD enzyme. The accumulation of H_2O_2 , which results from the canalisation reaction of the SOD enzyme and is a powerful oxidant, is prevented by the ascorbate–glutathione cycle. The hydroxyl radical (OH), which is very reactive and the most toxic oxide, can react with all macromolecules without discrimination. SOD and CAT, by combining their actions, can prevent or decrease

the formation of this oxide (Kusvuran *et al.*, 2016). Our results showed that both genotypes induced SOD and CAT activities upon drought, consistent with the increment in peroxidation levels. At the same time, these enzymatic activities were higher in the drought tolerant genotype than in the drought sensitive genotype.

APX and GR activities were determined in plants that were subjected to stress and plants that were not (Fig. 8-9). The results showed that APX and GR activities increased under stress conditions compared to the control. The highest enzyme activities (APX and GR) were determined to be 102.31 and $15.42 \text{ } \mu\text{mol min}^{-1} \text{ mg}^{-1} \text{ FW}$, respectively, in Bn-150 under S3 treatment. The GR and APX enzymes, which are a part of the defence mechanism of tolerant genotypes against salt, drought and chilling stress, are generally effective in the reduction of hydrogen peroxide to water in chloroplasts and mitochondria, thereby detoxifying them (Scandalios, 1993). APX uses ascorbate as an electron donor to reduce H_2O_2 to water. The main function of APX is the removal of toxic H_2O_2 and thereby protecting plants during oxidative stress. GR activity increased during severe water stress. GR catalyses the NADP-dependent reduction of GSSG to generate reduced glutathione which plays an important role during the removal of dioxygen under stress conditions. The regeneration of GSH from oxidized glutathione (GSSG) by GR is very important since only the reduced form of GSH can take part in the removal of active oxygen species (Slabbert and Krüger, 2014). Increased SOD, CAT, APX, and GR activities in tolerant plants could reduce the amount of damage caused by various stress conditions (Dawood *et al.*, 2014). Hence, it is proposed that these antioxidative enzymes may play important roles in the rapid defence responses of plant cells against oxidative stress.

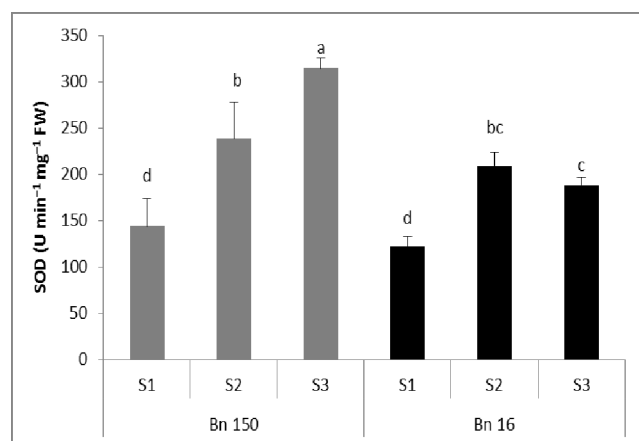


Fig 6: Changes in the SOD activity in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

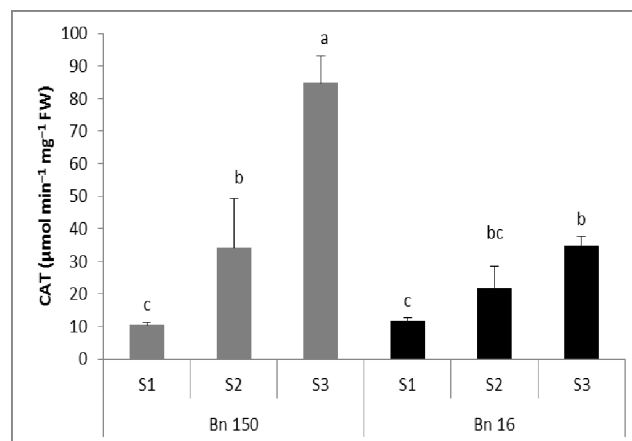


Fig 7: Changes in the CAT activity in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

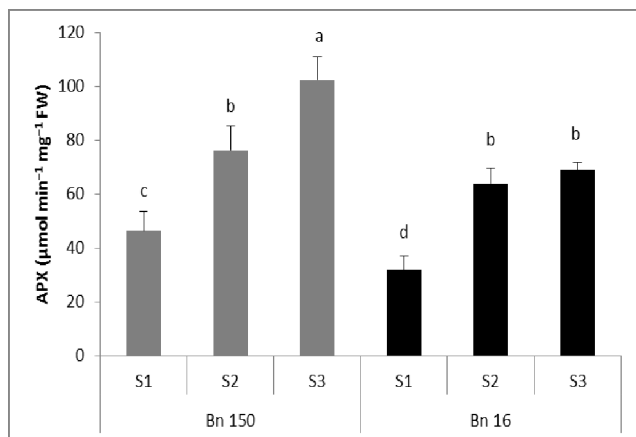


Fig 8: Changes in the APX activity in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

From the observations of physiological and biochemical analyses, we found that *Phaseolus vulgaris* L. genotypes could enhance their ability by up-regulating antioxidative systems and making osmotic adjustments in response to drought stress. It is possible that proline, secondary metabolite accumulation and antioxidative enzyme activities could be used as effective mechanisms for drought tolerance.

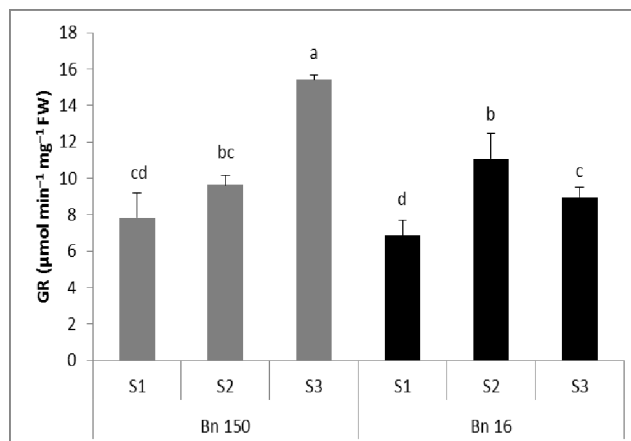


Fig 9: Changes in the GR activity in the leaf of two common bean genotypes treated for different drought stress (S1: 100% of field capacity- Control S2: 50% of field capacity-moderate stress; S3: 0% of field capacity-severe stress)

Gua *et al.* (2006) indicated that the tolerance against stresses such as drought, depends on the response of the antioxidative system. Our results showed that drought stress caused damage in the bean genotypes. However, this damage was at a lesser degree in Bn-150, which uses antioxidative response mechanisms more effectively and has significantly increased levels of enzyme activity.

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