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Biochemical Responses of Some Common Bean (*Phaseolus vulgaris* L.) Genotypes to Drought Stress

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ABSTRACT

In this study, biochemical responses of three common bean genotypes were investigated under three levels of water deficit (75, 50 and 25% FC) at pre- flowering stage. The results showed different effects of drought stress on photosynthetic pigments, total carotenoids, Electrolyte Leakage (EL), Relative Water Content (RWC), lipoxygenase enzyme activity, lipid peroxidation (MDA), total soluble proteins, proline and Abscisic Acid (ABA) content in K-S-31167, GE-288 and NAZ genotypes. Our results showed different patterns in antioxidant enzymes activity including Catalase (CAT), Guaicol PerOxidase (GPOX) and Ascorbate PerOxidase (APX) in K-S-31167 as drought tolerant, GE-288 and NAZ as semi tolerant and sensitive genotypes, respectively. Results showed that drought stress response in common beans is highly genotype dependent.

Keywords: ABA, Enzyme activity, Water deficit.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a major source of protein in developing countries, but plant growth and yield are often reduced by water stress (Pimentel *et al*., 1990). Bean varieties are not very tolerant to severe water stress. It was reported that about 60% of common bean yield decreases under drought stress (Singh, 1995). Thus, breeding of plant species manifests that drought tolerance is a tool for economic and efficient elimination of agricultural problems in arid regions. Stress result in several changes in the subjected plants including leaf Relative Water Content (RWC), leaf Electrolyte Leakage (EL), photosynthetic pigments, carotenoids, etc., which reduce the efficiency of photosynthesis and yield (Lonbani and Arzani, 2011). The primary sign of stress in plants is an overproduction of Reactive Oxygen Species (ROS), such as superoxide (O_2^-), Hydroxyl radical (•OH), Hydrogen peroxide (H_2O_2), and single Oxygen ($^1O_2$) (Apel *et al*., 2004). ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins, and Lipid PerOxidation (LPO) (Foyer *et al*., 2005). Lipid peroxidation is known as the most destructive process that occurs in every living organism. LipOxygenase enzyme (LOX, EC, 1.13. 11. 12) is multifunctional in the plant kingdom and involved in many physiological processes, such as flowering, seed germination and plant growth and development (Hildebrand *et al*., 1991). This enzyme catalyzes deoxygenation of multiple unsaturated membrane fatty acids and produces toxic molecules such as MaloneDiAldehyde (MDA). The increase of MDA content in seedlings of licorice (*Glycyrrhizin uralensis*) under drought and salt stresses was reported by Pan *et al*, (2006). Stress-induced accumulation of ROS can counteract antioxidant enzyme systems such as SuperOxide Dismutase (SOD), Ascorbate Peroxidase (APX),

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Guaiacol PerOxidase (GPOX), Catalase (CAT) and non-enzymatic and low molecular weight metabolites such as vitamin E, carotenoids and flavonoids (Mittler *et al*., 2004). Change in antioxidant enzyme activity was reported in various kinds of stresses. Many researchers reported a change in activity of APX, GPOX, and CAT enzymes under drought stresses in different kinds of crop plants (Badawi *et al*., 2004; Sreenivasulu *et al*., 2004). Plant phytohormones like ABA have important roles in stress signaling. ABA change under drought stress was reported by Nayyar and Gupa (2006). In addition, proline as osmolyte could be added to non-enzymatic antioxidants that microbes, animals, and plants need to counteract the effects of ROS formation (Chen *et al*., 2005). Plant responses to water stress are very complex, and may be influenced by changes in some factors including duration and severity of drought stress, stage of plant maturity, previous environmental conditions, and their interactions. Understanding plant responses to the external environment is important, and is a fundamental part of introducing stress tolerant crops (Farahani *et al*., 2011). Despite a large number of studies on drought stress, the primary effects of water deficit at biochemical levels are not well understood (Chaves *et al*., 2003; Chaitanya *et al*., 2003). Therefore, the main aim of this study was to investigate some biochemical responses of drought tolerant and sensitive common bean genotypes at the pre-flowering stage to drought stress.

MATERIALS AND METHODS

Greenhouse Experiments

Based on two years field experiments, among 90 genotypes, two genotypes, GE-288 and NAZ, were screened as semi tolerant and sensitive to drought stress, respectively (Keshavarz Nia *et al*, 2013). Additionally, K-S-31167 genotype, as drought tolerant, was kindly supplied by Legume Research Institute (Khomein, Arak-Iran). Seeds of each genotype were planted in plastic pots (20 cm high, 25 cm top and 12 cm bottom diameter) containing 3 kg clay loam soil and grown in a greenhouse at a temperature of 27±2°C, relative humidity of 60±2%, and a light intensity of 400 µmol m^{-2} s^{-1} for 50 days in factorial experiment based on a completely randomized design with three replicates. Pots were well watered (%100 FC) by daily irrigation until the pre-flowering stage of common bean growth. At this stage, irrigation regimes were performed based on Field Capacity (FC). *FC* was maintained stable by weighting and adding the needed water. Plants were subjected to four irrigation regimes including normal irrigation (%100 FC), 75, 50, and 25% *FC*. Leaves were harvested after seven days, frozen in liquid nitrogen, and stored at -80 °C for further analysis.

Sample Preparation for Biochemical Assay

After water stress treatments, leaves of each plant were harvested, washed with distilled water and homogenized in 0.16M Tris buffer (pH= 7.5) at 4°C. Then, 0.5 mL of total homogenized solution was used for protein determination according to the method described by Bradford (1976).

Electrolyte Leakage Measurement

In order to measure the Electrolyte Leakage (EL), plant material (0.3 g) was washed by placing in tubes with 15 mL of deionized water and incubated for 2 hours at 25°C. Subsequently, the electrical conductivity of the solution (L1) was determined. Samples were then autoclaved at 100°C for 30 minutes, and the final conductivity (L2) was measured at 25°C. Then, the *EL* was calculated by the following equation (Lutts *et al*., 1996):

$$EL\ (\%) = (L_1/L_2) \times 100 \quad (1)$$

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Relative Water Content (RWC) Measurement

Relative water content was measured by soaking leaf samples (0.5 g) in 100 mL of distilled water at 20°C in the dark for 16-18 hours. The turgid leaves were quickly blotted dry prior to the turgid weight measurement. The dry weight of leaves was determined after oven-drying at 70°C for 72 hours. *RWC* was calculated according to Schonfeld *et al.* (1988), using the following equation:

$$RWC = [(Fresh\ weight - Dry\ weight)/(Turgid\ weight - Dry\ weight)] \times 100 \quad (2)$$

Chlorophyll and Carotenoid Contents

Leaf samples (0.1 g) were homogenized in a mortar with 80% acetone. The extract was centrifuged at 5,000×g for 5 min. The absorbance of the supernatants was recorded at 663, 645 and 450 nm. Total Chlorophyll (Chl), chlorophyll a, chlorophyll b and Carotenoid (CRT) contents were estimated according to Arnon (1949) and Jaspars (1965).

Lipid Peroxidation

Lipid peroxidation was measured in the term of MDA content (ε= 155 mM^{-1} cm^{-1}), a product of lipid peroxidation, following the method of Heath and Packer (1968). Leaf samples (0.5 g) were homogenized in 10 mL of 0.1% (w/v) TriChloroacetic Acid (TCA). The homogenate was centrifuged at 15,000×g for 5 minutes. Four mL of 0.5% (w/v) ThioBarbituric Acid (TBA) in 20% (w/v) TCA was added to the one mL aliquot of the supernatant. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000×g for 10 minutes, the absorbance of the supernatant was recorded at 532 and 600 nm. The MDA content was expressed as nmol MDA per one gram fresh leaf weight.

Proline Content

Free proline content was determined following the method of Bates *et al*. (1973). Leaf samples (0.5 g) were homogenized in 5 mL of sulphosalicylic acid (3%) using mortar and pestle. Two mL of extract was transferred to the new test tube, then, two mL of glacial acetic acid and 2 mL of ninhydrin reagent were added. The reaction mixture was boiled in a water bath at 100°C for 30 minutes. After cooling the reaction mixture, six mL of toluene was added and then transferred to a separating funnel. By vortex, the chromophore-containing toluene was separated and absorbance read at 520 nm in a spectrophotometer against toluene blank. The concentration of proline was estimated by referring to a standard curve of proline.

Total Soluble Protein Determination

Fresh leaf material (0.2 g) was homogenized in the liquid nitrogen and transferred to the 15 mL tubes, then 2.5 mL extract buffer (0.1M Tris and 30% glycol) was added to the sample. It was centrifuged at 15,000×g for 15 minutes at 4°C. Supernatant was saved at -80°C for further analysis. Total soluble protein determination was performed based on Bradford (1976).

Enzyme Assays

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed by the method of Nakano and Asada (1987). A decrease in the absorbance of ASC at 290 nm (ε= 2.8 mM^{-1} cm^{-1}) was measured in 1 mL of a reaction mixture containing an aliquot of the supernatant, 0.25 mM ASC, and 5 mM hydrogen peroxide in 50 mM phosphate buffer (pH 7.0).

Guaiacol PerOxidase (GPOX, EC 1.11.1.7) activity was measured by monitoring the increase in absorbance at 470 nm in 100 mM potassium phosphate buffer

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(pH 7.0) containing 0.1 mM EDTA, 5 mM guaiacol, 15 mM H_2O_2, and 50 µL of the enzyme extract (Urbanek *et al*., 1991). GPOX activity was calculated using and extinction coefficient of 26.6 (mM cm) for tetraguaiacol at 470 nm.

Catalase (CAT, EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 (ε= 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min (Aebi, 1983). The reaction mixture 1 mL in volume contained 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, and 10 µL of the enzyme extract.

LOX (LOX, E.C. 1.13.11.12) enzyme activity measured according to the Ederli *et al*. (1997) method. As 300 mg of frozen leaf samples was homogenized in 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 0.1 mM Phenyl Methyl Sulfonyl Fluoride (PMSF), 2% (w/v) polyvinylpyrrolidone (PVP), 1% (v/v) glycerol, and 0.1% (v/v) tween 20. The extract was centrifuged at 15,000×g for 20 minutes and the supernatant was immediately used to assay for lipoxygenase activity. LOX activity was spectrophotometrically measured at room temperature by the addition of 1 mM linoleic acid in 0.1M sodium acetate buffer (pH 5.6) to the extract and by reading the increase in absorbance at 234 nm.

ABA Assay

HPLC analysis of abscisic acid was performed based on Lee (2010) method. Leaf Tissue (1 g) homogenized at 4°C in 2 mL 80% methanol. Then, 0.01 g polyvinylpyrrolidone and 0.01 g vitamin C were added and homogenized at 4°C. After 10 minutes, homogenized solution was centrifuged at 4,000×g for 15 minutes. Then, upper layer was separated and pH was increased to pH= 8. This stage was repeated. Finally, pH was reduced to pH= 2.5 and 10 mL acetate ethyl was added. Acetate ethyl was evaporated and then 1 mL 80% methanol and 0.1 mL acetic acid were added to the pellet and homogenized. For quantitative assay, 20 µL of final solution was injected to small columns (10 cm length and 3 mm internal diameter) filled with 3 g reverse phase material of Chrompack-Nederland (CP Micros-Phere) attached to HPLC model Unickam- Crystal 200. The detector was UV type with absorption at 260 nm and the solvent flow was 4 mL/min (methanol: acetic acid 97/3 v/v).

Statistical Analysis

Analysis Of Variance (ANOVA) was performed with multiple comparison tests, using SAS software (version 9.2) on three replicates. Statistical significance was determined at the 1% level ($\alpha \leq 0.01$).

RESULTS

Chlorophyll and Carotenoids Content

Effect of water stress regimes including 75, 50, and 25% FC on the chlorophyll and carotenoids content was evaluated. Results showed an increase followed by a decrease in photosynthetic pigments (Figure 1, a-d). In comparison with the other water stress regimes, 25% FC caused the maximum change in chlorophyll and carotenoids. Drought sensitive genotype, NAZ, manifested maximum change in photosynthetic pigments in comparison with both drought tolerant genotype, K-S-31167, and drought semi-tolerant genotype, GE-288 (Figure 1, a-d).

Effects of Water Stress on Lipid Peroxidation

Under stress conditions, lipid peroxidation was significantly activated in genotypes. On the subject of water stress regimes, 25% FC caused the maximum increase in the MDA content (Figure 2, a). In the case of genotypes, the ones more tolerant to water

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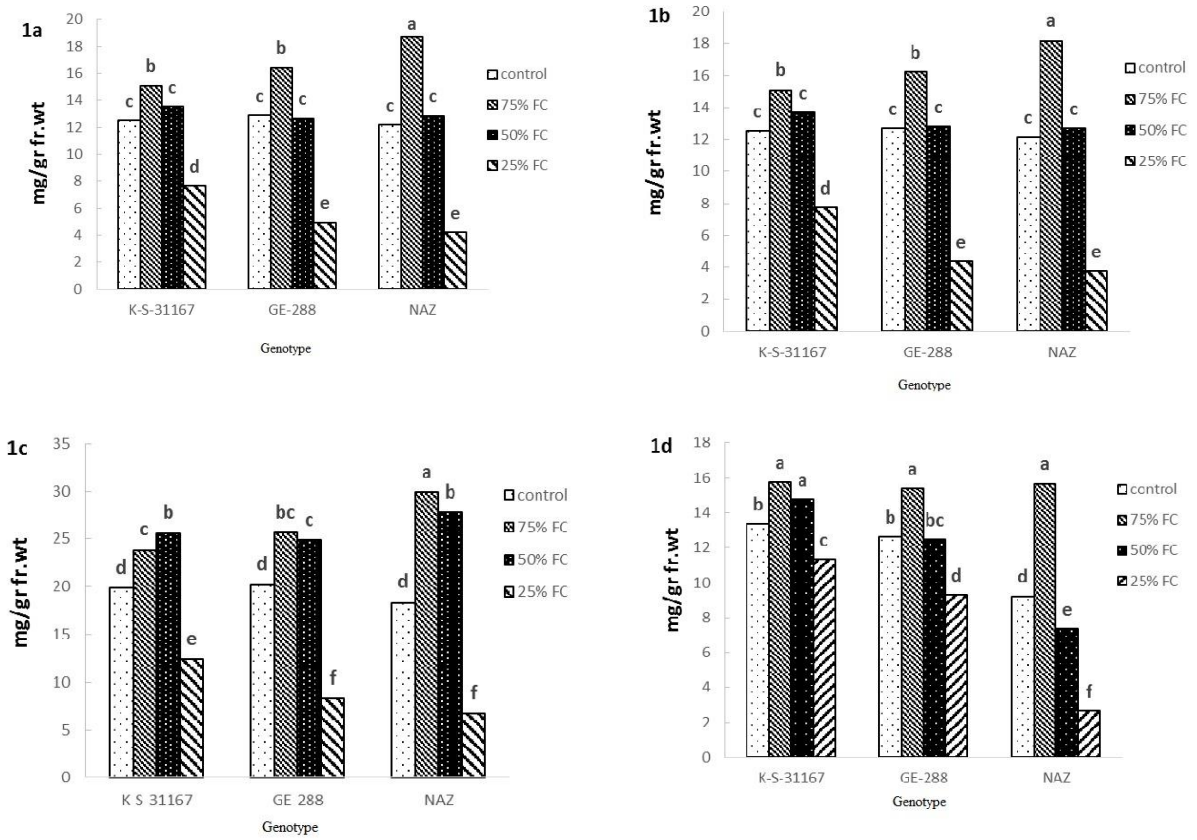

Figure 1. Effect of drought stress on chlorophyll a (1a), chlorophyll b (1b), total chlorophyll (1c) and carotenoids (1d) in K-S-31167(drought tolerant), GE.288 (drought semi tolerant) and NAZ (drought sensitive) common bean genotypes. (Columns with same letters have no significant difference at P< 0.01 based on Duncan test).

stress, GE-288 and K-S-31167, showed more similar pattern in MDA change in comparison with a sensitive genotype, NAZ.

Effects of Drought Stress on Electrolyte Leakage (EL)

Change in electrolyte leakage was both genotype and stress conditions dependent. In comparison with the other water stress regimes, 25% *FC* caused the maximum increase in *EL*. On the subject of 75 and 50% *FC*, K-S-31167 and GE-288 genotypes showed more similarity in the pattern. The amount of *EL* increase was higher in NAZ genotype than K-S-31167 and GE-288 genotypes (Figure 2, b). Genotypes comparison showed that from the tolerant genotype to sensitive genotype by increasing water stress a sharp increase happened in *EL* percentage. So, NAZ genotype showed the maximum change in *EL* in comparison with the other genotypes.

Effect of Water Stress on RWC

Change in *RWC* content was affected based on genotype and water stress conditions. In comparison with the other water stress regimes, 25% *FC* caused a remarkable decrease in *RWC* content (Figure 2, c). In the case of 75 and 50% FC, in spite of a significant decrease in *RWC* content of NAZ genotype, K-S-31167 and GE-288 genotypes showed no significant change in comparison with the control. Results showed no significant

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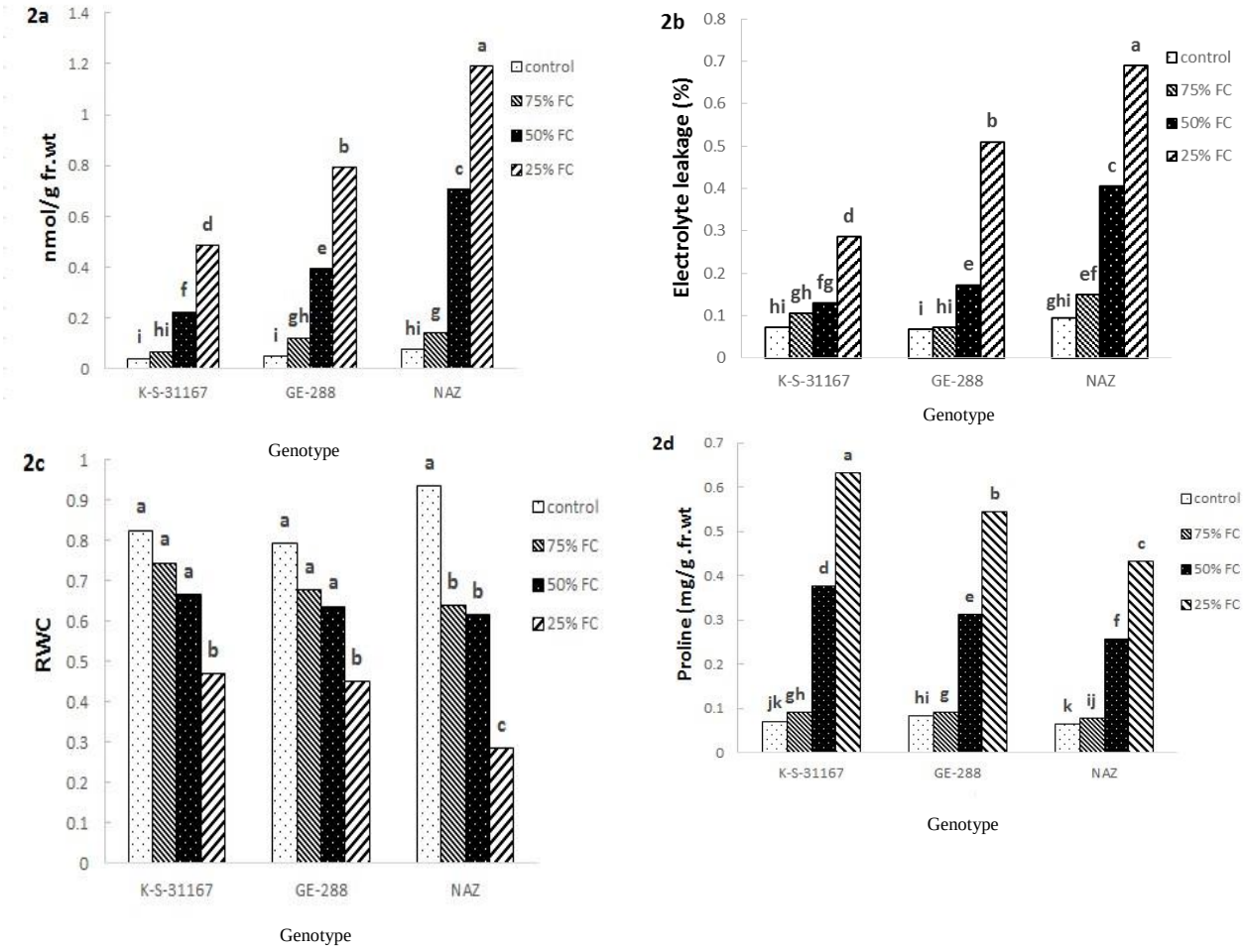

Figure 2. Effect of drought stress on MaloneDiAldehyde (MDA) (2a), Electrolyte Leakage (EL) (2b), Relative Water Content (RWC) (2c) and proline content (2d) in K-S-31167, GE.288, and NAZ, common bean genotypes. (Columns with same letters have no significant difference at P< 0.01 based on Duncan test).

difference between K-S-31167 and GE-288 genotypes, but NAZ genotype was different with the other genotypes by consistent reduction trend in *RWC* content.

Effect of Water Stress on Proline Content

Responses of genotypes to water stress treatments were a constant increase in proline content. In all three genotypes, with increasing levels of water stress, proline content was constantly increased (Figure 2, d). Each genotype showed maximum proline content at 25% *FC* in comparison with the control. Our results showed genotype dependent proline content. In control conditions, there was no significant difference in proline content between K-S-31167and NAZ, but GE.288 showed significantly more proline content. At 75% FC, there was no significant difference between K-S-31167 and GE.288 genotypes but these two genotypes showed significantly more proline content compared to NAZ genotype. At 50 and 25% *FC*, a significant difference in proline content was observed among the three genotypes while K-S-31167 genotype had maximum proline content at 50 and 25% *FC* (Figure 2, d).

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Effect of Water Stress on Total Soluble Proteins

In K-S-31167 genotype, there was no significant difference at 75 and 50% *FC*, but a significant decrease was observed at 25% *FC* (Figure 3). In GE.288 genotype, there was a significant increase in total soluble proteins at 75 and 25% *FC* in comparison with the control, but there was no significant difference at 50% *FC* compared to the control. In NAZ cultivar, a significant increase was observed in 75 and 50% *FC*, but there was no significant difference in total soluble proteins at 25% *FC* in comparison with the control (Figure 3).

Enzyme Activity under Water Stress

In K-S-31167 genotype, there was no significant increase in APX enzyme activity at 75% *FC* in comparison with the control (Figure 4, a). A significant increase in the enzyme activity was measured at 50 and 25% *FC* compared to the control. Ge-288 genotype showed steadily increase in the enzyme activity by increasing water deficit (Figure 4, a). NAZ genotype showed different APX enzyme activity. This genotype showed no significant difference, increase and finally decrease in the enzyme activity at 75, 50 and 25% FC, respectively. By reducing soil water content, GPOX enzyme activity in all three genotypes showed a significant increase, compared to the control. GPOX enzyme activity in NAZ cultivar was increased up to 50% *FC* but significantly decreased at 25% *FC*, interestingly, still showed more activity than the control. CAT enzyme activity was different among genotypes (Figure 4, c). This enzyme showed an increase, no significant change, and, finally, decreases in activity at 75, 50 and 25%. In Ge-288 genotype, a significant increase in CAT enzyme activity was observed at 75 and 50% *FC* (Figure 4, c). There was no significant difference in the enzyme activity at 25% *FC*, in comparison with the control (Figure 4, c). In NAZ genotype, at 75 and 50% *FC*, the enzyme activity increased significantly, but at 25% FC, the enzyme activity showed no significant difference in comparison with the control. LOX enzyme activity was different among genotypes. In all three genotypes, there was no different in LOX enzyme activity at 75% *FC*, but a significant increase in the enzyme activity was observed at 50 and 25% *FC* in comparison with the control (Figure 4, d). Comparison among genotypes showed a

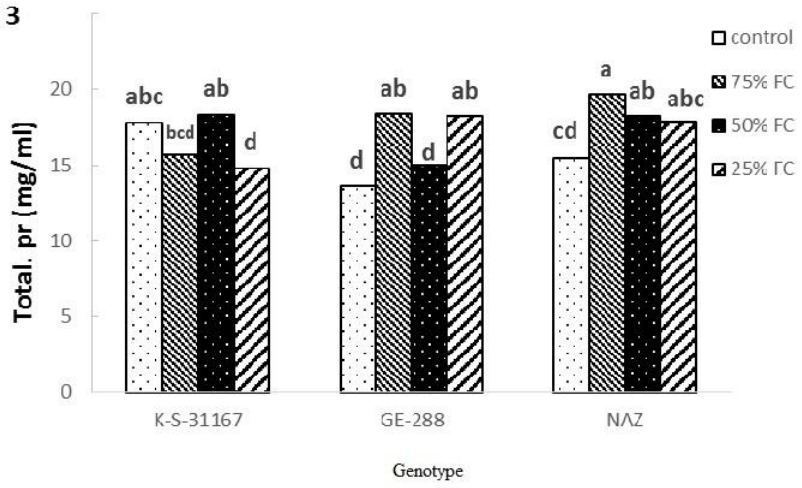

Figure 3. Total soluble protein in K-S-31167, GE.288, and NAZ common bean genotypes under drought stress. (Columns with same letters have no significant difference at P< 0.01 based on Duncan test)

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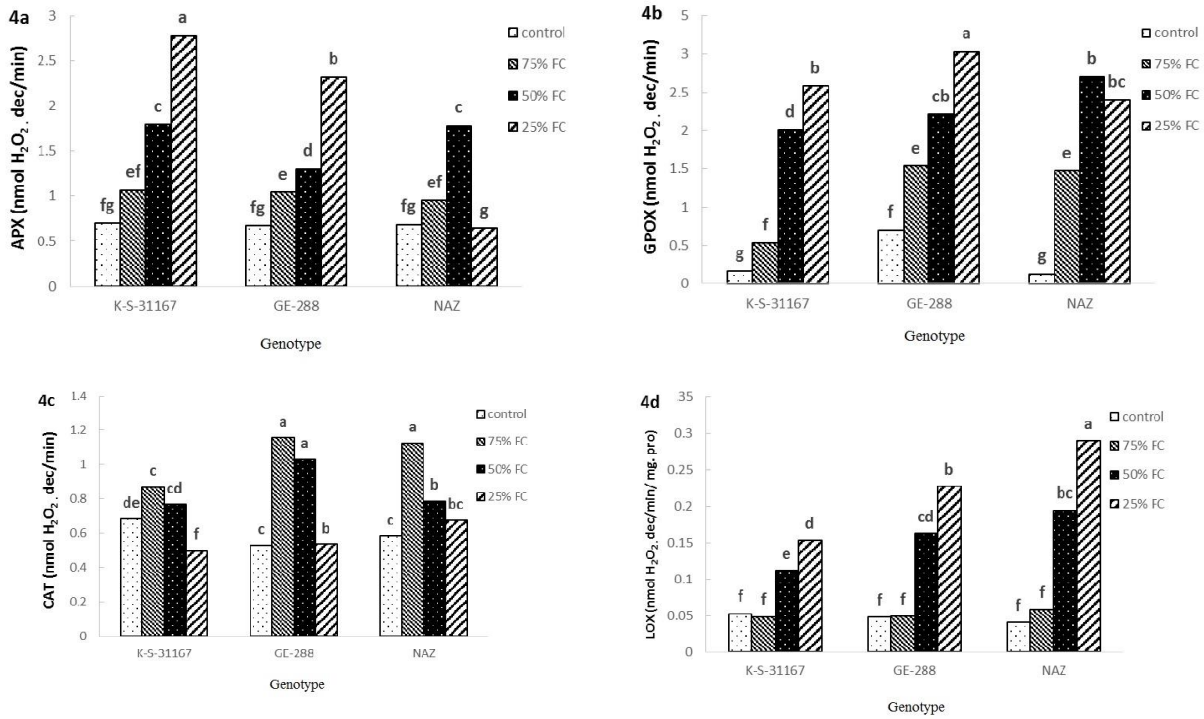

Figure 4. Effect of water stress on ascorbate peroxidase (4a), gauicole peroxidase (4b), catalase (4c) and lipoxigenase (4d) in K-S-31167, GE.288 and NAZ common bean genotypes under drought stress. (Columns with same letters have no significant difference at P< 0.01 based on Duncan test).

significant increase in enzyme activity followed by increase in water stress severity from drought tolerant genotype (K-S-31167) to drought sensitive genotype (NAZ).

As antioxidant response mechanism is complex and genotype dependent, for a better understanding of antioxidant activity, we drew new graphs for each genotype (Figure 5, a-c). Our result showed that APX and GPOX enzymes activities relatively resembled in each genotype. In K-S-31167 and GE.288 genotypes, this activity increased but in NAZ genotype, the activities increased and then decreased (Figure 5, a-c). CAT enzyme showed an increase and then decrease in activity in all three genotypes (Figure 5, a-c). LOX enzyme showed a constant increase in activity (Figure 5, a-c).

Effect of Stress on ABA

Our results showed steady increases in ABA content of three genotypes by increase in water stress (Figure 6). On the subject of water stress regimes, 25% *FC* caused the highest ABA accumulation in the genotypes. Based on our results, K-S-31167 genotype (as drought tolerant) showed more ABA content than GE-288 and NAZ genotypes in all four experimental treatments (Figure 6).

DISCUSSION

Plant responses to water stress are very complex, and may be influenced by changes in some factors including degree and time of

encounter to drought stress, stage of plant maturity, previous environmental condition, and their interactions. Understanding plant responses to the external environment is of great importance, and is also a fundamental part of making stress tolerant crops (Farahani *et al*., 2011). Decrease of *RWC* is one of the early symptoms of water deficiency in plant tissues (Valentovic *et al*.,

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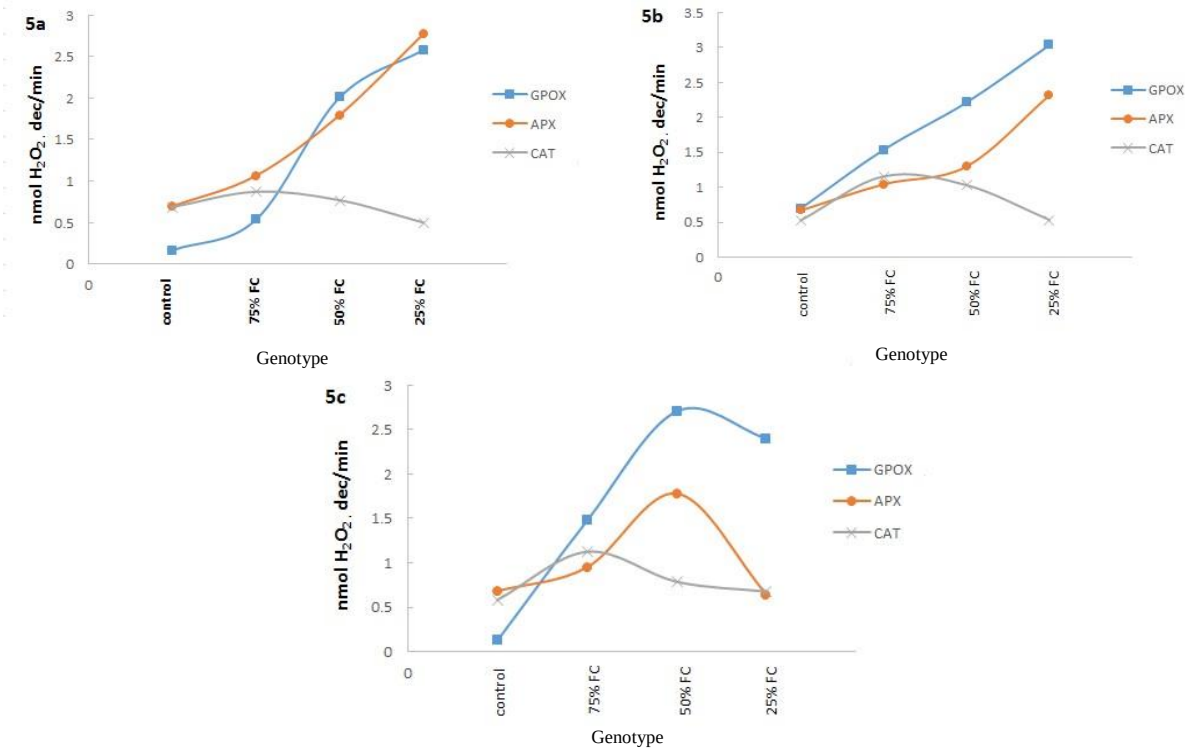

Figure 5. Antioxidant enzyme activity pattern in K-S-31167 (5a), GE.288 (5b) and NAZ (5c) common bean genotypes under water stress.

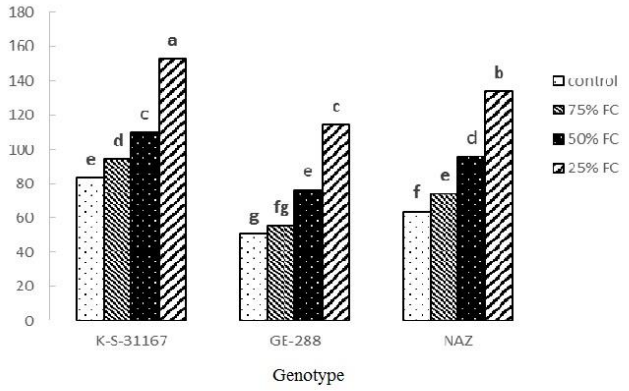

Figure 6. Effect of drought stress on ABA content of K-S-31167, GE-288, and NAZ common bean genotypes. (Columns with the same index have no significant difference at P< 0.01 based on Duncan test).

2006) and many researchers have reported decrease in *RWC* under drought stress (Siddique *et al*., 2000; Valentovic *et al*., 2006, Ghaderi *et al*., 2011). Siddique *et al* (2000) reported 43 percent (from 88 to 45%) *RWC* reduction under water stress in four cultivars of bread wheat. Mationn *et al*. (1989) presented a similar report as regards a drop in *RWC* in the tolerant and sensitive cultivars of barley. Our results showed lower *RWC* change in K-S-31167 and GE.288 genotypes in comparison with NAZ genotype. It is reported that high relative water content is a resistant mechanism to drought, and high relative water content is the result of more osmotic regulation or less

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elasticity of tissue cell wall (Ritchie *et al*., 1990). Valentovic *et al*. (2006) reported that the Electrolyte Leakage (EL) from a sensitive maize cultivar increased about 11% to 54% more than that of a tolerant cultivar after water stress treatment. Present results expressed lower *EL* change in K-S-31167 and GE.288 genotypes in comparison with NAZ genotype. It is generally accepted that the maintenance of cell membrane integrity and stability under water stress conditions is a major component of drought tolerance in plants (Bajji *et al*., 2002). Often, plant cell membranes are subjected to changes associated with increase in permeability and loss of integrity under environmental stresses (Blokhina *et al*., 2003). Therefore, the ability of cell membranes to control the rate of ion movement in and out of cells is used as a test of damage to a great range of tissues. It is well known that drought enhanced free radicals production, which induces lipid peroxidation of biomembranes, reflect stress-induced damage in tissues. The content of MDA is often used as an indicator of the lipid peroxidation extension resulting from oxidative stress (Smirnoff, 1993). The increase of MDA in genotypes under drought stress conditions suggests that the repairing mechanisms do not keep pace with damage and water deficit can influence the composition and turnover of membrane lipids. In particular, a peroxidation of thylakoid glycolipids and the subsequent production of diacylglycerol, triacylglycerol and free fatty acids occur (Smirnoff, 1993). Turkan *et al*. (2005) found that MDA content was lower in the leaves of drought-tolerant *Phaseolus acutifolius* Gray than drought-sensitive *P. vulgaris* L. In general, MDA change in K-S-31167 as drought tolerant genotype was lower than NAZ as drought sensitive genotype and in agreement with Turkan *et al*. (2005). Our results showed constant *EL*, high *RWC* and low MDA content under moderate water stress in K-S-31167 and GE.288 genotypes that indicate efficient cellular cell wall stability compared to drought sensitive NAZ cultivar. Our data supported a direct relation between the degree of drought stress and proline content. Results showed that K-S-31167 as drought tolerant genotype had a maximum accumulation of proline under severe water deficit status. The role of proline in response to osmotic stress include a very important part in the biosynthesis of cell-wall matrix proteins, such as extensins, that have important roles in cell morphology and provide mechanical support for the cell under stressed conditions (Nanjo *et al*., 1999). A neglected aspect of proline metabolism concerns its importance during the stress relief phase. In fact, its rapid oxidation is equally important in recycling the free amino acid accumulated during the stress conditions with the production of reducing power, amino nitrogen and energy, all needed in the restoration of cellular homeostasis during the recovery from osmotic stress (Verbruggen *et al*., 1996). Finally, its unique capacity to consume high levels of reductants during its biosynthetic pathway, paralleled by high-energy output during its degradation justifies the main role played in many different species as a great resource either in the acclimation to stress or in plant recovery upon relief from it (Hare and Cress, 1997). Another plant response to drought stress is a change in photosynthetic pigment content. Photosynthetic pigments play important roles in harvesting light. The content of both chlorophyll a and b changed under drought stress (Farooq *et al*., 2009). The carotenoids play fundamental roles and help plants to resist drought stress (Jaleel *et al*., 2009). Resistant cultivar to drought and thermal stress conditions had high chlorophyll content (Sairam *et al*., 2002). Pastori and Trippi, (1992) expressed that resistant genotypes of wheat and corn had higher chlorophyll content than sensitive genotypes under the oxidative stress. Our results showed an increase and then decrease in chlorophyll and carotenoids content in experimental treatments. The higher amount of pigment is attributed to the accumulation of solutes in the cell sap through passive accumulation resulting from reduced cell size (Ranjbarfordoei *et al*., 2000). Decrease

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in pigment contents in drought-stressed plants might possibly be due to changes in the lipid-protein ratio of pigment–protein complexes or increased chlorophyllase activity (Iyengar and Reddy, 1996; Parida *et al*., 2004). There are several reports of change in protein synthesis or degradation of protein in plant species in response to drought. Drought stress results in a decrease in total soluble proteins in sunflower (Abdel-Nasser and Abdel-Aal, 2000; Valifard *et al*., 2012). Accumulation of dehydrin protein family in a wide range of plant species under water stress has been also reported (Cellier *et al*., 1998). Our data indicated that increase or decrease of total protein in plants subjected to drought stress was genotype-dependent to some degree. However, quantitative changes in protein content may be responsible for adjustments in metabolic pathways under stress condition (Sarhan and Perras, 1987). Accumulation of proteins in stressed plants may provide a storage form of nitrogen that is re-utilized when the stress is over, and may play a role in osmotic adjustment (Amini and Ehsanpour, 2005). Increase in soluble proteins may be also due to the synthesis of osmotin-like proteins or structural proteins and, in particular, due to the synthesis of those proteins that are involved in the modification of cell wall (Amini and Ehsanpour, 2005). Stress may lead to stomatal closure, which reduces CO_2 availability in the leaves, and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of ROS and induce oxidative stress (Gossett *et al*., 1994). Accumulation of the ROS, H_2O_2, induced by various environmental stresses result in the combined activity of APX, CAT and GPOX. Our results are also in agreement with heat stressed mustard (Dat *et al*., 1998) and drought stressed pea (Moran *et al*., 1994), which exhibited a significant increase in endogenous APX, GPOX and increase followed by a marked decline in CAT. APX plays a crucial role in the management of ROS in higher plants during stress due to its higher affinity for H_2O_2 than CAT. In the present research, APX activity increased during experimental treatments, similarly, it was shown that APX activities were also enhanced during drought stress in cotton (Ratnayaka *et al*., 2003). Our results show an increase and then decrease in CAT activity. APX, GPOX and CAT are crucial enzymes for H_2O_2 scavenging; in fact, the high activities of APX, GPOX and CAT would have contributed to the small increase of H_2O_2 under the imposed drought conditions. Under drought condition, CAT activities can increase, decrease, or remain unchanged in different genotypes, which could be related to the different genetic background (Zhang and Kirkham, 1996). On the other hand, a significant decrease in CAT activity under water stress conditions (Figure 2, c) may have contributed to the higher H_2O_2 accumulation. Du and Klessig (1997) proposed that CAT may be inactivated by binding to salicylic acid or to other cellular components, but the relevance of these data towards physiological conditions is difficult to assess. Our results are in agreement with Ye *et al.* (2000) who reported the activating role of drought stress on the specific LOX isoenzymes and membrane lipid peroxidation in Arabidopsis plant. The high degree of lipid peroxidation (MDA) can play as secondary messenger role by activating specific transcription factors that can induce some drought-stress-associated genes (Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997). Another response of stressed plants is a change in ABA phytohormone. Our results show an increase in ABA content in all three genotypes. Many researchers reported ABA accumulation under drought stress (Nayyar and Gupta, 2006). Our results showed higher ABA in drought tolerant genotype (K-S-31167) in agreement with Beebe *et al* (2003) that reported higher ABA content in common bean drought tolerant genotypes under drought stress. ABA has a crucial role in stress signaling, inducing antioxidant gene expression like APX and SOD, stomata closure (Guan *et al*, 2009). Therefore, higher

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RWC and more powerful anti-oxidative enzyme activity in K-S-31167 may be the result of higher ABA in this genotype. Many researchers conclude one or more complex mechanism to cope with drought stress in plants (Noctor *et al*., 1998; Reddy *et al*., 2004). These results indicated that plant water stress responses are complicated and depend on genotype and stress type. Biotechnology, as a new tool to engineer crops against drought stress, needs biochemical information about responses to stress, so, the mentioned results can be useful to achieve some new information about biochemical responses of drought tolerant and sensitive genotypes.

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پاسخ های بیوشیمیایی برخی از ژنوتیپ های لوبیا قرمز (*Phaseolus vulgaris* L.) به تنش خشکی

م. ممبنی، و ع. عباسی

چکیده

در این مطالعه پاسخ های بیوشیمیایی سه ژنوتیپ لوبیا قرمز تحت سه سطح تنش خشکی (۷۵٪، ۵۰٪ و ۲۵٪ ظرفیت زراعی) در مرحله قبل از گلدهی مورد بررسی قرار گرفت. نتایج تاثیرات مختلف تنش خشکی بر روی رنگدانه های فتوسنتزی، کاروتنوئید کل، نشت یونی(EL)، محتوای نسبی آب (RWC)، فعالیت آنزیم لیپوکسی ژناز،پروکسیداسیون لیپید(MDA) ، پروتئین کل محلول، پرولین و اسید آبسزیک را در ژنوتیپ های K-S-31167 ، GE-288و NAZ را نشان داد. نتایج ما الگوهای مختلف فعالیت آنزیم های آنتی اکسیدانت کاتالاز(CAT) ، گایوکول پراکسیداز (GPOX)، و اسکوربات پرکسی داز (APX) را در ژنوتیپ های K-S-31167 بعنوان ژنوتیپ مقاوم، GE-288 و NAZ به ترتیب بعنوان ژنوتیپ های نسبتاً مقاوم و حساس را نشان داد. نتایج نشان داد که پاسخ به تنش خشکی در لوبیا به نسبت بالایی وابسته به ژنوتیپ است.

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