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Full Length Research Paper

Use of biochemical indices and antioxidant enzymes as a screening technique for drought tolerance in Chickpea genotypes (*Cicer arietinum* L.)

Rahbarian Raheleh[1]*, Khavari-Nejad Ramazanali[2], Ganjeali Ali[3], Bagheri Abdolreza[4], Najafi Farzaneh[2] and Roshanfekr Masoud[5,6]

[1]Department of Biology, Islamic Azad university of Neyshabur branch, Iran.
[2]Department of Biology, Tarbiat moallem University of Tehran, Iran.
[3]Department of Biology, Ferdowsi University of Mashhad, Mashhad, Iran.
[4]College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
[5]Department of science, Farhangian University, Daneshvar Tarbiat moalem of Neyshabur, Iran.
[6]College of science, Islamci Azad University of Neyshabur branch, Iran.

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In order to evaluate the physiological and biochemical traits regarding drought tolerance and further to determine the best criteria for screening and identification of drought tolerant chickpea genotypes, an experiment was conducted in controlled conditions in the Plant Science Research Center of Ferdowsi University of Mashhad in Iran. In this study, two tolerant genotypes (MCC392 and MCC877) and two susceptible genotypes (MCC68 and MCC448) were grown under controlled (field capacity) and drought stress (25% field capacity) conditions. In this experiment, tolerant and susceptible genotypes were compared with each other for proline, malondialdehyde and soluble protein content. We also compared these genotypes with each other for catalase, ascorbat peroxidase, peroxidase and superoxide dismutase during the stages of seedling, flowering and podding. The results showed that drought stress significantly increased proline content in the flowering and podding stages and also increased catalase activity in the three investigated stages. By contrast, the effects of drought stress on ascorbat peroxidase, peroxidase and malondialdehyde were not significant. In the flowering stage, tolerant genotype (MCC877) had higher catalase activity as well as, higher proline contents in comparison with susceptible genotypes (MCC68 and MCC448). Also, drought stress had significant effects on superoxide dismutase activity in the flowering stage. These results indicated that catalase and superoxide dismutase activity and proline content can be effective markers in the identification of drought tolerant chickpea genotypes. Also, the flowering and podding stages can be more suitable than seedling stage for comparing susceptible and tolerant genotypes under drought stress and also to classify adapted cultivars of chickpea under drought stress.

Key words: Antioxidant enzymes, Chickpea (*Cicer arietinum* L.), drought, malondialdehyde, proline.

INTRODUCTION

One of the important legume crops found in the semi-arid areas is Chickpea (*Cicer arietinum* L.) (Toker and Cagirgan, 1998). Chickpea is an important source of protein supply in the human diet. Drought lessens the yield and has the potential for leading into a total crop failure. However, chickpea is known for its better drought tolerance when compared to most of the other cool-season legumes (Gunes et al., 2006). Furthermore, drought stress is one of the fundamental reasons for reducing the amount of growth and yield of chickpea

*Corresponding author. E-mail: ra_rahbarian@yahoo.com. Tel: 09153518157. Fax: 98 0551 2224661.

Abbreviations: MDA, Malondialdehyde; **CAT,** catalase; **POX,** peroxidase; **SOD,** superoxide dismutase; **APX,** ascorbat peroxidase.

(Jaleel et al., 2009). Plants show a lot of morphophysiological and metabolic changes in response to drought stress. Consequently, these changes lead them to adapting to drought stress conditions. As Sanchez et al. (2008) mentioned, response mechanisms to drought stress are very complicated because in addition to morphophysiological and metabolic changes, interactions of these factors are also important in resistance to drought stress. Therefore, to investigate the mechanisms of resistance to drought stress and to identify drought tolerant genotypes, reviewing many physiological and biochemical studies and ultimately molecular research can be helpful (Chopra and Selote, 2007).

The increase in proline is usually considered as a plant response to drought stress. Higher proline content in tolerant genotypes as compared to susceptible genotypes helps them to improve their cellular osmotic adjustment and also the stabilization of enzymes proteins under drought stress (Kumar et al., 2006).

An alternative defensive system called antioxidative is also activated to protect cells against oxidative stress and support plants against oxidative hurt. The endogenous supportive mechanisms consist of some enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) as well as, peroxidase (POX) that can effectively scavenge the toxic oxygen species. If the plants are confronted to environmental stress situations, such as drought conditions, the capacity of leaves for active oxygen generation will increase. This situation mostly results in oxidative damages (Chopra and Selote, 2007).

Furthermore, Bian and Jiang (2008) reported that there is a close relationship between increased antioxidant enzyme activity and enhanced resistance to environmental stresses. Simova et al. (2008) reported that the effects of drought stress on wheat species depends on variety, intensity and duration of the stress as well as, on the developmental stages. Masoumi et al. (2010) reported that drought stress caused a significant increase in antioxidant enzyme activities such as CAT, APX and glutathione redoctase, but it had no significant effects on SOD and POX activity. Keles and Unyayar (2004) reported that drought stress had different effects on antioxidant enzymes.

They further believed that the relative tolerance of a genotype to water stress as reflected by its comparatively lower lipid peroxidation, higher proline and chlorophyll contents was closely associated with its antioxidant enzyme system. Nevertheless, according to Muhammad Zia-Ul-Haq (2008) antioxidant enzymes activity and production of active oxygen species under drought stress and their relationship with photosynthetic rate and cell membrane destruction is not yet clear, and therefore, it has been the subject of intensive study for a long time. Identification of biochemical markers along with improved field performance under drought stress aid plant breeding efforts to improve drought tolerance (Yordanov et al., 2003). Furthermore, Bray (1997) contended that the responses to drought stress depend on the species and genotype, the length and severity of water deficit and the age and stage of development.

This study is designed to investigate the effects of drought stress on the production of malondialdehyde (MDA) and proline.

In addition, the study aims at determining the role of enzymes involved in antioxidative defense mechanism, including CAT, APX, POX and SOD in drought stressed chickpea plants. According to Bray (1997), plant responses to drought stress can be different in each growth stage as compared to other stage. So, in this study we tried to investigate differences of chickpea genotypes in responses to drought stress in the seedling, flowering and podding stages.

This paper explored further the possibility of using physiological and biochemical traits measured at different growth stages as screening tools for introducing drought tolerance chickpea genotypes.

MATERIALS AND METHODS

Plant materials

This study was conducted in control condition at the Research Center for Plant science in Ferdowsi University of Mashhad. Seeds of two tolerant genotypes (MCC392 and MCC877) and two susceptible genotypes (MCC68 and MCC448) that was introduced by Ganjeali et al. (2009), were grown in pots containing 3 kg of soil mixture composed of sand and the farmyard manure at 2: 3 ratio in drought stress (25% field capacity) and controlled conditions (field capacity). In each pot, four seeds were planted. Growth chamber temperatures were maintained at 21°C during the day and 8°C during the night (12.5/11.5 h- day/night) for 30 days and then were changed into 27°C during the day and 12°C during the night (13 /11 h. day/night), light intensity was 200 µmol m^{-2} s^{-1} similar to normal field situations of chickpea growth region. Biochemical indicators and activity of antioxidant enzymes were measured in seedling, flowering and podding stages to determine fast and easy techniques for screening chickpea genotypes for drought tolerance.

Biochemical measurements

Proline assay

Proline was determined in fully expanded leaves according to Bates et al. (1973). The samples (0.5 g fresh weight) were homogenized with 10 ml of 3% sulphosalicylic acid solution and were then filtered on Whatman fiber glass paper. Then 2 ml of the extract was added to 2 ml ninhydrin reactive and 2 ml of acetic acid and was incubated in boiling water for 1 hour. After the process of fast cooling in ice, the samples were added to 4 ml of toluene and were strongly shaken. Subsequently, the toluene phase, which was characterized by a colored complex, was used to measure the absorbance at 520 nm versus toluene by spectrophotometer UV 2100 model. Finally, the proline amount of each sample was calculated by means of a calibration curve, made by starting from known amount of proline.

Lipid peroxidation level

Malondialdehyde in the leaf samples was measured to assess the

membrane damage. MDA was measured in leaf tissue by following the procedure described by Heath and Packer (1968). Firstly, fresh leaf tissue (0.2 g) was homogenized in 0.1% trichloroacetic acid (TCA). Afterwards, the extracts were centrifuged at 10000 × g for 5 min and then 1 ml of the supernatant was mixed with 4 ml of a 0.5% thiobarbituric acid (TBA) and 20% TCA mixture. Then, the extracts were incubated at 95°C for 30 min and were then centrifuged at 10000 × g for 10 min. Later, absorbance of the supernatant at 532 and 600 nm was also measured. For calculation, the value of absorbance at 600 nm was subtracted from the value of absorbance at 532 nm. At last, MDA concentration was calculated by using its molar extinction coefficient at 532 nm (155 mM^{-1} cm^{-1}).

Soluble protein content determination

For the assays of CAT, POX, APX and soluble protein content, about 0.1 g sample of young, fully expanded leaves were collected in the seedling, flowering and podding stages. After that, the samples were frozen immediately at −70°C until the time they were going to be used. As for the extraction of enzymes, frozen leaves were homogenized with 5 ml of 0.1 M buffer solution, which consisted of KH_2PO_4 and K_2HPO_4. They were then crushed with a mortar and pestle, and were centrifuged at 15000 × g for 20 min in a refrigerated centrifuge. The supernatant was collected in a bottle for the determination of soluble protein content and enzymes activity. The total soluble protein content was estimated by employing the method of Lowry et al. (1951).

Determinations of antioxidant enzymes activities

Catalase (EC 1.11.1.6, CAT) activity was measured according to Chandlee and Scandalios (1984) with modification. The assay mixture (3 ml) contained 2.5 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.3 ml of 3% H_2O_2 and 0.2 ml of enzyme extract. Thus, the decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. Peroxidase (EC 1.11.1.7, POX) activity was measured according to Holy (1972). The reaction mixture contained 0.2 M acetate buffer (pH 5) with H_2O_2 (3%), benzidin 0.2 mM in methanol (50%) and enzyme extract. The reaction rate was then identified by increasing in absorbance at 530 nm. One unit of POX and CAT was defined as µmol ml^{-1} H_2O_2 decomposed per min at 25°C. Ascorbate peroxidase (EC 1.11.1.11, APX) activity was measured according to Asada and Takahashi (1987). The reaction mixture contained 50 mM phosphate buffer (pH 6.5), 5 µM ascorbate, H_2O_2 (3%) and enzyme extract. Therefore, the H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 256 nm. One unit of this enzyme was defined as the amount of enzyme required to hydrolyze 1 mmol of the substrate per min at 25°C. Superoxide dismutase (EC 1.15.1.1, SOD) activity was assayed by the nitro blue tetrazolium (NBT) method (Beauchamp and Fridovich, 1971). The reaction mixture contained 50 mM phosphate buffer (pH 7.3), 13 mM methionine, 75 mM NBT, 0.1 mM EDTA, 4 mM riboflavin and enzyme extract. The reaction started by adding riboflavin, and the glass test tubes were shaken and placed under fluorescent lamps (60 mmol m^{-2} s^{-1}). The reaction was allowed to proceed for 15 min and was then stopped by switching off the light. The absorbance was measured at 560 nm. Moreover, blanks and controls were run in the same manner but without illumination and enzyme, respectively. Also, one unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions.

Statistical analysis

The experiment was carried out in a completely randomized design with four replications. Then, the analysis of the variance was conducted on the data, and significant differences among treatment means were calculated by using Duncan's multiple range tests ($p \leq 0.05$).

RESULTS

MDA content

During the seedling stage, the MDA content significantly increased in MCC68, MCC877 and MCC448 genotypes under drought stress, but MCC392 genotype did not show remarkable increase in MDA content in drought stress as compared with control condition (Figure 1a). On the contrary, MCC877 genotype had high MDA content in this stage under drought stress but, this genotype could decrease remarkably MDA content in the flowering and podding stages under drought stress. So, in the podding stage the lowest MDA content belonged to MCC877 genotype under drought stress condition (Figure 1a). In the seedling stage, there were not significant differences among genotypes in MDA content in control condition, but under drought stress, MCC392 genotype had significantly lower level of MDA than that of other genotypes ($p < 0.05$). The effects of drought stress on MDA content were significant in the seedling stage (Table 1), but they were not significant in both the flowering and podding stages (Tables 2 and 3).

Soluble protein content

Drought stress had no significant effects on soluble protein content in all three investigated stages (Tables 1, 2 and 3). During the seedling stage, there were no significant differences between genotypes in both drought stress and control conditions (Figure 1b). In the flowering stage, soluble protein content in MCC392 genotype was significantly higher than other genotypes under drought stress (Figure 1b). Also, in the podding stage, soluble protein content significantly decreased in MCC392 genotype whereas, it increased in MCC68 and MCC877 genotypes in drought stress. In the control condition, the highest and the lowest soluble protein contents belonged to MCC392 and MCC448 genotypes, respectively. However, under drought stress, the highest soluble protein content belonged to MCC877 genotype and this genotype showed significant differences with others (Figure 1b). In drought stress condition, the highest soluble protein content in MCC68, MCC877 and MCC448 genotypes belonged to the podding, but in MCC392 genotype to the flowering stage (Figure 1b).

Proline content

Drought stress increased remarkably proline content in

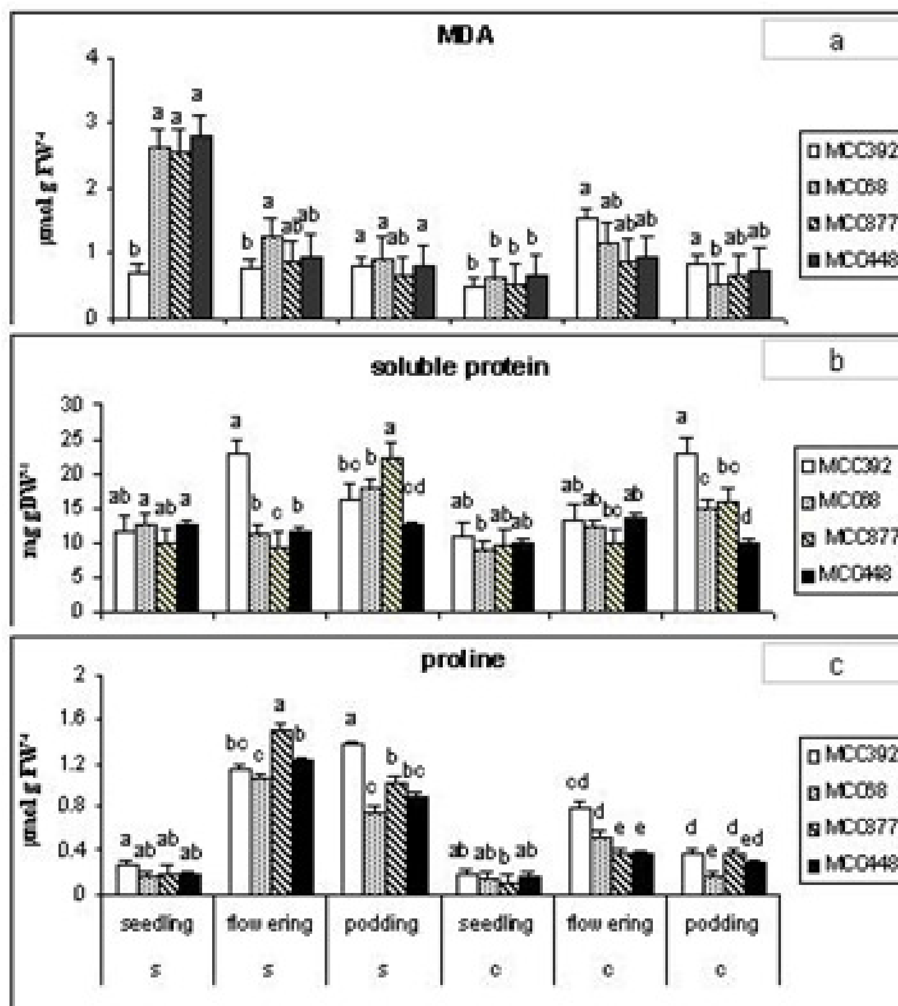

Figure 1. Effect of drought stress on (a) MDA (µmol g $F.W^{-1}$), (b) soluble protein (mg g $D.W^{-1}$), and (c) proline (µmol g $F.W.^{-1}$) content in the seedling, flowering and podding stages in chickpea genotypes, (s: drought stress (25% FC) and c: control (FC) conditions). Columns indicated with the same letters in each stage are not significantly different ($p < 0.05$). Error bars are SE of means (n = 4).

Table 1. Sum of squares from analyses of variance of data for peroxidase (POX), catalase (CAT), ascorbat peroxidase (APX), superoxide dismutase (SOD), soluble protein, malondialdehyde (MDA) and proline of four chickpea genotypes (MCC392, MCC68, MCC877 and MCC448) in the seedling stage under drought stress and control condition.

Proline	MDA	Soluble protein	SOD	APX	CAT	POX	Degrees of freedom	Source of variation
0.025ns	6.7**	1.1 e11^{ns}	0.023^{ns}	0.000007^{ns}	0.0005^{ns}	0.0005^{ns}	3	Genotype
0.020ns	20.7**	2.9 e11^{ns}	0.00006^{ns}	0.000001^{ns}	0.0007**	0.0001^{ns}	1	treatment
0.012ns	5.1**	1.7 e11^{ns}	0.022^{ns}	0.000009^{ns}	0.0004^{ns}	0.0003^{ns}	3	g × t
0.16	2.5	1.8 e12	0.068	0.00008	0.002	0.004	24	error

*, ** and ns significant at 0.05 and 0.01 levels and non significant, respectively.

the flowering and podding stages in all genotypes ($p < 0.01$) (Tables 2 and 3). But, in the seedling stage, there were no significant changes in proline content as compared with the control condition (Figure 1c). The highest level of proline content belonged to MCC877 and MCC392 genotypes in the flowering and podding stages respectively, under drought stress.

In drought stress, MCC877, MCC448 and MCC68

Table 2. Sum of squares from analyses of variance of data for peroxidase (POX), catalase (CAT), ascorbat peroxidase (APX), superoxide dismutase (SOD), soluble protein, malondialdehyde (MDA) and proline of four chickpea genotypes (MCC392, MCC68, MCC877 and MCC448) in the flowering stage under drought stress and control condition.

Proline	MDA	Soluble protein	SOD	APX	CAT	POX	Degrees of freedom	Source of variation
0.23ns	1.24^{ns}	0.24^{ns}	0.002^{ns}	0.00004*	0.0001^{ns}	0.00001^{ns}	3	Genotype
5.59**	0.67^{ns}	0.08^{ns}	0.006*	0.000000^{ns}	0.0002*	0.00000^{ns}	1	treatment
0.72ns	0.93^{ns}	0.39^{ns}	0.001^{ns}	0.000002^{ns}	0.0000^{ns}	0.0001^{ns}	3	g × t
2.32	6.9	2.9	0.026	0.00006	0.0007	0.0008	24	error

*, ** and ns significant at 0.05, 0.01 levels and non significant, respectively.

Table 3. Sum of squares from analyses of variance of data for peroxidase (POX), catalase (CAT), ascorbat peroxidase (APX), superoxide dismutase (SOD), soluble protein, malondialdehyde (MDA) and proline of four chickpea genotypes (MCC392, MCC68, MCC877 and MCC448) in the podding stage under drought stress and control condition.

Proline	MDA	Soluble protein	SOD	APX	CAT	POX	Degrees of freedom	Source of variation
1.2*	0.57^{ns}	3.6^{ns}	0.006*	0.00001*	0.0001^{ns}	0.0001^{ns}	3	Genotype
2.2**	0.22^{ns}	0.14^{ns}	0.001^{ns}	0.00000^{ns}	0.0002**	0.00002^{ns}	1	treatment
0.8ns	0.21^{ns}	1.7^{ns}	0.001^{ns}	0.00000^{ns}	0.0004ns	0.00006^{ns}	3	g × t
2.6	2.27	11.9	0.012	0.00002	0.0003	0.0005	24	error

*, ** and ns significant at 0.05, 0.01 levels and non significant, respectively.

demonstrated an increase of proline content from the seedling to the flowering stage (7.5, 7 and 9 folds) but, they showed a decrease of proline content from the flowering to the podding stage. Though, the proline content in MCC392 genotype increased from the seedling to the podding stage (5 fold) and this genotype had the highest level of proline content in the podding stage (Figure 1c).

Antioxidant enzyme activity

POX

The results from ANOVA showed that drought stress had no significant effects on POX activity in all three investigated stages (Tables 1, 2 and 3). Further, we observed that the leaf POX activity significantly decreased in MCC392 and MCC68 genotypes under drought stress as opposed to control plants in the seedling stage (Figure 2a). In drought stress condition, MCC392 genotype had the lowest level of POX activity than other genotypes (Figure 2a). In the flowering stage, POX activity significantly decreased in MCC448 genotype in drought stress (Figure 2a). Therefore, MCC448 genotype had significantly lower POX activity than other genotypes in this stage under drought stress ($p < 0.05$) (Figure 2a). In the podding stage, POX activity significantly decreased in MCC877 genotype in drought stress condition. Therefore, POX activity in MCC448 genotypes was higher than that of MCC877 and MCC68 genotypes in drought stress condition ($p < 0.05$) (Figure 2a).

In this experiment, MCC392 and MCC448 showed an increase in their POX activity from seedling to podding stages (20 and 9%) respectively, whereas, MCC877 and MCC68 had a decrease in their POX activity from seedling to podding stage (36 and 33%) respectively (Figure 2a).

APX

In the seedling stage, the leaf APX activity increased significantly in MCC392 and MCC877 genotypes under drought stress, as compared to the control group ($p < 0.05$) (Figure 2b). In this stage, MCC877 and MCC68 genotypes had significantly higher APX activity than MCC392 and MCC448 genotypes under drought stress condition ($p < 0.05$) (Figure 2b).

In the flowering stage, APX activity significantly decreased in MCC68 genotype in drought stress. In the control condition, the highest and the lowest APX activity was observed in MCC68 and MCC448 genotypes respectively.

The genotype, MCC877 had much higher APX activity than MCC68 and MCC448 genotypes in drought stressed plants (Figure 2b). In the podding stage, drought stress increased APX activity in MCC392 genotype. Further, APX activity in tolerant genotypes MCC877 and MCC392 was significantly higher than the susceptible genotypes MCC68 and MCC448 genotypes under drought condition

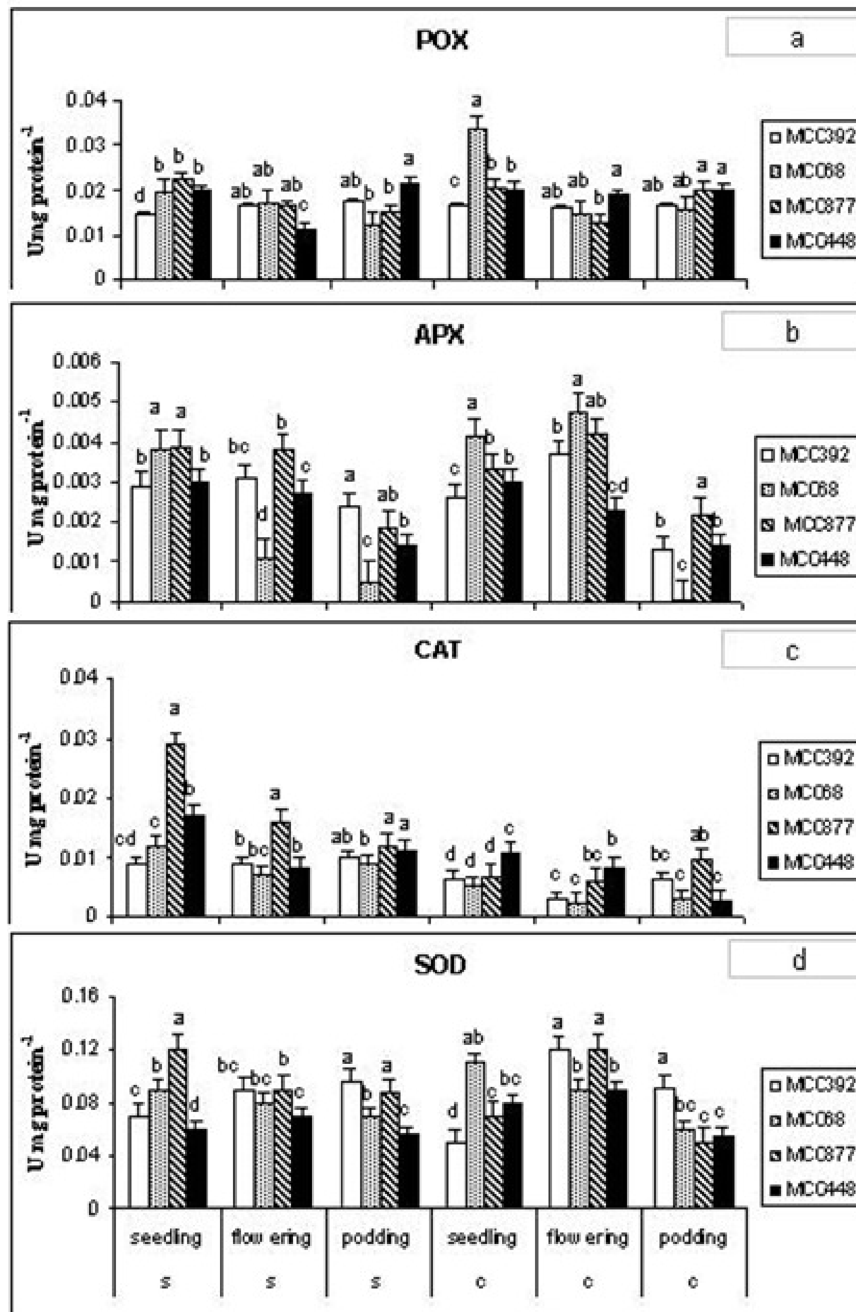

Figure 2. Effect of drought stress on (a) POX, (b) APX, (c), CAT, and (d) SOD activity in the seedling, flowering and podding stages in chickpea genotypes (s: drought stress (25% FC) and c: control (FC) conditions). Columns indicated with the same letters in each stage are not significantly different ($p < 0.05$). Error bars are SE of means (n = 4).

($p < 0.05$) (Figure 2b). Among the three investigated stages, the lowest level of APX activity belonged to the podding stage in all genotypes.

CAT

Another enzyme which we verified was CAT enzyme. In its ANOVA, it was observed that drought stress had significant effects on CAT activity in all three investigated stages ($p < 0.05$) (Tables 1, 2 and 3). Drought stress significantly increased CAT activity in MCC392 genotype only under flowering stage, and as such, this genotype showed significant differences compared with other investigated genotypes in this stage ($p < 0.05$) (Figure 2c). Also in MCC68 and MCC448 genotypes, a

remarkable increase in CAT activity was observed in both seedling and podding stages under drought stress condition ($p < 0.05$). In addition, MCC877 genotype showed a significant increase in CAT activity in both seedling and flowering stages, therefore, this genotype had significant differences compared with other investigated genotypes under drought condition ($p < 0.05$) (Figure 2c).

Among the three investigated stages, the highest CAT activity in MCC68, MCC877 and MCC448 belonged to the seedling stage under both drought stress and control conditions. But in MCC392 genotype, there were no remarkable changes in CAT activity between three investigated stages (Figure 2c).

SOD

SOD activity increased in MCC392 and MCC877 genotypes and decreased in MCC448 genotype under drought stress as opposed to the control (Figure 2d). SOD activity in MCC877 was significantly higher than other investigated genotypes under drought stress ($p < 0.05$) (Figure 2d). In the flowering stage, SOD activity significantly decreased in MCC392, MCC877 and MCC448 genotypes, however, MCC877 genotype had higher SOD activity than MCC68 and MCC448 genotypes in drought stress (Figure 2d). In this stage, drought stress effects on SOD activity was significant (Table 2). In the podding stage, SOD activity showed a noticeable increase in MCC877 genotype under drought stress. As a result, MCC392 and MCC877 (tolerant genotypes) had much higher SOD activity than that of MCC68 and MCC448 genotypes under drought stress ($p < 0.05$). In this experiment, MCC392 showed an increase of SOD activity from the seedling to the podding stage (30%), whereas, MCC68, MCC877 and MCC448 genotypes had a decline of SOD activity from the seedling to the podding stage (22, 30 and 10%) respectively (Figure 2d).

DISCUSSION

According to Jaleel et al. (2009) although, the effects of drought stress on growth and development of plants have been studied in a large-scale, still, the physiological and biochemical responses of plants to drought stress are not well understood. Overwhelming evidence showed that drought induces oxidative stress through the production of active oxygen species such as superoxide, hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen ($^1O_2$) (Fu and Huang, 2001). They will then react to O_2 in the absence of other acceptors. Afterwards, antioxidative defense system was activated in response to oxidative stress. Furthermore, drought stress increased certain ROS production in leaves and induced lipid peroxidation in chickpea (Gunes et al., 2006).

The results from this study showed that in the seedling stage, MDA contents significantly increased in MCC877, MCC68 and MCC448 genotypes under drought stress. But it had no change in MCC392 genotype. However, drought stress had no significant effects on MDA content in the flowering and podding stages. In addition, MDA content decreased in the flowering and podding stages when compared with the seedling stage. Lipid peroxidation is an indicator of the prevalence of free radical reaction in tissues (Helal and Samir, 2008). Accumulation of O_2^- and H_2O_2 in the leaves contributed to the lipid peroxidation and increase in lipid peroxidation and increased in drought stress (Yordanov et al., 2003).

Our findings suggested that oxidative injury due to drought stress in the seedling stage has more severity than flowering and podding. Similarly to these findings, a higher increase in MDA was observed in the maize susceptible genotype as compared to tolerant genotype in drought stress (Helal and Samir, 2008). In this study, drought stress had no significant effects in proline content and antioxidant enzymes activity in the seedling stage but, only CAT activity increased in this condition. These results showed that in the flowering and podding stages defenses responses to drought stress such as proline content and antioxidant enzymes activity were more remarkable than the seedling stage. Therefore, in this study, tolerant genotypes could decrease damaged effects of drought stress via an increase in proline content and antioxidant enzymes activity such as CAT and SOD enzymes, in the flowering and podding stages.

In addition, in the flowering stage, tolerant genotype (MCC877) had higher proline content and CAT activity than susceptible genotypes (MCC68 and MCC448) under drought stress. Also, in the podding stage, tolerant genotypes (MCC392 and MCC877) had higher proline content and SOD activity than susceptible genotypes (MCC68 and MCC448) under drought stress. Higher superoxide dismutase activity during drought stress might protect plants from oxidative injury (Arora et al., 2002). Higher SOD activity in *Aatharanthus roseus* tolerant genotypes was observed under drought stress (Jaleel et al., 2007). Also, Helal and Samir (2008) reported that SOD activity was higher in tolerant genotype of maize than susceptible genotype. In this study, drought stress significantly increased proline content in the flowering and podding stages. Therefore, according to Ashraf and Iram (2005), accumulation of proline is an important indicator of drought stress tolerance in chickpea plants. Further, higher concentration of proline in tolerant genotype versus susceptible genotype was observed in maize genotypes in drought stress (Helal and Samir, 2008). Of the several biochemical indices of drought stress, proline accumulation has been widely reported (Ashraf and Iram, 2005). Increase of proline causes the mediation of osmotic adjustment and thus, the plant will keep growing under drought stress. In addition to this, proline has a good impact on maintaining the structure of the enzyme and removal of reactive oxygen species

(Kumar et al., 2006). These genotypes which have high proline content might increase the synthesis ability of osmotic regulators (proline) for protection from the damage of drought stress. Since proline has hydrophilic property, it might replace water molecules around nucleic acid, protein and membranes during water shortage. It might also prevent interaction between destabilize ions and cellular components by replacing the water molecules around these components, thereby, protecting it against destabilization during drought (Bayoumi et al., 2008).

Furthermore, our results showed that there were significant positive correlations between SOD and APX activity, during the seedling and flowering stages ($r^2 = 0.28$ and 0.15, respectively). The experiment moreover, suggested that CAT activity increased in tolerant genotypes as compared to susceptible genotypes under drought stress conditions. These results are in accordance with the findings in *Catharanthus roseus* (Jaleel et al., 2007) and mungbean (Ahmed et al., 2002). Helal and Samir (2008) also reported that the increased activity of CAT enzyme develops a potential for defense against damage in maize genotypes.

In this study, there were significant positive correlations between CAT and SOD ($r^2 = 0.17$ and 0.16) during the seedling and the podding stages, respectively. CAT enzyme converts the toxic H_2O_2 (produced by SOD activity) to water and molecular oxygen, thus, averting the cellular damage under unfavorable conditions like water stress (Jaleel et al. 2007).

According to these results, we found that chickpea tolerant genotype protected themselves from damage effects of drought stress by increasing in proline content and CAT and SOD activity. Also, it can be concluded from this study, that chickpea genotype showed greater proline content and more CAT and SOD activity which helps to tolerate drought stress. Therefore, considering all the results obtained from this study, proline content, CAT and SOD activity can be useful biochemical markers for identifying tolerant genotypes. In addition, defense responses to drought stress were not remarkable in the seedling stage. As a result, the highest level of MDA and the lowest level of proline content belonged to the seedling stage as compared with the flowering and the podding stages. Therefore, the flowering and the podding were more suitable stages for investigation tolerance to drought stress and comparing susceptible and tolerant chickpea genotypes. However, the data presented here reflects the importance of a physiological and biochemical analysis of plant response, which must be accompanied with field experiments and further evaluation. Therefore, more investigations are required to ascertain this conclusion.

Conclusions

This study is designed to investigate the effects of drought stress on the production of malondialdehyde (MDA) and proline. In addition, the study aims at determining the role of enzymes involved in antioxidative defense mechanism, including CAT, APX, POX and SOD in drought stressed chickpea plants. In this study, it was observed that increased antioxidant enzyme activities (SOD, CAT and APX), and also accumulation of proline are involved as part of the defenses against drought stress. Therefore, proline content, CAT and SOD activity can be used as biochemical markers for identifying tolerant genotypes of chickpea. Based on this research, the combination of molecular biology and plant physiology is the key of mechanism of drought tolerance. Thus, further work is required to identify and manipulate the genes controlling the physiological and molecular traits and to gear our research to the right direction of drought tolerance.

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