# Differential Antioxidative Responses to Environmental Constraints in Shoots and Roots of Wild Legumes

Taha Ramadan*, Mohamed Hemida Abd-Alla, Abdelwahab E. Elenany, Manal Alzohri and Ibrahim M. Nafady

*Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71516, Egypt*

**Abstract:** The current study aimed to explore the antioxidant system of five legumes inhabiting regions with different conditions. In these legumes, $H_2O_2$ generation and lipid peroxidation enhanced in roots of plants inhabiting the Mediterranean region (MR) and Sinai (S) where high soluble salts and low water content in the soil were estimated. High levels of phenols and ascorbic acid were detected in shoots of these plants compared with those inhabiting the Nile region (NR) or Oases (O), which characterized by low soluble salts and high water content. There were great variations between species in their responses to adverse conditions, and enhanced activities of antioxidant enzymes were recorded in plants inhabiting the more stressful habitats. Roots and shoots of legumes responded differentially to oxidative stresses regarding the induction of enhanced or suppressed activities of a definite antioxidative enzym. While CAT activity increased in shoots, GP activity greatly stimulated in roots of legumes at different habitats. The activity of APX decreased in roots but increased in shoots by the harsh conditions of habitate showing minimum and maximum activities in roots and shoots, respectively, in plants inhabiting S. The activity of CAT and APX increased in shoots by increasing the concentration of $H_2O_2$, while the over expression of GP gene in roots enhanced scavenging $H_2O_2$ to a level between 6% to 37% of its concentration in shoots. Genes expression of the antioxidant enzymes (CAT, GP and APX) more regulated, especially in shoots, by the environmental constraints than the differences between species.

**Keywords:** Antioxidation, *melilotus indicus, medicago polymorpha*, *trifolium resupinatum, trigonella hamosa, Vicia sativa.*

## 1. INTRODUCTION

Exposure of plants to unfavorable environmental conditions such as temperature extremes, heavy metals, drought, water availability, air pollutants, nutrient deficiency, or salt stress can increase the production of ROS e.g., $^1O_2$, $O_2^-$, $OH^-$ and $H_2O_2$. To protect themselves against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems. A great deal of research has established that the induction of the cellular antioxidant machinery is important for protection against various stresses [1, 2, 3]. Response and adaptation to environmental stresses are probably complex phenomena involving many physiological and biochemical processes that likely reflect changes in gene expression and in the activity of enzymes and transport proteins [4, 5].

Abiotic stress induces the accumulation of ROS in the cells, which can cause harsh oxidative damage to the plants, thus inhibiting growth and grain yield. The equilibrium between the production and scavenging of ROS is commonly known as redox homeostasis.

*Address correspondence to this author at the Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71516, Egypt;
Tel: 020-01111125123; Fax: 020-88-2342708; E-mail: taha60@yahoo.com; taha60@aun.edu.eg

However, when ROS production prevails the cellular scavenging capacity, thus unbalancing the cellular redox equilibrium, the result is a rapid and transient excess of ROS, known as oxidative stress [6, 7]. To avoid potential damage caused by ROS to cellular components, as well as to maintain growth, metabolism, development, and overall productivity, the balance between production and elimination of ROS at the intracellular level must be tightly regulated and/or efficiently metabolized. This equilibrium between the production and detoxification of ROS is sustained by enzymatic and nonenzymatic antioxidants [8, 9, 10]. The enzymatic components comprise several antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, guaiacol peroxidase, peroxiredoxins, and enzymes of the ascorbate-glutathione cycle, such as ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase [8, 11]. $H_2O_2$, which is not a free radical, toxicity is reduced by removing it enzymatically (i.e. by catalase or ascorbate peroxidase) or by complexing Fe (III) and Fe(II) with compounds such as tannic acid and proanthocyanidins and thus prevent $OH^-$ generation [12, 13].

Nonenzymatic components include the major cellular redox buffers ascorbate and glutathione as well as tocopherol, carotenoids and phenolic compounds

E-ISSN: 2311-858X/17 

[9, 14, 15]. The degree to which the activities of antioxidant enzymes and the amount of antioxidants increase under different stress conditions is extremely variable among several plant species. The level of response depends on the species, kind of stress, and the metabolic state of the plant. Many stress situations cause an increase in the total antioxidant activity [16].

The purpose of the current study was to verify the differential antioxidative responses to the harsh conditions in five wild legumes inhabiting four different Egyptian phytogeographical regions. Also, the study aimed to achieve the differences between shoots and roots of these legumes in responses to environmental stresses and their antioxidation systems.

## 2. MATERIAL AND METHODS

### 2.1. Sampling

Locations inhabited by five legumes *Melilotus indicus* L., *Medicago polymorpha* L., *Trifolium resupinatum* L., *Trigonella hamosa* L. and *Vicia sativa* L. were chosen for sampling. These species represent the most common legumes, and so they selected to be reoccurring in the studied sites. The locations were representing four distinctive Egyptian phytogeographical regions: Nile region (NR; at Assiut city), Oases (O; at Al-Kharga), Mediterranean region (MR; at Burg Al-Arab) and South Sinai (S; at Saint Katherine). On the bases of average temperature across the sampling season, soil water content and total soluble salts, S and MR considered more stressful than O and NR. The average maximum temperature of the sampling season was 23 ± 2, 23 ± 2, 19 ± 1, 15 ± 3 ºC; while average minimum temperature was 8 ± 2, 10 ± 2, 10 ± 1, and 4 ± 2 ºC at NR, O, MR and S, respectively. The percentage of water content of soil was 30.8, 20.8, 13.7 and 8.5, while the percentage of total soluble salts in the dry soil was 0.12, 0.14, 0.11 and 0.18 at NR, O, MR and S, respectively [17].

At each site, three individuals (as replicates) of every plant species were taken out, washed thoroughly by distilled water to get rid of soil particles and dropped dry quickly by absorbent tissue. Sufficient tissues from the shoots and roots were taken, each sample was wrapped up in aluminium foil, and the three replicates for each species were rolled in another strip of aluminium foil and immersed immediately in liquid nitrogen until transferring it to Lab. for analyses.

### 2.2. Concentration of Hydrogen Peroxide

The hydrogen peroxide content in the shoot and root tissues were colorimetrically measured as described by Mukherjee and Choudhuri [18]. The samples were removed from liquid nitrogen and a defined weight (0.1 g) of shoot or root was extracted with 5 ml cold acetone. From this extraction, 3 ml was mixed with 1 ml of 0.1% titanium dioxide in 20% (v/v) $H_2SO_4$ and the mixture centrifuged at 6000 rpm for 15 min. The intensity of yellow color of the supernatant was measured at 415 nm. The concentration of $H_2O_2$ was calculated from a standard curve plotted with known concentration of $H_2O_2$ and expressed as mg/g FW.

### 2.3. Lipid Peroxidation (Malondialdehyde "MDA" Content)

Lipid peroxidation in roots of legumes was determined as 2-thiobarbituric acid (TBA) reactive metabolites as described by Madhava Rao and Sresty [19]. The root tissues (0.45 g from the liquid nitrogen-immersed samples) were homogenized with 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged for 10 min. at 10,000 rpm. For every one ml aliquot, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95 ºC for 30 min., and then cooled quickly on an ice-bath. After that, the mixture was centrifuged at 10,000 for 15 min. and the absorbance of the supernatant monitored at 532 nm. A calibration curve was made using 1,1,3,3,Tetramethoxypropane, and the level of lipid peroxidation was expressed as nmol MDA/g FW.

### 2.4. Determination of Total Phenols

Free phenols were determined according to Kofalvi and Nassuth [20]. The tissues of shoots (0.5 g) were extracted in 5 ml of 50% methanol for 90 min. at 80 ºC. The extract was centrifuged at 14,000 rpm for 15 min and the total phenols were determined in the supernatant using Folin-Ciocalteu's reagent. One hundred microliters of the extract were diluted to 1 ml with water and mixed with 0.5 ml 2 N Folin-Ciocalteu's reagent and 2.5 ml of 20% $Na_2CO_3$. After 20 min. at room temperature, absorbance of samples was measured at 725 nm with a Unico UV-2100 spectrophotometer. Concentration of total phenols in the extract was estimated from a standard curve prepared with gallic acid and expressed as mg gallic acid equivalent (µ g GAE /g FW).

### 2.5. Determination of Ascorbic Acid

Ascorbic acid was determined according to Jagota and Dani [21]. Leaves (0.2 g) were ground with liquid nitrogen and suspended in 2 ml 5% TCA. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 ºC and to 0.2 ml of this homogenate, 0.8 ml of 10% TCA was added. After vigorous shaking the tubes were kept in an ice bath for 5 min and centrifuged at 3000 rpm for 5 min. The extract (0.5 ml) was diluted to 2.0 ml using bi-distilled water, thereafter 0.2 ml of diluted Folin reagent (Folin-Ciocalteu's reagent of 2 M concentration was diluted 10-fold with bi-distilled water) added to the extract and the tubes vigorously shaken. After 10 min, the absorbance of the blue color developed was measured using Unico UV-2100 spectrophotometer at 760 nm. According to this method, the dilution of Folin reagent and acidic pH may prevent the interference of those substances that can react with Folin (proteins and phenols react with Folin in alkaline media, pH ≈10). The concentration of ascorbic acid was calculated from a standard curve (in the range of 5-50 µg AsA) and expressed in mg $g^{-1}$ FW.

### 2.6. Extraction and Determination of Antioxidant Enzymes

Root or shoot tissues (0.5 g from the liquid nitrogen-immersed samples) were ground to a fine powder in liquid $N_2$ then homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM ethylenediamine tetraacetic acid (EDTA) and 0.1 g polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 18,000 rpm for 10 min at 4 ºC and the supernatants were collected and used for the assays of catalase, guaiacol peroxidase and ascorbate peroxidase. All colorimetric measurements were made at 20ºC using a Unico UV-2100 spectrophotometer. The specific activity was expressed as units/mg protein. Protein concentrations in the enzyme extract were determined by the method of Lowry *et al*. [22].

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the rate of $H_2O_2$ conversion to $O_2$ for one minute according to modified method of Aebi [23]. The assay medium (3 ml) consisted of 2.8 ml 50 mM potassium phosphate buffer (pH 7), 100 µl enzyme extract and the reaction was initiated by addition of 100 µl 10 mM $H_2O_2$. The decrease in absorbance at 240 nm was recorded for one minute.

Guaiacol peroxidase (GP; EC 1.11.1.7) activity was determined according to the method of Tatiana *et al*. [24]. The assay medium (3 ml) consisted of 2.7 ml 30 mM potassium phosphate (pH 7), 200 µl 6.5 mM $H_2O_2$, and 100 µl enzyme extract. The reaction was started by addition 100 µl of 9 mM catechol. The increase in absorbance at 470 nm was recorded for one minute.

Estimation of ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to the method described by Nakano and Asada [25]. The rate of hydrogen peroxide-dependent oxidation of ascorbic acid was determined in reaction mixture contained 2.7 ml of 50 mM potassium phosphate buffer (pH 7), 100 µl 5 mM $H_2O_2$, and 100 µl enzyme extract. The reaction was initiated by addition of 200 µl (0.5 mM) ascorbic acid. The oxidation rate of ascorbic acid was estimated from the decrease in absorbance at 290 nm for one minute.

### 2.7. Statistical Analysis

Data were subjected to statistical analysis using SPSS (version 19). One-way ANOVA was performed followed, when the effect was significant ($P$< 0.05), by the post hoc Duncan's multiple-range test for comparison between means at $P$ < 0.05. Factorial ANOVA was carried to achieve the effect of regions, species and their interaction on different parameters estimated in plants and partial eta square "$\eta^2$" was calculated as: $\eta^2 = SS_{between} / SS_{total}$. Correlation analysis (Pearson correlation) was performed to obtain the relation between some parameters.

## 3. RESULTS

### 3.1. $H_2O_2$ and MDA

The data recorded in figure **1** revealed that the contents of $H_2O_2$ in both of shoots and roots and MDA in roots of all studied legumes are significantly different due to different magnitudes of oxidative stress at their habitats. High $H_2O_2$ and MDA contents were generally recorded in legumes inhabiting MR and S, while low contents were detected in plants inhabiting NR and O. The extremely highest content of $H_2O_2$ was estimated in shoots of *M. indicus* at different habitats (2.12 - 4.12 mg/g FW), while the lowest content was found in shoots of *M. polymorpha*. In general, the concentrations of $H_2O_2$ in shoots of all studied species were greatly higher than that detected in roots (Figure **1a**). Amongst roots, those of *M. polymorpha*

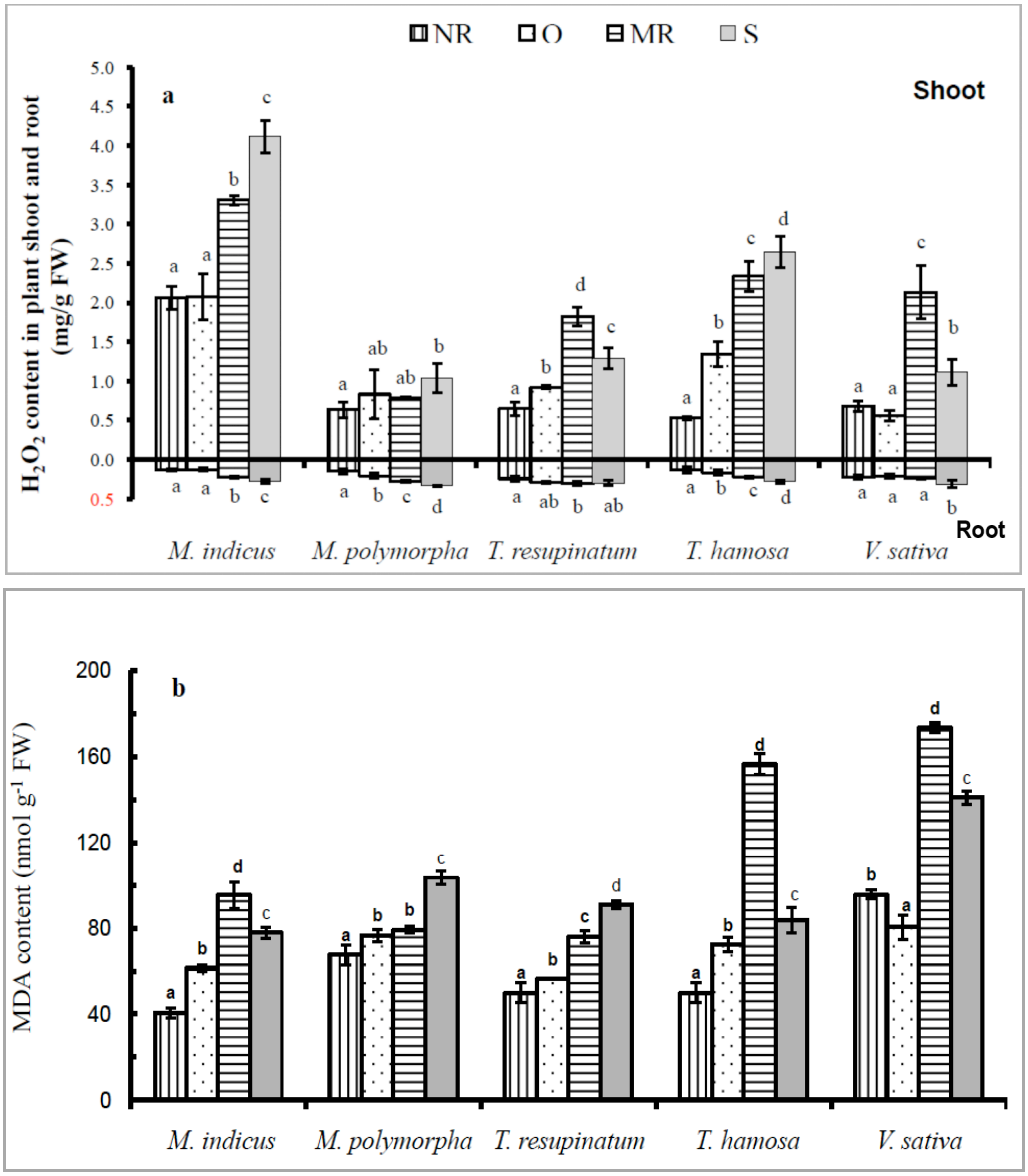


**Figure 1:** Content of Hydrogen peroxide (mg $H_2O_2$ $g^{-1}$ FW) in shoots and roots (**a**) and lipid per oxidation (nmol MDA $g^{-1}$ FW) in roots (**b**) of five wild legumes inhabiting four different habitats. NR, Nile Region; O, Kharga Oases; MR, Mediterranean Region and S, Sinai. Data are means ± SD, n= 3. For each species, means with different letters are significantly different at $P<0.05$ according to Duncan test.

inhabiting Sinai contained the highest concentration of $H_2O_2$ (0.33 mg/g FW). The content of MDA in roots of *V. sativa* inhabiting MR was significantly high (173.39 nmol/g FW) as compared with the other species (Figure **1b**). Concentrations of $H_2O_2$ were correlated with MDA in legumes roots; significant correlation in *M. polymorpha* (r-value= 0.91*), strong in *M. indica* (0.74) and *T. resupinatum* (0.75), and weak in *T. hamosa* (0.44) and *V. sativa* (0.49). As shown in Table **1**, differences between species have the greatest magnitude of effect on differences in $H_2O_2$ contents in shoots ($\eta^2 > 0.56$), while interaction between species and regions have the greatest magnitude of effect on contents of MDA and $H_2O_2$ in roots ($\eta^2 > 0.52$).

**Table 1: The Partial Eta Square ($\eta^2$) for the Magnitude of Effect of Species, Region or their Interaction on Different Parameters**

| Parameter | Species | Region | Species * Region |
|---|---|---|---|
| $H_2O_2$ in shoot | 0.556 | 0.178 | 0.246 |
| $H_2O_2$ in root | 0.263 | 0.113 | 0.522 |
| MDA in root | 0.341 | 0.105 | 0.546 |
| Phenols in shoot | 0.043 | 0.441 | 0.492 |
| AsA in shoot | 0.221 | 0.414 | 0.322 |
| $CAT_{activity}$ in shoot | 0.112 | 0.705 | 0.154 |
| $CAT_{activity}$ in root | 0.361 | 0.106 | 0.475 |
| $GP_{activity}$ in shoot | 0.047 | 0.464 | 0.465 |
| $GP_{activity}$ in root | 0.185 | 0.132 | 0.627 |
| $APX_{activity}$ in shoot | 0.101 | 0.512 | 0.372 |
| $APX_{activity}$ in root | 0.027 | 0.417 | 0.500 |

### 3.2. Non-Enzymatic Antioxidants: Free Phenols and Ascorbic Acid

Contents of free phenols (Figure **2**) and ascorbic acid (Figure **3**) differed significantly among the studied legumes as affected by abiotic variations between habitats. The highest content of free phenols and ascorbic acid were recorded in legumes inhabiting MR and S compared with those inhabiting NR or O. Amongst the studied plants, *T. resupinatum* contained the lowest level of free phenols (25.83 µg/g FW) and the highest level of ascorbic acid (2.61 mg/g FW). In contrast, *M. polymorpha* achieved higher content of free phenols (251.67µg/g FW) and low content of ascorbic acid (0.64 mg/g FW). As indicated by $\eta^2$ (Table **1**), variation between habitats have a magnitude of effect on contents of phenolic compounds and As A higher than that for species.

### 3.3. Antioxidant Enzymes

The present study proved that the activity of antioxidant enzymes differ significantly amongst the studied legumes (Figure **4a-f**). The highest activities of CAT, GP and APX in shoots were estimated in most legumes inhabiting MR and S compared with those inhabiting NR and O (Figure **4a**, **c**, **e**). It is very interesting to note that the activity of CAT (in contrast to GP) in shoots (Figure **4a**) was more effective than in roots (Figure **4b**) of legumes; in roots the activity was weak and variable. *T. resupinatum* showed the highest CAT activity (1.34 unit /mg protein) compared with other studied species (Figure **4a**). The minimum GP activity was found in *M. indicus* (0.002 unit /mg protein). In *M. indicus,* the activity of CAT in shoots of plants collected from NR, O and MR decreased by about 81%, 73% and 66%, respectively, as compared

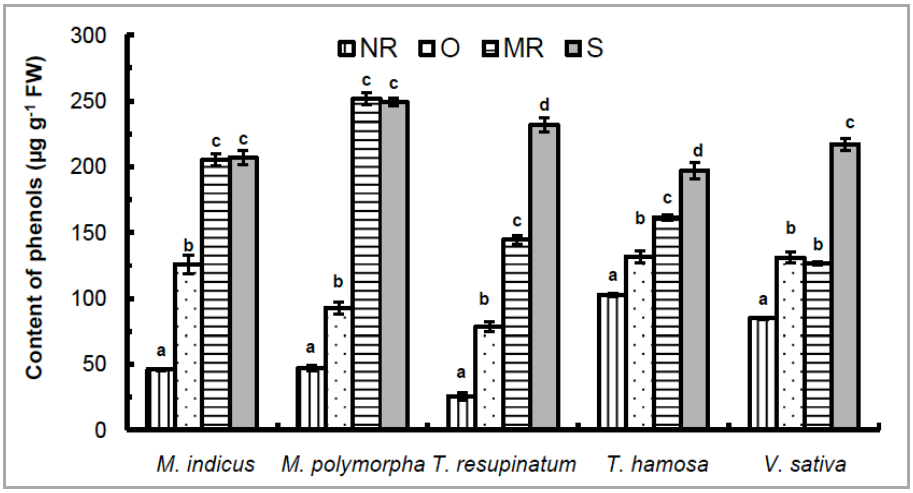


**Figure 2:** Content of free phenolic compounds (µg GAE $g^{-1}$ FW) in the shoots of five legumes. Statistics as in Figure **1**.

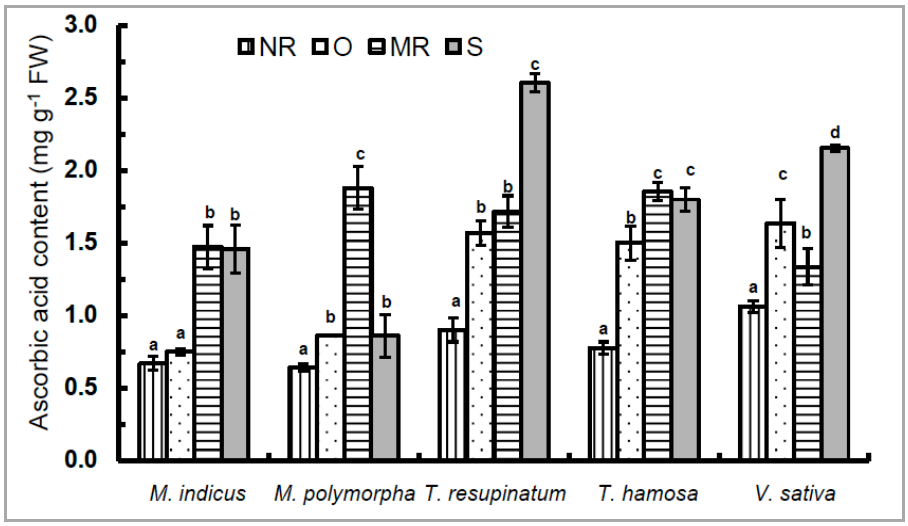


**Figure 3:** Content of ascorbic acid (mg $g^{-1}$ FW) in the shoots of five legumes. Statistics as in Figure **1**.

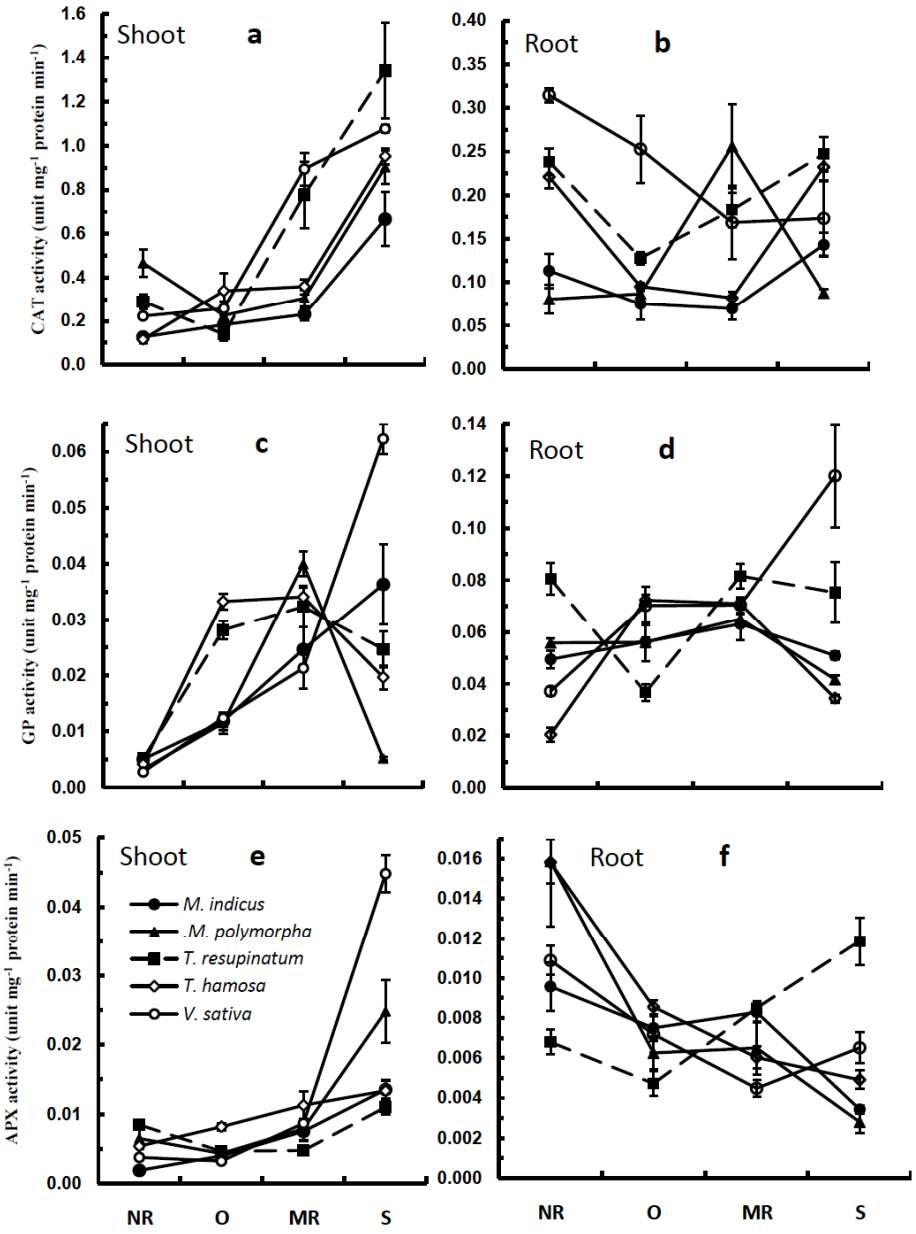


**Figure 4:** Catalase (CAT, **a** and **b**), guiacole peroxidise (GP, **c** and **d**) and ascorbate peroxidise (APX, **e** and **f**) activities (unit $mg^{-1}$ protein $min^{-1}$) in shoots and roots, respectively, of the five-studied wild legumes at different habitats. For each species, means ± SD (n= 3) with different letters are significantly different at $P < 0.05$ according to Duncan test.

with individuals inhabiting S. The GP enzyme in all shoots of legumes inhabiting NR achieved the minimum activity, but at the three other regions the enzymatic activities were variable from species to another (Figure **4c**). In *T. hamosa,* the activity of APX in shoots of plants collected from NR, O and MR was reduced by 54%, 38% and 15%, respectively, as compared to those inhabiting S (Figure **4e**). In shoots of *T. resupinatum*, the APX activity reduced by about 84%, 13% and 22% in plants inhabiting NR, O and S, respectively, as compared with those inhabiting MR (Figure **4e**). Variation between habitats has the main effect on the activity of antioxidant enzymes in shoots, while the interaction between species and regions has the highest magnitude of effect in roots of legumes (Table **1**).

The averages of pooled data of all species indicated that CAT activity increased in shoots by increasing stress conditions of the habitat, while it approximately unchanged in roots (Figure **5a**). Regardless the habitat conditions, CAT activity in shoots was significantly higher than that in roots reaching to more than 5-fold in plants inhabiting Sinai. Guaiacole peroxidase activity in

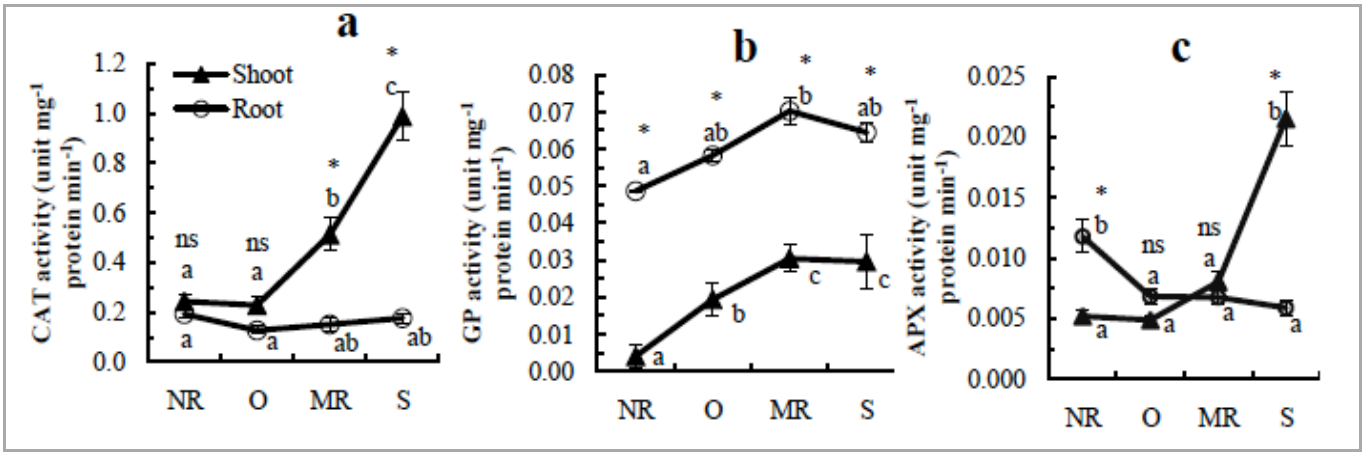


**Figure 5:** Catalase (CAT, **a**), guiacole peroxidise (GP, **b**) and ascorbate peroxidise (APX, **c**) activities in shoots and roots of legumes at different habitats. The data are averages across the five legumes at each habitat, n= 15. For each line, values with different letters are significantly different at *P*< 0.05 according to Duncan test. Shoot and root values that show statistically significant differences are marked with asterisks, ns= not significant.

plants sampled from MR and S was significantly higher than that sampled from NR or O. The GP activity in roots was ranging between 2- to 12-fold of that in shoots (Figure **5b**). A different trend is shown for APX activity where it increased in shoots and decreased in roots by increasing stress conditions (Figure **5c**). In plants inhabiting NR and O (the less stressful habitats), APX activity in roots was significantly higher than that in shoots and vice versa in plants inhabiting the more stressful habitats, MR and S.

## 4. DISSCUSION

The studied annual legumes were selected to be common in four phytogeographical regions of Egypt with significant variations in temperature, soil salts, EC, organic matter, water content and pH [17]. The present study clearly indicated that there is a critical role of the environmental conditions on antioxidant systems of wild legumes. There is remarkably differentiation in activities of enzymatic and non-enzymatic antioxidant systems in studied plants as influenced by differences in abiotic factors of their native habitats. Amongst ROS, $H_2O_2$ is one of the most abundant in aerobic biological systems in higher plants, being highly reactive and toxic. On the other hand, $H_2O_2$ is considered a signaling molecule in plants that mediates responses to various biotic and abiotic stresses [26]. However, the harmful or benefit effects of $H_2O_2$ on plants are depending on its concentration. Hydrogen peroxide can spread cross cell membranes and be transported to other compartments, where it can carry out as a signaling molecule or be eliminated [27]. In shoots of the five legumes studied in different habitats, the concentrations of $H_2O_2$ were very high and ranging between 3- and 32-fold of that in roots. The highest $H_2O_2$ content was estimated in shoots of legumes inhabiting the arid regions MR and S. In addition, $H_2O_2$ did not lead to increasing phenols or AsA in shoots, instead there were very weak or –ve correlation in most studied legumes. McKersie *et al*. [28] found that levels of ascorbate in leaves of alfalfa, generally declined with mild water stress.

Determination of MDA which is a secondary end product of polyunsaturated fatty acid oxidation is widely used to measure the extent of lipid peroxidation as response of oxidative stress. The rate of lipid peroxidation is used as an indicator to evaluate the tolerance of plants to oxidative stress as well as the sensitivity of plants to salt stress [29]. Our results showed that oxidation of lipids were affected by environmental stress such as heat, drought and salinity. However, there was a significant decline of MDA content in roots of legumes inhabiting NR and O compared with those inhabiting MR or S. A similar results were obtained from studies on rice [30], alfalfa [31], cotton [32] and wheat [33, 34]. Increasing MDA content in roots of *T. hamosa* and *V. sativa*, despite the concentration of $H_2O_2$ unchanged significantly, have lead to a suggestion that both species more sensitive to their habitat conditions than the other studied species. Although the level of $H_2O_2$ in roots of *T. resupinatum, T. hamosa* and *V. sativa* inhabiting S were high (compared with those inhabiting NR), it caused less damage to cell membranes as reflected by decreasing MDA.

Legumes principally produce and transport fixed nitrogen in the form of amides or ureides such as allantoin or allantoic acid [35]. The oxidation of purines to ureides occurs in the peroxisomes in two reactions where purine xanthine is firstly converted to uric acid which is secondly converted to allantoin by uricase. Convertion of uric acid to allantoin liberates $H_2O_2$ which requires antioxidant enzymes for destruction [36]. The ureides producers, that are common in tropical legumes, use less organic carbon to transport the same amount of nitrogen as do the amides [37]. In this study, the five species are indeterminate nodule-forming and amides exporter; so no $H_2O_2$ is further liberated. In addition, the amide transporter legumes are more tolerant to stressful conditions. The comparison between amides and ureides exporter legumes [38] also implies that the water use efficiency for amides-nitrogen exporter is higher. This may be due to the acceleration of nitrogen fixation system at roots by the rapid incorporation of amides passed to sinks of the plant (especially proteins). In addition, the solubility of amides is higher than that of ureides and the latter serve more as nitrogen storage forms.

Ascorbic acid is a strong antioxidant and abundantly exists in plants [39]. It plays many important roles in several cellular processes. It is involved in cell division and cell wall expansion, and regulates the plant growth and development [40]. The results in this study indicates that ascorbic acid level was high in most wild plants collected from S due to increasing stresses and harsh conditions and gradually the level decreased in wild plants collected from the less arid regions MR, O and NR. A wealth of information suggests that ascorbic acid plays a significant role in protection of plants against several environmental circumstances [41], such as salt stress [42], ozone [43], UV-B and pathogenesis [44], drought [45], and in heavy metal stress [46]. In this study, a negative correlation between contents of $H_2O_2$ and AsA were resulted in four studied legumes out of five (r-value ranged between -0.45 and -0.72). This indicates that AsA is an effective scavenging agent for ROS.

Plants have endogenous systems to protect cellular and sub cellular structures from the cytotoxic effects of active oxy-free radicals [47, 48, 49, 50]. The activities of antioxidant enzymes were variable among the studied legumes, and the level of response depends on the species and the extent of oxidative stresses. Exposure of plants to harsh conditions leads to generation of ROS, which stimulates the specific genes to encode antioxidant enzymes. In this study we found differential activity of enzymes in between legumes shoots and roots according to the type of species in the same region. For instance in S, GP enzyme activity in *M. indicus*, *M. polymorpha, T. resupinatum*, *T. hamosa* and *V. sativa* was 0.0364, 0.005, 0.025, 0.020 and 0.062 unit $mg^{-1}$ protein $min^{-1}$, respectively, while CAT activity was 0.667, 0.902, 1.3416, 0.953 and 1.078 unit $mg^{-1}$ protein $min^{-1}$. Mundree *et al*. [51] concluded that the effect of climatic factors on enzyme activity was high in CAT and APX enzymes and low in GP enzyme, where in periods of more severe stress in desiccation-sensitive plants, the scavenging system becomes saturated by the increased rate of radical production, and damage is inevitable. Changes of antioxidants reflect the impact of environmental stresses on plant metabolism [52, 53] and modulation of the activities of these enzymes may be important in the resistance of a plant to environmental stresses [54]. The results of this study prove that by increasing edaphic and climatic stress, as in S and MR, the activity of antioxidant enzymes of shoot and root systems increased but inhibited in plants of NR and O due to the advantage of favourable conditions. In root system, the activity of antioxidant enzymes CAT and APX was weak and variable, while GP activity was high. This may be due to the less generation of ROS in the roots, less ROS transported to the roots, or increasing stability of GP. Roots of wild plants in the soils of studied regions are exposed to great changes in the environmental conditions, especially the temperature. Abdel Gadir and Alexander [55] reported that in the Egyptian sandy soils, the temperature during day hours at surface was exceeding by about 20 ºC from the air temperature. However, presence of enzymes known for their stability like peroxidases will have an advantage in protecting the root cells from ROS. In addition, the plant enzymatic and non-enzymatic antioxidants play the crucial role to eliminate ROS, or reduce them to less harmful forms before reaching to the nodulated roots with the transported metabolites. The statistical analysis for $\eta^2$ showed that the variation in the environmental constraints of habitats or species-habitat interaction have the greatest magnitude of effect in regulation of gene expression of the antioxidant enzymes CAT, GP and APX. In contrast, differences between species have a weak effect in the gene expression of these antioxidant enzymes, as $\eta^2$ was less than 0.12 in shoots and less than 0.36 in roots of legumes.

The data of this study refer to a great variation between the concentration of $H_2O_2$ in shoots of plants and their modulated roots; however, the quation is why the antioxidation systems did not function efficiently in shoots as in roots. It was suggested by Willekens *et al*., [56] that CAT have lower affinity for $H_2O_2$ than APX, and both are only efficient at high levels of $H_2O_2$ and are essential for maintaining the redox balance during oxidative stress. However, as the concentration of $H_2O_2$ increased in the shoots of the studied 5 legumes compared with roots, the activity of CAT and APX increased especially at the more stressful habitats (MR and O). The GP activity in roots of the studied legumes was 2- to 12-fold of that estimated in shoots, and the over expression of GP gene in legumes roots enhanced scavenging $H_2O_2$ to be ranging between 6% and 37% of its concentration in the shoots. Roots are the first organs interacting with the environment and mediating signal exchange and communication between legumes and their associated Rhizobia. However, increasing GP gene expression in the roots under environmental constraints will assist Rhizobia to cope with the plant oxidative burst and ameliorate the legume stresses by protecting its cells from oxidative damages.

## CONCULSION

The present work revealed that the enzymatic and non enzymatic antioxidants of wild legumes are strongly affected by their native habitat conditions as evidenced by changing $H_2O_2$ and lipid peroxidation. Different species as will as different organs, shoots and roots here, differantially responded to the oxidative stress of the environmental constraints. The activity or expression of a difined antioxidant enzyme is regulated by the severity of stressful conditions, the plant species and organ, and by the stability of the enzyme itself. Enhanced activities of CAT and APX were reported in shoots of all the studied 5 legumes, while high activity of GP was reported in their roots. More induction of all assayed antioxidant enzymes activities were found in plants inhabiting the more stressful habitats.

## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

## FUNDING

This research was financially supported by Assiut University Fund.

## REFERENCES

[1] Tuteja N. Mechanisms of high salinity tolerance in plants. Meth Enzymol Osmosens Osmosignal 2007; 428: 419-438. https://doi.org/10.1016/S0076-6879(07)28024-3

[2] Khan S and Singh S. Abiotic Stress and Plant Responses. IK International, New Delhi 2008l; 159-189.

[3] Singh S, Khan NA, Nazar R and Anjum NA. Photosynthetic traits and activities of antioxidant enzymes in blackgram (Vigna mungo L. Hepper) under cadmium stress. Am J Plant Physiol 2008; 3: 25-32. https://doi.org/10.3923/ajpp.2008.25.32

[4] Botsford JL and Lewis TA. Osmoregulation in Rhizobium meliloti: production of glutamic acid in response to osmotic stress. Appl Environ Microbiol 1990; 56: 488- 494.

[5] Abid G, Hessini K, Marwa Aouida, et al. Agro-physiological and biochemical responses of faba bean (Vicia faba L. Var, 'minor') genotypes to water deficit stress. Biotechnol Agron Soc Environ 2017; 21(2).

[6] Mullineaux PM and Baker NR. Oxidative stress: antagonistic signaling for acclimation or cell death. Plant Physiol 2010; 154: 521-525. https://doi.org/10.1104/pp.110.161406

[7] Sharma P, Jha AB, Dubey RS and Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot Volume 2012, Article ID 217037.

[8] Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 2002; 7: 405-410. https://doi.org/10.1016/S1360-1385(02)02312-9

[9] Mittler R, Vanderauwera S, Gollery M and Van Breusegem F. Reactive oxygen gene network of lants. Trends Plant Sci 2004; 9: 490- 498. https://doi.org/10.1016/j.tplants.2004.08.009

[10] Caverzan A, Casassola A and Brammer SP. Antioxidant responses of wheat plants under stress. Genetics and Molecular Biology 2016; 39(1): 1-6. https://doi.org/10.1590/1678-4685-GMB-2015-0109

[11] Asada K. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Ann Rev Plant Physiol Plant Mol Biol 1999; 50: 601- 639. https://doi.org/10.1146/annurev.arplant.50.1.601

[12] Toda S. Antioxidative effects of polyphenols in leaves of Houttuynia cordata on protein fragmentation by copper-hydrogen peroxide in vitro. Journal of Medicinal Food 2005; 8: 266-268. https://doi.org/10.1089/jmf.2005.8.266

[13] Andrade RG, Ginani JS, Lopes GKB, Dutra F, Alonso A, et al. Tannic acid inhibits in vitro iron-dependent free radical formation. Biochimie 2006; 88: 1287-1296. https://doi.org/10.1016/j.biochi.2006.02.006

[14] Gratão PL, Polle A, Lea PJ and Azevedo RA. Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 2005; 32: 481-494. https://doi.org/10.1071/FP05016

[15] Scandalios JG. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz J Med Biol Res 2005; 38: 995-1014. https://doi.org/10.1590/S0100-879X2005000700003

[16] Pastori G, Foyer CH and Mullineaux P. Low temperature-induced changes in the distribution of H2O2 and antioxidants between the bundle sheath and mesophyll cells of maize leaves. J Exp Bot 2000; 51: 107-113. https://doi.org/10.1093/jexbot/51.342.107

[17] Abd-Alla MH, Elenany AE, Ramadan T, El Zohri M and Nafady IM. Nodulation and nitrogen fixation of some wild legumes from differing habitats in Egypt. European Journal of Biological Research 2017; 7(1): 9-21.

[18] Mukherjee S and Choudhuri M. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiologia Plantarum 1983; 58: 166-170. https://doi.org/10.1111/j.1399-3054.1983.tb04162.x

[19] Madhava Rao K and Sresty T. Antioxidative parameters in the seedlings of pigeonpea (Cajanus cajan (L.) Millspaugh) in response to Zn and Ni stresses. Plant Science 2000; 157: 113-128. https://doi.org/10.1016/S0168-9452(00)00273-9

[20] Kofalvi S and Nassuth A. Influence of wheat streak mosaic virus infection on phenylpropanoid metabolism and the accumulation of phenolics and lignin in wheat. Physiological and Molecular plant pathology 1995; 47: 365-377. https://doi.org/10.1006/pmpp.1995.1065

[21] Jagota S and Dani H. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Analytical biochemistry 1982; 127: 178-182. https://doi.org/10.1016/0003-2697(82)90162-2

[22] Lowry O, Rosebrough N, Farr A and Randall R. Protein measurement with the Folin phenol reagent. J Bil Chem 1951; 193: 265- 275.

[23] Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

[24] Tatiana Z, Yamashita K and Matsumoto H. Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber roots. Plant Cell Physiol 1999; 40: 273-280. https://doi.org/10.1093/oxfordjournals.pcp.a029538

[25] Nakano Y and Asada K. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant and cell physiology 1981; 28: 131-140.

[26] Szymańska R, Ślesak I, et al. Physiological and biochemical responses to high light and temperature stress in plants. Environmental and Experimental Botany 2017; 139: 165-177. https://doi.org/10.1016/j.envexpbot.2017.05.002

[27] Neill S, Desikan R and Hancock J. Hydrogen peroxide signalling. Curr Opin Plant Biol 2002; 5: 388-395. https://doi.org/10.1016/S1369-5266(02)00282-0

[28] McKersie BD, Bowley SR, Harjanto E and Leprince O. Water-deficite tolerance and field performance of transgenic alfalfa overexpresing superoxide dismutase. Plant Physiol 1996; 111: 1177-1181. https://doi.org/10.1104/pp.111.4.1177

[29] Jain M, Mathur G, Koul S and Sarin NB. Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (Arachis hypogea L.). Plant Cell Rep 2001; 20: 463-468. https://doi.org/10.1007/s002990100353

[30] Tijen D and Ìsmail T. Comparative lipid peroxidation, antioxidant defense systems and praline content n roots of two rice cultivars differing in salt tolerance. Environ Exp Bot 2005; 53: 247-257. https://doi.org/10.1016/j.envexpbot.2004.03.017

[31] Wang XS and Han JG. Effects of NaCl and silicon on ion distribution in the roots, shoots and leaves of two alfalfa cultivars with different salt tolerance. Soil Sci Plant Nutr 2007; 53(3): 278-285. https://doi.org/10.1111/j.1747-0765.2007.00135.x

[32] Diego AM, Marco AO, Carlos AM and José C. Photosynthesis and activity of superoxide dismutase peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 2003; 49: 69-76. https://doi.org/10.1016/S0098-8472(02)00058-8

[33] Sairam RK, Rao KV and Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci 2002; 163: 1037-1046. https://doi.org/10.1016/S0168-9452(02)00278-9

[34] Milone MT, Sgherri C, Clijters H and Navari-Izzo F. Antioxidative responses of wheat treated with realistic concentrations of cadmium. Env Exp Bot 2003; 50: 265-273. https://doi.org/10.1016/S0098-8472(03)00037-6

[35] Taiz L and Zeiger E. Plant Physiology. 2nd Edition, Sinauer Associates Publishers, Sunderland, Massachusetts 1998. http://dx.doi.org/10.1071/PP9840361. https://doi.org/10.1071/PP9840361

[36] Newcomb EH. Ultrastructure and cytochemistry of plant peroxisomes and glyoxysomes. In: Kindl H, Lazarow PB (eds) Peroxisomes and Glyoxysomes. Annals of the New York Academy of Science 1982; 386: 228-241. https://doi.org/10.1111/j.1749-6632.1982.tb21419.x

[37] Pate JS, Atkins CA and Rainbird RM. Theoretical and experimental costing of nitrogen fixation and related processes in nodules of legumes. In: Gibson AH, Newton WE (eds). Current Perspectives in Nitrogen Fixation. Aust Acad Sci, Canberra 1981; 105-116.

[38] Pate JS, Layzell DB and MacNeill DL. Modeling the transport and utilization of carbon in a nodulated legume. Plant Physiol 1979; 63: 730-738. https://doi.org/10.1104/pp.63.4.730

[39] Smirnoff N. Ascorbic acid: metabolism and functions of a multi-facetted molecule. Curr Opin Plant Biol 2000; 3: 229-235. https://doi.org/10.1016/S1369-5266(00)00069-8

[40] Pignococchi C and Foyer CH. Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. Curr Opin Plant Biol 2003; 6: 379-389. https://doi.org/10.1016/S1369-5266(03)00069-4

[41] Paital B and Chainy GBN. Antioxidant defenses and oxidative stress parameters in tissues of mud crab (Scylla serrata) with reference to change salinity. Original Research Article. Comparative Biochemistry and Physiology, Part C: Toxicol Pharmacol 2010; 151: 142-147. https://doi.org/10.1016/j.cbpc.2009.09.007

[42] Shalata A and Neumann PM. Exogenous ascorbic acid (vitamin C) increases resistance of salt stress and reduces lipid peroxidation. J Exp Bot 2001; 52: 2207-2211. https://doi.org/10.1093/jexbot/52.364.2207

[43] Sanmartin M, Drogoudi PD, Lyons T, Pateraki I, Barnes J, et al. Overexpression of ascorbate oxidase in the apoplast of transgenic tobacco results in altered ascorbate and glutathione redox states and increased sensitivity to ozone. Planta 2003; 216: 918-928.

[44] Fotopoulos V, Sanmartin M and Kanellis AK. Effect of ascorbate oxidase over expression on ascorbate recycling gene expression in response to agents imposing oxidative stress. J Exp Bot 2006; 57: 3933-3943. https://doi.org/10.1093/jxb/erl147

[45] Fotopoulos V, Tullio D, Barnes J and Kanellis AK. Altered stomatal dynamics in ascorbate oxidase over-expressing tobacco plants suggest a role for dehydroascorbate signaling. J Exp Bot 2008; 59: 729-737. https://doi.org/10.1093/jxb/erm359

[46] Vwioko ED, Osawaru ME and Eruogun OL. Evaluation of okra (Abelmoschus esculentus L. Moench.) exposed to paint waste contaminated soil for growth, ascorbic acid and metal concentration. Afr J Gen Agric 2008; 4: 39-48.

[47] Liebler DC, Kling DS and Reed DJ. Antioxidant protection of phospholipid bilayers by α-tocopherol. Control of α-tocopherol status and lipid peroxidation by ascorbic acid and glutathione. J Biol Chem 1986; 261: 12114-12119.

[48] Elstner EF. Metabolism of activated oxygen species. In: Davies DD (ed) The Biochemistry of Plants, Biochemistry of Metabolism, Vol. 11. Academic Press, San Diego, USA 1987; 253-315. https://doi.org/10.1016/B978-0-12-675411-7.50014-8

[49] Larson RA. The antioxidants of higher plants. Phytochemistry 1988; 27: 969-978. https://doi.org/10.1016/0031-9422(88)80254-1

[50] Omprakash, Gobu R, Bisen P, Baghel M and Chourasia KN. Resistance/tolerance mechanism under water deficit (drought) condition in plants. Int J Curr Microbiol App Sci 2017; 6(4): 66-78. https://doi.org/10.20546/ijcmas.2017.604.009

[51] Mundree SG, Baker B, Mowla S, Peters S, Marais S, et al. Physiological and molecular insights into drought tolerance. African J Biotech 2002; 1(2): 28-38. https://doi.org/10.5897/AJB2002.000-006

[52] Herbinger KM, Tausz A, Wonisch G, Soja A and Sorger Grill D. Complex interactive effects of drought and ozone stress on the antioxidant defense systems of two wheat cultivars. Plant Physiol Biochem 2002; 40: 691-696. https://doi.org/10.1016/S0981-9428(02)01410-9

[53] Cho U and Seo N. Oxidative stress in Arabidopsis thaliana exposed to cadmium is due to hydrogen peroxide accumulation, Plant Sci 2005; 168: 113-120. https://doi.org/10.1016/j.plantsci.2004.07.021

[54] Allen RD. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol 1995; 107: 1049-1057. https://doi.org/10.1104/pp.107.4.1049

[55] Abdel Gadir AH and Alexander M. Procedures to enhance heat resistance of Rhizobium. Plant Soil 1997; 188: 93-100. https://doi.org/10.1023/A:1004200329070

[56] Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, et al. Catalase is a sink for H2O2 and is indispensable for stress defence in C-3 plants. EMBO Journal 1997; 16: 4806-4816. https://doi.org/10.1093/emboj/16.16.4806

Received on 25-09-2017 Accepted on 03-10-2017 Published on 31-12-2017

DOI: https://doi.org/10.12974/2311-858X.2017.05.02.4