

# Differential physiological responses of three Sesame genotypes to drought stress and the expression of antioxidant genes

Hiba Fouad Abdulfatah<sup>1</sup>, Dhia S. Hassawi<sup>1</sup> and Saeid Abu-Romman<sup>2</sup>

<sup>1</sup>Departement of Biology, College of Sciences, University of Anbar, Al-Anbar, Iraq

<sup>2</sup>Departement of Biotechnology, Faculty of Agricultural Technology, Al-Balqa Applied University, Al-Salt, 19117 Jordan

(Received 12 November, 2020; accepted 19 March, 2021)

## ABSTRACT

This study was conducted to determine the physiological responses and the expression of major antioxidant genes (*Cu/Zn-SOD*, *CAT*, and *APX*) to drought stress in three sesame (*Sesamum indicum* L.) genotypes. Drought conditions were imposed at three levels: D1 (100%), D2 (50%), and D3 (25%) of field capacity on three major Iraqi genotypes of sesame, namely Wadaa, Sumer, and Local. The physiological results indicated that variety Wadaa was characterized by the highest relative water content (82%). The expression of *Cu/Zn-SOD* gene was influenced by drought in Wadaa variety and recorded the lowest gene expression compared to non-stress plants at 50% of field capacity. Gradual increase of expression was recorded in Sumer and Local varieties to reach 43.63 and 50.98-fold over, respectively of the normal expression. The expression of *CAT* gene was up-regulated in both Wadaa and Sumer for treatments D2 and D3, and up-expression for treatment D2 with the Local variety while down-regulated from normalized expression for D3 treatment (1.56-fold). The expression levels of *APX* gene increased in Wadaa variety for the treatment D2 and decreased for the treatment D3 by 83.33-fold. While its expression up-regulated for the treatments D2 and D3 in Sumer variety as well as in treatment D2 in the Local variety. Severe drought at 25% of field capacity caused down-regulated in *APX* in Local variety and scored 1.26-fold than non-stress plants.

**Key word :** Sesame, Antioxidant enzymes, Gene expression, Drought tolerance, RWC

## Introduction

Sesame (*Sesamum indicum* L.  $2n = 26$ ) is an essential oil and food crops in many countries in warm and semi-warm temperate regions. Globally, demand for sesame is growing mainly due to three reasons: first sesame has a high medicinal and nutritional value (Anastasi *et al.*, 2017). Second, it is consumed in both the oil and confectionary industries (Peter, 2012), and third, it has been identified as a good source of high-grade oil (high proportion of unsaturated fatty acids, protein content and antioxidants) (Bahrami *et*

*al.*, 2012).

In a persistently changing environment, plants are constantly challenged by various abiotic stresses (Abu-Romman, 2016), such as salinity, drought, temperature extremes, heavy metal toxicity, high light intensity, nutrient deficiency, UV-B radiation, ozone, etc., which cause significant crop yield and quality losses. Drought stress is one of the most prevailing abiotic stress affecting plant growth and development (Abu-Romman and Suwwan, 2012). Drought stress happens when the water available in the soil is limited to such low levels and atmospheric

conditions contribute to the constant loss of water. Under water stress conditions, growth is inhibited and a variety of molecular, biochemical and physiological changes are produced (Hsu *et al.*, 2003). However, unlike most of the important food crops, sesame can survive drought but severe and repeated drought episodes, especially occurring during the reproductive stage, significantly curtail the productivity of this high oil crop (Dossa *et al.*, 2019).

According to Gill and Tuteja, (2010), drought stress led to release ROS, which is mainly produced by aerobic respiration in mitochondria, photosynthesis in chloroplasts, and photorespiration in peroxisomes such as singlet oxygen (O), superoxide (O<sub>2</sub><sup>•-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (OH). Du *et al.*, (2013) reported that ROS may inactivate biomolecules and initiate autocatalytic peroxidation of the membrane and other macromolecules, such as photosynthetic pigments, proteins, lipids and nucleic acids, resulting in loss of membrane integrity and some functional alterations. Thus, their levels must be closely and carefully monitored inside the cell so that the injury caused by ROS is known as "oxidative stress." Increased production of ROS induces molecular reactions, which are key factors in the determination of plant tolerance to stress (Dudziak *et al.*, 2019). ROS appears to have a dual effect under abiotic stress conditions that depend on their total cell count. If maintained at relatively low levels, they are likely to function as components of the stress-signaling pathway, triggering a stress defense/ acclimation response.

Enhancing antioxidant defense in plants can therefore increase tolerance to different types of reactive oxygen (ROS) stress factors. Defending mechanisms against free radical oxidative stress include: (i) precautionary mechanisms; (ii) repair mechanisms; (iii) physiological defenses; and (iv) antioxidant defenses (Mittova *et al.*, 2003). Antioxidant defense systems that work together to regulate uncontrolled oxidation cascades and protect plant cells from oxidative damage by scavenging ROS (Gill and Tuteja, 2010). In oxidative stress, redox homeostasis is maintained by enzymatic antioxidants such as superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), guaiacol peroxidase (POD EC 1.11.1.7), glutathionereductase (GR, EC 1.6.4.2), catalase (CAT, EC 1.11.1.6), Dehydroascorbatereductase and monodehydroascorbatereductase) (Noctor and Foyer, 1998), and non-enzymatic low molecular

compounds such as ascorbic acid (AA), glutathione, carotenoids and anthocyanins. Additional compounds, such as osmolytes, proteins (e.g. peroxiredoxin) and amphiphilic molecules (e.g.  $\alpha$ -tocopherol), may also function as ROS scavengers, reduced glutathione (GSH), carotenoids, phenolics, flavonoids, and proline (Gill and Tuteja, 2010).

The aim of this study was to explore the physiological responses of sesame varieties to different drought levels. Also, gene expression profile of selected antioxidant genes was investigated.

## Materials and Methods

The experiment was conducted in the laboratory of College of Science/University of Anbar during the season of 2019. The plant materials included three sesame genotypes: Wadaa, Sumer and Local that were obtained from College of Agriculture/Department of Field Crops and from the Center of Desert Studies/University of Anbar. The soil was mixed with peatmoss in 5:1 ratio. The seeds were sterilized with 10% sodium hypochlorite for 1-2 min and washed two times with sterile distilled water. Five sesame seeds were planted in each of six pits that made in (25 × 25 cm) pots containing 9 Kg of the prepared soil. After germination, the seedlings were thinned and the strongest and healthiest one was kept in each pit. The plants were irrigated and fertilized with urea at a concentration of 100 mg/kg soil. The urea fertilizer was divided and applied at planting date and after one month from the first application.

The three sesame genotypes were subjected to drought stress after 55 days from planting date by using tap water for three treatments: 100% irrigation (D1, control), 50% of field capacity (D2), and 25% of field capacity (D3). Irrigation was applied daily at 7-8 AM by using graduated cylinder. For physiological and molecular analysis, the samples were collected after 100 days of the planting date.

## Physiological indices

The RWC of the leaves was measured as an indicator of leaf hydration at reproductive stages based on fresh (FW), turgid (TW) and dry (DW) weights (Barr and Weatherley, 1962). RWC was determined according to the formula: (FW-DW)/ (TW-DW) × 100.

Cell membrane permeability was measured as membrane stability index (MSI); electrolyte leakage measured at the reproductive stage. Leaf samples

were cut into six equal disk sizes of 12 mm and immersed in 10 ml of distilled water for 12 h. The electrical conductivity (EC1) of the solution was measured by EC meter (Trans-BC3020, China). The samples were then heated in a 60°C water bath for 20 minutes, and the second electrical conductivity (EC2) of the heated samples was measured. Electrolyte leakage was estimation by using the del Carmen Rodríguez-Hernández *et al.*, (2013) formula:

$$\text{MSI}\% = (\text{EC1}/\text{EC2}) \times 100$$

The proline content was estimated according to Bates *et al.*, (1973) from 0.2 gm of fully expanded leaf collected at reproductive stage. Standard curve of pure proline (0.1 mg. ml<sup>-1</sup>) was prepared taking different concentrations of proline, using toluene as a blank. The proline content of plant material was calculated on a fresh weight using standard curve as follows:

$$[(\mu\text{g proline} / \text{ml} \times \text{ml toluene}) / 115.5 \mu\text{g} / \mu\text{moles}] / [(g \text{ sample}) / 5] = \mu\text{moles proline} / g \text{ of fresh weight material.}$$

### Expression analysis of antioxidant genes

Total RNA was isolated from sesame leaf samples using the QIAzol® reagent and the concentration was quantified using Qubit 4 fluorometer. The transcription levels of all genes were analyzed using quantitative real-time PCR (RT-PCR, Bioer, Japan) and the assessment of the melting curves confirmed that all primers generated only a single amplicon and that no primer dimers or unexpected products were found. Actin's gene (*SiActin*) was used as an internal reference to verify the equivalent loading of cDNA. Primers were designed using Primer3 program (Table 1).

A total of 20 µl of PCR reaction mixture has been prepared for the reaction mix by combining 10 µl of GoTaq® Probe qPCR Master Mix with dUTP with

0.4 µl of GoScript™ RT Mix for 1-Step RT-qPCR, 2 µl of each gene-specific primer, 5 µl of 10 pg RNA template and Nucleas-free water was added to make up the final volume of 20 µl. Amplifications were performed for 1 cycle of 15 minutes at 37 °C for reverse transcription, 1 minute at 95°C for activation of GoTaq® DNA polymerase. For denaturation 40 cycles of 10 seconds at 95 °C, 40 cycle at 60 °C for 30 seconds for the Anneal/Collect data, 40 cycles at 72 °C for 30 seconds to Extend, Melting Stage 1 cycle at 95 °C for 5 minutes, 1 cycles at 60 °C for 5 minutes to annealing and final denaturation (Read) step were performed by 1 cycle 10 minute at 95 °C. The relative quantification of the differential expression was carried out using the comparative threshold cycle method as described by Livak and Schmittgen, (2001).

## Results and Discussion

### Relative water content (RWC)

The decrease in RWC leaves under drought conditions was shown in Table 2. Wadaa variety showed the highest ratio of 82.0%, while Sumer and Local showed the lowest ratio of 77.7% and 77.0 %, respectively and was therefore identified as an indicator of drought tolerance in crops (Jones, 2007). The effect of the interaction between the drought and varieties of the RWC of treatments in the reproductive stage shows the distinction of the Local variety in D2 irrigation level with the highest percentage was 91.7%.

High relative water content (RWC) has previously been reported to improve the ability of plants to extract soil water for increased water uptake associated with PIP1 and RWC3 aquaporin gene in maize, resulting in improved root water uptake and, as a result, increased drought tolerance (Comas *et al.*, 2013). Hassanzadeh *et al.*, (2009) also reported that the rate of RWC in plants with high resistance to

**Table 1.** List of primers used in the current study.

Gene	Accession Number	Sequence (5'→3')	Amplicon size
<i>Cu/Zn-SOD</i>	XM_011090495.2	Forward-CCTCTGACTGGTCCTGACTC Reverse-ACAGGGGAGTCAAACCAACA	151 bp
<i>CAT</i>	NM_001304397.1	Forward-GCCCTGCTATTATCGTCCCT Reverse-ACCTCTTCATCCCTGTGCAT	196 bp
<i>APX</i>	XM_011091553.2	Forward-GCTGGAGTTGTTGCTGTTGA Reverse-CGCAACAATATCCTGGTCCG	177 bp
<i>SiActin</i>	XM_011076839.2	Forward-CCCAGGTATTGCTGACAGGA Reverse-GCCAGCCTCATCATACTCT	178 bp

drought is higher than in others. In other words, plants with higher yields under drought stress should have a high RWC. Therefore, on the basis of the results, the genotypes mentioned, which are classified as high and medium yielding genotypes, should be of high RWC content.

Drought treatment appears to have a significant decrease in the RWC ratio with decreased water irrigation at reproductive stage and a clear decrease in the D3 value of 68.5 % compared to the control and D2 treatment levels were 83.8% and 84.4%. The increase in RWC in irrigation level D2 may be due to the great role of water in increasing the activity of antioxidant enzymes (*CAT* and *APX*), which were over expressed in this treatment, it may also have significance in the acquisition by the plant of an adaptive characteristic in order to resist the shortage of water in this treatment. On the contrary decrease in RWC leaf in D3 may be due to limited availability of water for plants use.

RWC also establishes a close relationship with plant water potential, in such a way that the decreasing RWC can be attributed to the imbalance between re-watering and water losses due to canopy evapotranspiration (Jones, 2007). In the study of sesame, Anee *et al.*, (2019); Behzadnejad *et al.*, (2020) reported similar results.

### Electrolyte leakage (EL)

Electrolyte leakage results (Table 3) showed no significant differences among sesame varieties. For D3 irrigation water level Wadaa variety recorded highest ratio (59.3%). The degree of cell membrane injury caused by water stress can be easily estimated by measuring electrolyte leakage from the cells (Bajji *et al.*, 2002); In fact, it was assumed that sugars, particularly non-reducing disaccharides such as sucrose and trehalose (in a few species) interact with cell membranes to increase cellular membranes stability of the lipid layers (Nilsen and Orcutt, 1996).

The protection mechanism of these solutes is still uncertain, but one hypothesis suggests that, under desiccation, molecules normally associated with phospholipids head-groups are replaced by sugars (Leopold and Vertucci, 1986). This may prevent the lateral phase transition and the formation of lipid domains that have the potential to form inverted lipid domains and thus increase membrane leakage, increased accumulation of such compatible solutes in drought resistant cultivars' leaf tissues would reduce dehydration damage and promote growth during and after water stress (Bajji *et al.*, 2000).

Significant differences were existed among drought treatments; D3 recorded 51.8% compared with D1 that recorded 28.3%. The increase in electro-

**Table 2.** Relative water content (%) for sesame varieties that treated with different drought levels

Variety name	RWC (%) in reproductive stage			
	D1 (100% Field Capacity)	D2 (50% Field Capacity)	D3 (25% Field Capacity)	Average
Wadaa	87.6	90.9	67.4	82.0 <sup>a</sup>
Sumer	81.0	70.6	81.3	77.7 <sup>b</sup>
Local	82.7	91.7	56.6	77.0 <sup>b</sup>
LSD P≤0.05	10.83			
Average	83.8 <sup>a</sup>	84.4 <sup>a</sup>	68.5 <sup>b</sup>	
LSD P≤0.05	6.26	6.26		

**Table 3.** Electrolyte Leakage (%) for sesame varieties that treated with different drought levels

Variety name	EL % in reproductive stage			
	D1 (100% Field Capacity)	D2 (50% Field Capacity)	D3 (25% Field Capacity)	Average
Wadaa	26.1	54.6	59.3	46.7 <sup>N.S</sup>
Sumer	32.5	48.9	49.5	43.6 <sup>N.S</sup>
Local	26.4	39.9	46.7	37.6 <sup>N.S</sup>
LSD P≤0.05	36.58			
Average	28.3 <sup>b</sup>	47.8 <sup>ab</sup>	51.8 <sup>a</sup>	
LSD P≤0.05	21.12	21.12		

lyte leakage as soil water content decreased can be attributed to a consequence of the limitation in photo synthesis imposed by the water deficit, as a result of the disruption of membrane integrity caused by overproduction of reactive oxygen species (ROS). High levels of these compounds may reduce the activity of enzymes, cause lipid peroxidation, oxidize proteins, and kill cells at the most advanced stage (Du *et al.*, 2013). Lima *et al.*, (2018) has found similar result.

### Proline content

As shown in Table 4, no significant differences were observed among sesame varieties for proline content. Sumer scored elevated proline content with D3 treatment (8.88  $\mu\text{mole.g plant}^{-1}$ ). Proline acts as an osmoprotective molecule, maintaining and improving the water status of plants (Anee *et al.*, 2019); also, proline acts as an antioxidant, defending cells from free radical damage and maintaining the cellular environment for better synthesis of biomolecules that play a major role in stress adaptation (Barickman *et al.*, 2019). Dossa *et al.*, (2017) reported that proline accumulation was significantly higher in drought tolerance plants compared to drought sensitivity, especially when drought stress was severe.

The irrigation water treatments increased proline content in D3 (7.83  $\mu\text{mole.g plant}^{-1}$ ) compared to D1 (5.49  $\mu\text{mole.g plant}^{-1}$ ). Increases proline proportionately faster than other amino acids in plants under water stress have been suggested as an assessment parameter for irrigation planning and selection of drought-resistant (Bates *et al.*, 1973). Gill and Tuteja, (2010) found that proline is involved in oxidative stress tolerance mechanisms, and this was the plant's main strategy to avoid harmful oxidative stress effects due to water stress.

### Expression analysis of antioxidant genes

Insufficient availability of the electron acceptor  $\text{CO}_2$  slows down the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) in the Calvin-Benson cycle. Lack of  $\text{NADP}^+$  causes a backlog of electrons and over-reduction of the photosynthetic electron transport which in turn increases the reduction rate of oxygen as alternative electron acceptor in the Mehler reaction at photosystem I (PSI) and enhanced release of superoxide anion ( $\text{O}_2^{\cdot-}$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in drought-treated cells during photosynthesis ((Cruz de Carvalho, 2008).

To study the molecular impacts drought stress and the associated ROS release and oxidative damage on sesame, gene expression analyses of *Cu/Zn-SOD*, *CAT*, and *APX* genes were investigated. SOD is considered as a key enzyme for maintaining normal physiological conditions and coping with oxidative stress in the regulation of intracellular levels of ROS, it is the first enzyme in the detoxifying process, converts  $\text{O}_2$  radicals to  $\text{H}_2\text{O}_2$  in plants (Mittler, 2002).

The results presented in Table 5 showed down-regulation *Cu/Zn-SOD* gene in Wadaa after treated with drought stress. The treatments of drought led to gradual increase in its expression for Sumer and Local, as the highest expression was in D2 treatment (43.631-fold and 50.984-fold, respectively) and in D3 treatment (7.514-fold and 21.008-fold, respectively). SOD is one of several important antioxidant enzymes with the ability to repair oxidative damage on drought tolerant variety of sesame (*Sesamum indicum* L.) under water stress (Fazeli *et al.*, 2007). Mehrabad Pour-Benab *et al.*, (2019) showed that SOD expression was increased in all species of wheat under the drought stress treatment compared to the well-watered conditions.

Under drought condition, CAT turns over

**Table 4.** Plants proline content for sesame varieties that treated with different drought levels

Variety name	Proline ( $\mu\text{mole.g plant}^{-1}$ ) in reproductive stage			
	D1 (100% Field Capacity)	D2 (50% Field Capacity)	D3 (25% Field Capacity)	Average
Wadaa	5.90	6.52	7.00	6.47 <sup>N.S</sup>
Sumer	4.74	6.87	8.88	6.83 <sup>N.S</sup>
Local	5.82	8.16	7.62	7.20 <sup>N.S</sup>
LSD $P \leq 0.05$	3.392			
Average	5.49 <sup>b</sup>	7.18 <sup>ab</sup>	7.83 <sup>a</sup>	
LSD $P \leq 0.05$	1.958	1.958		

N.S: Non Significant

quickly in leaf cells and is vital for the eliminating of free oxygen radicals formed in the peroxisomes by photorespiration (Mittler, 2002). CAT is a tetrameric, heme-containing enzyme that catalyzes the dismutation of  $H_2O_2$  into  $H_2O$  and  $O_2$  in the peroxisome. The results (Table 5) revealed that the expression level CAT gene in Wadaa was increased by 17.895-fold in response to D2 treatment, while the expression level was slightly increased by 1.77-fold in response to D3 treatment. The same applies to the cultivar Sumer, which showed an over expression of CAT gene in D2 treatment (6.453 fold) while in D3 treatment, the expression scored 4.723 fold. CAT gene expression was elevated in D2 treatment for the Local variety by 1.531-fold while it decreased by 1.569-fold than normalized for treatment D3. The increasing trend in  $H_2O_2$  accumulation due to increased stress duration is due to decreased CAT activity as an  $H_2O_2$  scavenging enzyme (Hasanuzzaman *et al.*, 2012). Uzilday *et al.*, (2012) reported that high levels of CAT expression were observed in *Cleome gynandra* and *Cleome spinosa* during prolonged drought exposure (10 days). Dudziak *et al.*, (2019) suggested that short-term drought has increased CAT expression as a rapid plant response. However, prolonged exposure to drought could lead to inhibition of expression and lower transcription levels.

APX is widely dispersed in plant cells, and different isoforms are more efficient in removing  $H_2O_2$  under stressor conditions (Sharma *et al.*, 2012). The expression of the APX gene (Table 5) varied among the three varieties of sesame under the influence of drought, as the expression in Wadaa reached 4512.403-fold in D2 treatment while it was down-regulated in D3 treatment. APX gene expression increased in Sumer variety in both D2 treatment and D3 treatment as it scored a high expression in D2 treatment, 825.001-fold, while it was characterized by a little increase in D3 treatment with 7.693-fold

compared to non-stress plants. APX gene of Local variety showed an increase in the expression with 50% irrigation level and scored 12.466 fold, while it decreased from normal for D3 treatment to record a down-regulated with 1.265-fold compared to D1 treatment.

The pattern for APX gene expression was characterized by an initial increase, followed by a decrease with increase drought period (Dudziak *et al.*, 2019). APX, as the main component in the ascorbate-gluthathione cycle, has a crucial role in the balancing of the intercellular ROS accumulation. Under the non-stress and drought stress conditions, the expression of APX gene was significantly variable among species (Mehrabad Pour-Benab *et al.*, 2019). Ren *et al.*, (2016) reported that the expression of cytosolic APX and dehydroascorbatereductase (DHAR) was followed by significant up-regulation, indicating that they were regulated at transcriptional level for drought resistant plants exposed to long-term soil drought. In contrast, drought susceptible plants exhibited high ROS levels and poor antioxidant enzyme response, leading to increased membrane damage during long-term soil drought conditions.

Several studies in plants have shown that relatively low levels of ROS act as signaling molecules that induce abiotic stress tolerance by controlling the expression of defense genes. In addition, numerous results have shown that plants with higher levels of antioxidants, whether constitutive or induced, show greater resistance to different types of environmental stress (Hasanuzzaman *et al.*, 2012). Guan and Scandalios, (2000) reported that expression patterns of APX, CAT and SOD in barley under drought conditions depend on the stage of plant development and genotype. The comparison of tolerant with sensitive phenotypes highlights the role of the antioxidant system in drought tolerance. For instance, CAT, APX and SOD levels are up-regulated in the drought-tolerant CE704 genotype (maize), while

**Table 5.** Relative expression of Cu/Zn-SOD, CAT and APX genes in sesame varieties subjected to different drought levels. Values were normalized to sesame to reference gene using the  $2^{-\Delta\Delta Ct}$  method and expressed as ratios relative to D1 treatment (set as 1).

Variety	Cu/Zn-SOD		CAT		APX	
	D2 (50% Field Capacity)	D3 (25% Field Capacity)	D2 (50% Field Capacity)	D3 (25% Field Capacity)	D2 (50% Field Capacity)	D3 (25% Field Capacity)
Wadaa	0.0002	0	18.895	2.770	4513.403	0.012
Sumer	44.631	8.514	6.453	4.723	826.001	8.693
Local	51.984	22.008	2.531	0.637	12.466	0.790

CAT and APX levels decreased in the drought-sensitive 2023 genotype (Benešová *et al.*, 2012).

## Conclusion

The results showed a significant increase in the distinction of the variety Wadaa for RWC. The results also showed differential response of antioxidant enzymes at gene expression, (*Cu-Zn-SOD*) down-regulated in Wadaa variety; the gene expression of CAT and APX over expression at D2 treatment for the three varieties, indicating that sesame plants tolerance to this level of drought. SOD, CAT, and APX activities particularly in tolerant genotypes, protected them against oxidative damage, as indicated by the lower ROS production, and also specified their role in drought tolerance. This observation warrants further exploration to clarify the complex mechanism underlying the antioxidant defense system of plants for enhanced drought tolerance.

## References

- Abu-Romman, S. and Suwwan, M. 2012. Effect of phosphorus on osmotic-stress responses of cucumber microshoots. *Advances in Environmental Biology*. 6(5) : 1626-1632.
- Abu-Romman, S. 2016. Genotypic response to heat stress in durum wheat and the expression of small HSP genes. *Rendiconti Lincei*. 27(2) : 261-267.
- Anastasi, U., Sortino, O., Tuttobene, R., Gresta, F., Giuffrè, A.M. and Santonoceto, C. 2017. Agronomic performance and grain quality of sesame (*Sesamum indicum* L.) landraces and improved varieties grown in a Mediterranean environment. *Genetic Resources and Crop Evolution*. 64(1) : 127-137.
- Anee, T.I., Nahar, K., Rahman, A., Mahmud, J.A., Bhuiyan, T.F., Alam, M.U., Fujita, M. and Hasanuzzaman, M. 2019. Oxidative damage and antioxidant defense in *Sesamum indicum* after different waterlogging durations. *Plants*. 8(7) : 196. [www.mdpi.com/journal/plants](http://www.mdpi.com/journal/plants)
- Bahrami, H., Razmjoo, J. and Jafari, A.O. 2012. Effect of drought stress on germination and seedling growth of sesame cultivars (*Sesamum indicum* L.). *International Journal of AgriScience*. 2(5) : 423-428.
- Bajji, M., Kinet, J.M. and Lutts, S. 2002. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation*. 36(1) : 61-70.
- Bajji, M., Lutts, S. and Kinet, J.M. 2000. Resistance to water stress in durum wheat: comparison of reactions of cells and whole seedlings. *Resistance to water stress in durum wheat: comparison of reactions of cells and whole seedlings*. (40) : 227-231.
- Barickman, T.C., Simpson, C.R. and Sams, C.E. 2019. Waterlogging causes early modification in the physiological performance, carotenoids, chlorophylls, proline, and soluble sugars of cucumber plants. *Plants*. 8(6) : 160.
- Barrs, H.D. and Weatherley, P.E. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*. 15(3) : 413-428.
- Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*. 39(1) : 205-207.
- Behzadnejad, J., Tahmasebi-Sarvestani, Z., Aein, A. and Mokhtassi-Bidgoli, A. 2020. Wheat straw mulching helps improve yield in sesame (*Sesamum indicum* L.) under drought stress. *International Journal of Plant Production*, pp.1-12. <https://doi.org/10.1007/s42106-020-00091-8>
- Benešová, M., Hola, D., Fischer, L., Jedelský, P.L., Hnilička, F., Wilhelmová, N., Rothova, O., Kočová, M., Prochazkova, D., Honnerova, J. and Fridrichova, L. 2012. The physiology and proteomics of drought tolerance in maize: early stomatal closure as a cause of lower tolerance to short-term dehydration?. *PLoS One*. 7(6) : p.e38017. <https://doi.org/10.1371/journal.pone.0038017>
- Comas, L., Becker, S., Cruz, V.M.V., Byrne, P.F. and Dierig, D.A. 2013. Root traits contributing to plant productivity under drought. *Frontiers in Plant Science*. 4 : 442.
- Cruz de Carvalho, M.H. 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. *Plant Signaling & Behavior*. 3(3) : 156-165.
- del Carmen Rodríguez-Hernández, M., Moreno, D.A., Carvajal, M. and Ballesta, M.D.C.M. 2013. Interactive effects of boron and NaCl stress on water and nutrient transport in two broccoli cultivars. *Functional Plant Biology*. 40(7) : 739-748.
- Dossa, K., Diouf, D., Wang, L., Wei, X., Zhang, Y., Niang, M., Fonceka, D., Yu, J., Mmadi, M.A., Yehouessi, L.W. and Liao, B. 2017. The emerging oilseed crop *Sesamum indicum* enters the "Omics" era. *Frontiers in Plant Science*. 8 : 1154. <https://doi.org/10.3389/fpls.2017.01154>
- Dossa, K., Li, D., Zhou, R., Yu, J., Wang, L., Zhang, Y., You, J., Liu, A., Mmadi, M.A., Fonceka, D. and Diouf, D. 2019. The genetic basis of drought tolerance in the high oil crop *Sesamum indicum*. *Plant Biotechnology Journal*. 17 (9) : 1788-1803. <https://doi.org/10.1111/pbi.13100>
- Du, H., Zhou, P. and Huang, B. 2013. Antioxidant enzymatic activities and gene expression associated with heat tolerance in a cool-season perennial grass species. *Environmental and Experimental Botany*. 87: 159-166.

- Dudziak, K., Zapalska, M., Börner, A., Szczerba, H., Kowalczyk, K. and Nowak, M. 2019. Analysis of wheat gene expression related to the oxidative stress response and signal transduction under short-term osmotic stress. *Scientific Reports*. 9(1) : 1-14. <https://doi.org/10.1038/s41598-019-39154-w>.
- Fazeli, F., Ghorbanli, M. and Niknam, V. 2007. Effect of drought on biomass, protein content, lipid peroxidation and antioxidant enzymes in two sesame cultivars. *Biologia Plantarum*. 51(1) : 98-103. <https://doi.org/10.1007/s10535-007-0020-1>
- Gill, S.S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*. 48(12): 909-930.
- Guan, L.M. and Scandalios, J.G. 2000. Hydrogen peroxide-mediated catalase gene expression in response to wounding. *Free Radical Biology and Medicine*. 28(8) : 1182-1190.
- Hasanuzzaman, M., Hossain, M.A., da Silva, J.A.T. and Fujita, M. 2012. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: *Crop stress and Its Management: Perspectives and Strategies* (pp. 261-315). Springer, Dordrecht.
- Hassanzadeh, M., Asghari, A., Jamaati-e-Somarin, S.H., Saeidi, M., Zabihi-e-Mahmoodabad, R. and Hokmalipour, S. 2009. Effects of water deficit on drought tolerance indices of sesame (*Sesamum indicum* L.) genotypes in Moghan region. *Research Journal of Environmental Sciences*. 3(1) : 116-121.
- Hsu, S.Y., Hsu, Y.T. and Kao, C.H. 2003. The effect of polyethylene glycol on proline accumulation in rice leaves. *Biologia Plantarum*. 46(1) : 73-78.
- Jones, H.G. 2007. Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. *Journal of Experimental Botany*. 58(2) : 119-130.
- Leopold, A.C. and Vertucci, C.W. 1986. Physical attributes of desiccated seeds. *Membranes, Metabolism and Dry Organisms*. Cornell University Press, Ithaca, NY, pp.22-34.
- Lima, M.S., Rocha, M.D.S., Melo, A.S.D. and Dutra, W.F. 2018. Physiological, biochemical and productive changes in sesame genotypes subjected to different rates of water replenishment. *Revista Brasileira de Engenharia Agrícola e Ambiental*. 22(3) : 176-182.
- Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*. 25 (4) : 402-408.
- Mehrabad Pour-Benab, S., Fabriki-Ourang, S. and Mehrabi, A.A. 2019. Expression of dehydrin and antioxidant genes and enzymatic antioxidant defense under drought stress in wild relatives of wheat. *Biotechnology & Biotechnological Equipment*. 33(1) : 1063-1073. <https://doi.org/10.1080/13102818.2019.1638300>
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*. 7 (9) : 405-410.
- Mittova, V., Tal, M., Volokita, M. and Guy, M. 2003. Upregulation of the leaf mitochondrial and peroxisomal antioxidant systems in response to salt induced oxidative stress in the wild salt tolerant tomato species *Lycopersicon pennellii*. *Plant, Cell & Environment*. 26(6) : 845-856.
- Nilsen, E.T. and Orcutt, D.M. 1996. Physiology of plants under stress. Abiotic factors. *Physiology of Plants under Stress. Abiotic Factors*.
- Noctor, G. and Foyer, C.H. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Biology*. 49(1) : 249-279.
- Peter, K.V. ed. 2012. *Handbook of Herbs and Spices*. Elsevier.
- Ren, J., Sun, L.N., Zhang, Q.Y. and Song, X.S. 2016. Drought tolerance is correlated with the activity of antioxidant enzymes in *Cerasus humilis* seedlings. *Bio Med Research International*, 2016. <http://dx.doi.org/10.1155/2016/9851095>
- Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*. 2012. <https://doi.org/10.1155/2012/217037>
- Uzilday, B., Turkan, I., Sekmen, A.H., Ozgur, R.E.N.G.Ý.N. and Karakaya, H.C. 2012. Comparison of ROS formation and antioxidant enzymes in *Cleome gynandra* (C4) and *Cleome spinosa* (C3) under drought stress. *Plant Science*. 182 : 59-70.