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BIOCHEMICAL STUDIES OF DROUGHT TOLERANT AND SUSCEPTIBLE GENOTYPES OF *SORGHUM BICOLOR*

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Abstract

A comparative study of drought tolerant (CSV 15) and susceptible (PC 1080) genotypes of *Sorghum bicolor* was performed in present study. Activities of superoxide dismutase, guaiacol peroxidise and glutathione reductase was measured during drought and recovery periods in both drought tolerant and susceptible genotypes. A wide genotypic variation in the activity of antioxidant enzyme was observed under drought stress. As compared to PC 1080, high level expression of ROS-*scavenging enzymes was observed in* CSV 15. The results clearly indicate that drought tolerant CSV 15 genotype has high potential to cope with water stress condition by modulating the expression of antioxidant enzymes. The results also reveal that status of antioxidant enzymes can also be used as biochemical marker to determine drought tolerance potential in plants.

Key words: Drought, *Sorghum bicolor*, Superoxide dismutase, Guaiacol peroxidise, Gglutathione reductase

Introduction

Plants are constantly been exposed to several environmental stresses. Drought is one of the major cause of decreased growth and productivity of plants especially in arid and semiarid zones throughout the world (Bodner *et al.,* 2015). In the 21st century, drought is thought to be a major threat for plant productivity (UNESCO, 2012). It directly affects growth, productivity, integrity of membrane, osmotic adjustment, content of pigment, water relations and activity of photosynthesis (Benjamin & Nielsen, 2006; Praba *et al.,* 2009) and it leads to 50% decrease in the crop productivity (Zlatev & Lidon, 2012). Adverse drought stress may affect photosynthesis, inhibition of metabolism and finally leads to death of plant (Jaleel *et al.,* 2009).

Plants face drought stress because of two main reasons the first one is limited supply of water to plant roots and another cause is increased water loss because of increased transpiration rate (Anjum *et al.,* 2011). In the stressed cells of plants drought causes biochemical and physiological alterations, the major alteration occurring in plant is increased accumulation of reactive oxygen species (Wang *et al.,* 2011) for example hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radicals (HO^-) and singlet oxygen. Drought leads to oxidative stress caused due to disturbance in the balance of ratio between sink and source, for example decrease in the fixation of CO_2 and reducing equivalents were utilized, inadequate to the rate of electron transport, which further resulted in ROS accumulation in the chloroplasts (Sairam & Saxena, 2000; Edreva, 2005; Bacelar *et al.,* 2006). ROS causes destruction to several compounds like, protein, DNA, RNA, chlorophyll and lipids which causes destruction in membranes and alters metabolism of plant cell which finally leads to senescence. It further results in mutations which often leads to severe dysfunction in metabolic activity and causes death of plant cell (Karuppanapandian *et al.,* 2011).

There are various mechanisms in plants like biochemical, structural and functional which helps in counteracting oxidative stress induced through drought (Sairam & Srivastava 2001; Ashraf & Foolad 2007). To deal with this problem, a complex antioxidant machinery has been developed in plants, which includes a series of enzymatic antioxidants like, catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX) etc. and non-enzymatic antioxidants like, glutathione, ascorbate, tocopherol (Mittler *et al.,* 2004). Efficient use of these antioxidant enzymes is vital for plants to cope with oxidative stress that would otherwise be lethal. Tohidi-Moghaddam *et al.,* (2009)

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reported that during drought stress the increase in antioxidant activity helps in drought tolerance in *Brassica napus*.

The role of antioxidants in scavenging ROS is as follows: SOD provides the primary line of defense against increased ROS production by catalysing the O_2^- into molecular oxygen (O_2) and H_2O_2 within a plant cell. In plants increase in SOD activity is often linked with increase in tolerance against drought stress. Ahmed *et al.,* (2013) reported rise in the activity of SOD during drought in anthesis period of *Hordeum vulgare* genotypes. Zhang & Kirkham (1996) reported that activity of SOD was increased considerably in sunflower/sorghum during drought stress. Whereas GPOD catalyzes the elimination of H_2O_2. Under stressed conditions it also acts as a quencher of reactive intermediary forms of peroxy and O_2 radicals. Similarly, glutathione reductase (GR) have a major role in eliminating oxidative stress as they maintain balance between the intracellular glutathione pool preliminarily in the reduced state. *S. bicolor* L. (Moench) is a C_4 crop which is well adapted to arid and semiarid regions. World-wide it ranks fifth amongst other cereal crops as well as it is an important source of food, fodder and biofuel. This crop is known for its better adaption to drought than several other crops (Sanchez *et al.,* 2002). In present study, efforts were done to understand the biochemical basis of drought tolerance in tolerant (CSV 15) and susceptible (PC 1080) genotypes of *Sorghum bicolor*.

Materials and Methods

Growing conditions and drought treatment: Seeds of drought tolerant (CSV 15) and susceptible (PC 1080) genotypes of *S. bicolor* were obtained from Maharana Pratap Agriculture University and Technology, Udaipur, India and were sown *in vivo* in germination trays containing 50% clay, 25% sand, and 25% humus under controlled conditions at 15°C under a 12 h photoperiod (Fig. 1). The germinated plants were equally well watered for 2 weeks prior to exposure to drought stress treatment. Thereafter, the plants were divided into two sets (each of 50 plants), out of which one set was subjected to drought stress until the appearance of wilting symptoms by withholding of water supply, while the second set was watered regularly and served as a control.

Enzyme assay: SOD (EC 1.15.1.1) activity was measured through the method proposed by Kono (1978). GPOD (EC 1.11.1.7) activity was assayed as per Racusen and Foote (1965). GR (EC 1.8.1.7) was analysed spectrophotometrically according to Smith *et al.,* (1988).

Results and Discussion

In the current study, we aimed to elucidate the effect of drought stress on drought tolerant (CSV 15) and susceptible (PC 1080) sorghum genotypes. A wide difference in the activities of SOD, GPOD and GR was observed in both genotypes.

SOD activity: In CSV 15 the activity of SOD remained almost constant till day 4 of drought treatment. A significant increase in enzyme activity was recorded when plants were exposed to severe drought stress. The enzyme activity was decreased significantly and reached to control level on day 4 of rewatering. In contrast, drought treatment upto 6 days markedly enhanced the SOD

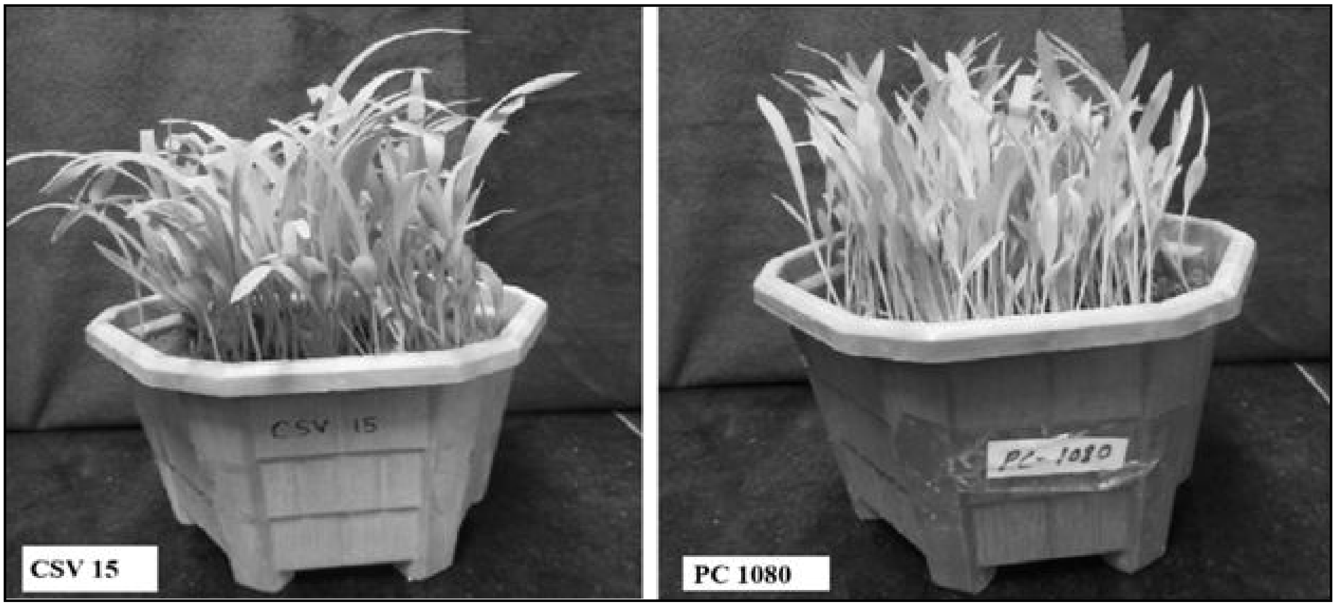

Fig. 1: Drought tolerant (CSV 15) and sensitive (PC 1080) genotypes of *S. bicolor*.

activity in PC 1080. Later on, the enzyme activity decreased with increasing the severity of drought stress. Upon rewatering, plants gradually up-regulated enzyme activity and recovered the control level on day 8 after re-supplying of water. Guo *et al.*, (2018) reported that drought-resistant varieties of sorghum could maintain higher SOD activity than varieties with weaker drought resistances. Similarly, in several studies it has been reported that SOD activity was increased in drought tolerant crops and decreased in drought sensitive crops like sugarbeet (Stajner *et al.*, 1995), wheat (Sairam & Saxena, 2000; Lascano *et al.*, 2001) which provides enzymatic defense against drought stress.

GPOD activity: GPOD activity in CSV 15 was enhanced with the progression of drought period (Fig. 3a). Highest enzyme activity was observed on day 10 of drought treatment. Upon rewatering, enzyme activity was significantly declined and reached to control level on day

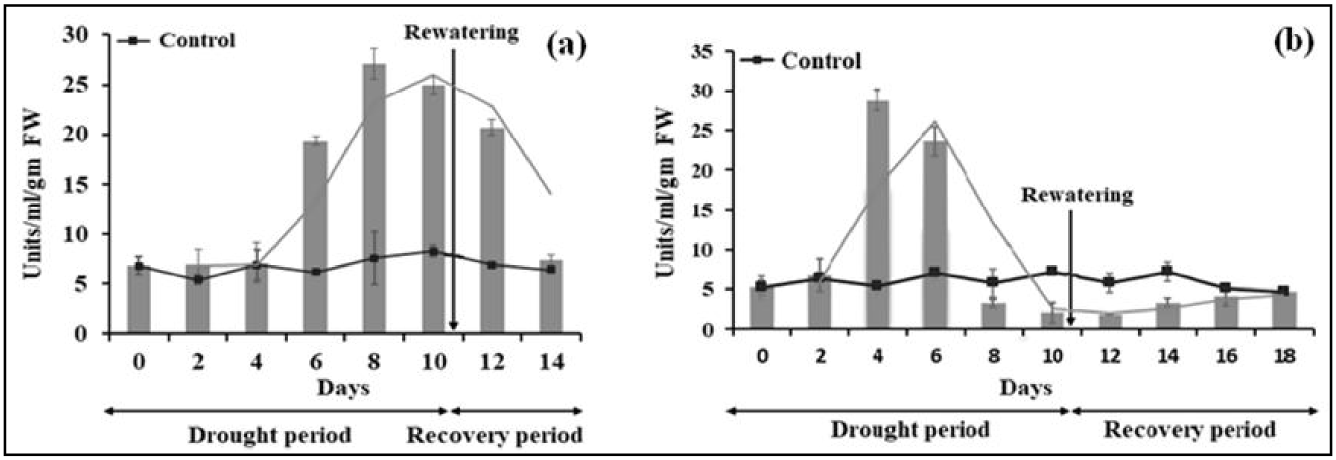

Fig. 2: SOD activity in the leaves of drought tolerant-CSV 15 (A) and sensitive-PC 1080 (B) genotypes of *S. bicolor*.

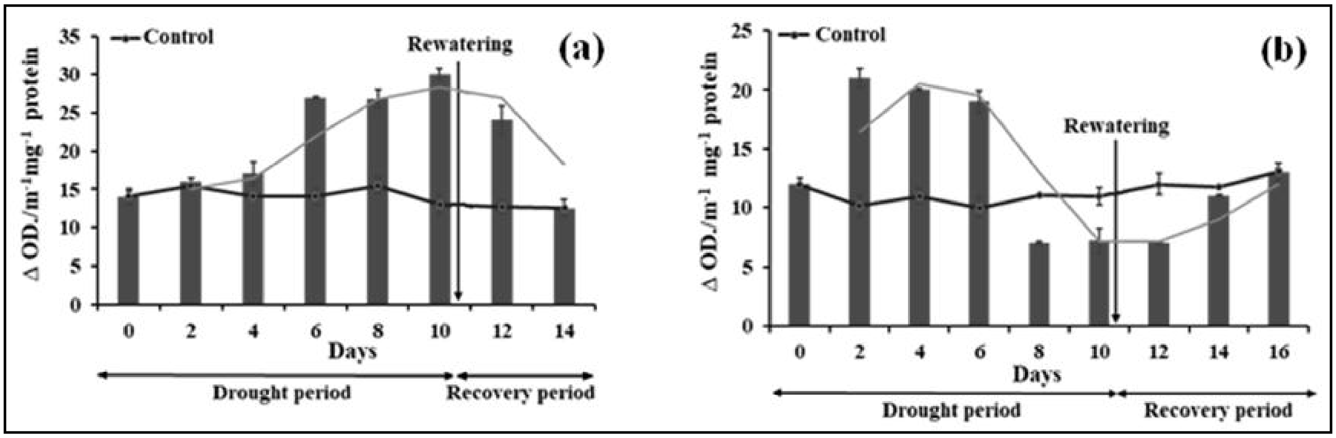

Fig. 3: GPOD activity in the leaves of drought tolerant-CSV 15 (A) and sensitive-PC 1080 (B) genotypes of *S. bicolor.*

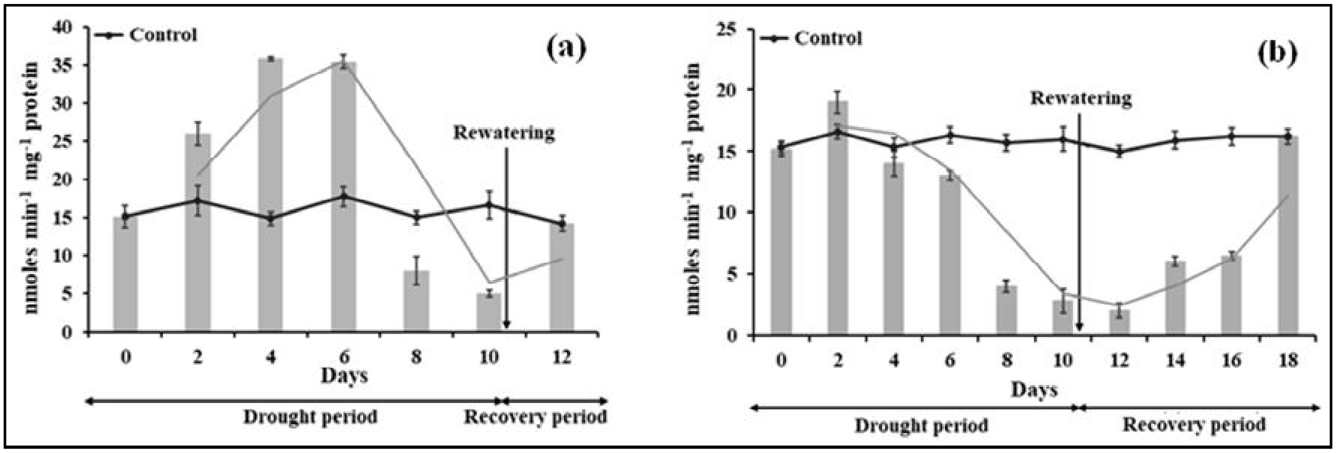

Fig. 4: GR activity in the leaves of drought tolerant-CSV 15 (A) and sensitive-PC 1080 (B) genotypes of *S. bicolor.*

4 day of recovery period. In PC 1080, the GPOD activity increased remarkably with increasing water stress period upto 6 days (Fig. 3b). Thereafter, at elevated drought stress, a significant decline in enzyme activity was noted. Re-watering led to a gradual increase in the enzyme activity. Vijayakumari & Puthur (2014) reported that GPOD activity was increased in drought tolerant variety of black pepper. Similarly, Mandi *et al.,* (2018) reported that GPOD activity was decreased under drought stress in two drought susceptible genotypes of *Vigna radiata.* Chugh *et al.,* (2010) also observed that peroxidase activity was increased in control seedlings of drought tolerant *Zea mays* as compared to drought sensitive genotype.

GR activity: It is well known that during stress GR have a key role in maintaining the pool of reduced glutathione (GSH) (Pastori *et al.,* 2000). In CSV 15 (Fig. 4a), the GR activity was increased till day 6 od drought treatment and then decreased during the exposure of severe drought treatment (Fig. 4a). Reirrigation quickly restored the enzyme activity on day 2 of rewatering. Turkan *et al.,* (2005) reported that GR activity was increased in drought tolerant *P. acutifolius*, but in *P. vulgaris*, it was increased at first and then decreased respectively. Sharma & Dubey (2005), also reported that GR activity was increased in *Oryza sativa* seedlings subjected to drought stress. In PC1080, the GR activity initially increased during the onset of mild drought stress condition. A remarkable decline in enzyme activity was noted when plants were subjected to moderate and severe drought stress (Fig. 4b). Similarly Pinheiro *et al.,* (2004) reported the involvement of GR in drought tolerance in *Coffea canephora*.

The results of biochemical studies clearly indicate that genotype CSV 15 has high drought tolerance due to the higher expression of antioxidant enzymes. The fast recovery, upon reirrigation, also makes this genotype suitable for cultivation at drought prone areas. The results also reveal that status of antioxidant enzymes could provide a meaningful tool for depicting drought tolerance in sorghum genotypes.

References

Ahmed, I.M., F. Cao, M. Zhang, X. Chen, G. Zhang and F. Wu (2013). Difference in yield and physiological features in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys. *PLoS ONE,* **8(10):**.

Anjum, S.A., L.C. Wang, M. Farooq, M. Hussain, L.L. Xue and C.M. Zou (2011). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *Journal of Agronomy and Crop Science,* **197:** 177–185.

Ashraf, M. and M.R. Foolad (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany,* **59:** 206–216.

Bacelar, E.A., D.L. Santos, J.M. Moutinho-Pereira, B.C. Goncalves, H.F. Ferreira and C.M. Correia (2006). Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. *Plant Science,* **170:** 596–605.

Benjamin, J.G. and D.C. Nielsen (2006). Water deficit effects on root distribution of soybean, field pea and chickpea. *Field Crops Research,* **97:** 248-253.

Bodner, G., A. Nakhforoosh and H.P. Kaul (2015). Management of crop water under drought: a review. *Agronomy for Sustainable Development,* **35(2):** 401-442.

Chugh, V., N. Kaur and A.K. Gupta (2011). Evaluation of oxidative stress tolerance in maize (*Zea mays* L.) seedlings in response to drought. *Indian Journal of Biochemistry and Biophysics,* **48:** 47-53.

Edreva, A. (2005). Generation and scavenging of reactive oxygen species in chloroplasts: a submolecular approach. *Agriculture Ecosystem and Environment,* **106:** 119–133.

Guo, Y.Y., S.S. Tian, S.S. Liu, W.Q. Wang and N. Sui (2018). Energy dissipation and antioxidant enzyme system protect photosystem II of sweet sorghum under drought stress. *Photosynthetica,* **56(3):** 861-872.

Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneerselvam (2009). Drought Stress in Plants: A Review on Morphological Characteristics and Pigments Composition. *Int. J. Agric. Biol.,* **11:** 100-105.

Karuppanapandian, T., H.W. Wang, N. Prabakaran, K. Jeyalakshmi, M. Kwon, K. Manoharan and W. Kim (2011). 2, 4- dichlorophenoxyacetic acid-induced leaf senescence in mung bean (*Vigna radiata* L. Wilczek) and senescence inhibition by co-treatment with silver nanoparticles. *Plant Physiology and Biochemistry,* **49:** 168-177.

Kono, Y. (1978). Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Archives of biochemistry and Biophysics,* **186(1):** 189-195.

Mandi, S., A.K. Pal, R. Nath and S. Hembram (2018). ROS scavenging and nitrate reductase enzyme activity in mungbean (*Vigna radiata* L. Wilczek) under drought stress. *International Journal Current Microbiological Applied Sciences,* **7:** 1031-1039.

Mittler, R., S. Vanderauwera, M. Gollery and F.V. Breusegem (2004). Reactive oxygen network of plants. *Trends Plant Sci.,* **9:** 490–8.

Pinheiro, H.A., F.M. DaMatta, A.R. Chaves, E.P. Fontes and M.E. Loureiro (2004). Drought tolerance in relation to protection against oxidative stress in clones of *Coffeacanephora* subjected to long-term drought. *Plant* *science*, **167(6):** 1307-1314.

Racusen, D. and M. Foote (1965). Protein synthesis in dark-grown bean leaves. *Canadian Journal of Botany*, **43(7):** 817-824.

Sairam, P.K. and D.K. Saxena (2000). Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. *Journal of Agronomy Crop Science*, **184:** 55–61.

Sairam, R.K. and G.C. Srivastava (2001). Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. *Journal of Agronomy Crop Science*, **186:** 63–70.

Sanchez, A.C., P.K. Subudhi, D.T. Rosenow and H.T. Nguyen (2002). Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). *Plant molecular biology*, **48:** 713-726.

Sharma, P. and R.S. Dubey (2005). Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. *Journal of Plant Physiology*, **162:** 854–864.

Smith, I.K., T.L. Vierheller and C.A. Thorne (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5¢-Dithiobis (2-nitrobenzoic acid). *Anal. Biochem.*, **175:** 408-413

Štajner, D., N. Mimica-Dukic and O. Gasic (1995). Adaptability to drought in sugar beet cultivars. *Biologia Plantarum*, **37(1):** 107.

Tohidi-Moghaddam, H.R., A.R. Shirani-Rad, G. Noormohammadi, D. Habibi and M.M.A. Boojar (2009). Effect of super absorbent application on antioxidant enzyme activities in canola (*Brassica napus* L.) cultivars under water stress conditions. *American Journal of Agriculturaland Biological Science*, **4:** 215-223.

Türkan, I., M. Bor, F. Ozdemir and H. Koca (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Science*, **168(1):** 223-231.

UNESCO (2012). Managing water under uncertainty and risk. UN World Water Development Report 4, Paris.

Vijayakumari, K. and J.T. Puthur (2014). Drought stress responses in tolerant and sensitive varieties of black pepper (*Piper nigrum* Linn.). *Journal of Plantation Crops*, 78-85.

Wang, B.Q., Q.F. Zhang, J.H. Liu and G.H. Li (2011). Overexpression of PtADC confers enhanced dehydration and drought tolerance in transgenic tobacco and tomato: effect on ROS elimination. *Biochemical and biophysical research communications*, **413(1):** 10-16.

Zhang, J.X. and M.B. Kirkham (1996). Enzymatic responses of the ascorbate-glutathione cycle to drought in sorghum and sunflower plants. *Plant Science*, **113:** 139–47.

Zlatev, Z. and F.C. Lidon (2012). An overview on drought induced changes in plant growth, water relations and photosynthesis. *Emirates Journal of Food and Agriculture*, **24:** 57-72.