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Changes in Antioxidant Enzymes Activities Mitigates Deleterious Effects of ROS in *Panicum miliaceum* (L.) under Drought Stress

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In nature, plants are always subjected to various abiotic stresses such as drought, salinity, high temperatures and so on. Among these drought is a worldwide problem, responsible for limiting the growth, physiology and productivity of plants, thus has become a challenge for global food security towards growing population. Proso millet (*Panicum miliaceum* L.) belonging to family Poaceae grows under wide environmental conditions and different soil profile. In this view, an experiment was conducted to investigate the range of tolerance and change of metabolic activities of Proso millet under drought stress. The control plants were irrigated regularly and treated plants were irrigated at 3, 5, 7, day intervals up to 60day interval drought (DAS). The root and leaf samples were collected on 30 DAS, 45 DAS and 60 DAS respectively for morphological and biochemical analysis. It was found that with increasing duration of water deficit, tremendous increases of antioxidants activities were recorded at all growth stages compared to control on 7DID at 60DAS. Furthermore, a decreased rate of growth, biomass and chlorophyll content was recorded in treated plants than control. Therefore, it can be concluded that proso millet has affinity to survive under prolonged drought stress and can help to understand the mechanism of photosynthetic efficacy for improving crop productivity.

Key words: Abiotic stress, antioxidants, drought stress, pigments, proso millet

Drought is the most severe environmental factor which results in global warming and affects almost all living organisms in the world. Water is an important factor in agricultural and food productivity, yet a highly limited resource (Wang *et al.*, 2012). Drought stress causes a major limitation to plant growth and development by affecting cell membrane integrity, osmotic adjustment and photosynthetic ability (Ravikumar *et al.*, 2014). Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high (Budak *et al.*, 2013). The climatic changes associated with global warming, drought stress may occur at any time during the growing season and thus may cause a profound decrease in biomass production and can lead to disastrous crop failure (Kabira and Muthoni, 2016).

When a plant is subjected to water deficit stress, it reacts by producing a range of reactive oxygen species (ROS) during photosynthesis, photorespiration and dark respiration. Moreover, overproduction of ROS causes damage to DNA, proteins and lipids in the plant cells that suffer from water deficit and adversely affects the crop production (Feng *et al.*, 2015). In order to re-establish redox homeostasis, plants have an internal ROS scavenging system known as antioxidants that alleviates oxidative damages, thus ensuring normal cellular function (Abass *et al.*, 2017). Among enzymatic antioxidants, Superoxide dismutase (SOD) acts as first defence line catalyzing superoxide radical to oxygen and hydrogen peroxide (H_2O_2). However, H2O2 can be further eliminated by catalase (CAT), ascorbate peroxidase (APX) and Proline oxidase (POX) present in the cell organelles (Hasheminasab et. al., 2012). On the other hand, non-enzymatic antioxidant such as ascorbic acid is the most abundant, powerful, and water-soluble antioxidant that can act to prevent or alleviate the damage caused by ROS in plant cells (Athar *et al.*, 2008). Besides ascorbic acid, the α-tocopherol, a flavonoid compound found in chloroplasts, also helps in quenching of oxygen, alleviation of oxidative damage in chloroplast and increases membrane rigidity, thereby plays a vital role in development, signal transduction, interacting or controlling phytohormonal regulation (Arrom and Munn´e-Bosch, 2010).

Millets are small-seeded annual cereals grown for food, forage, and fuel (Kothari *et al.*, 2005). Such crops are grown on marginal lands and under low-input agricultural conditions-situations in which major cereal crops often fail to grow or produce low yields (Amadou et. al., 2013). Proso millet (*Panicum miliaceum* L.) is one of the important species of the largest genus Panicum, which include more than 400 species is still widely cultivated in the arid and semi-arid areas of the world (Nazifi *et al.*, 2009). Undoubtedly, *Panicum miliaceum* is considered as relief and anti-famine crop; hence, it is a very efficient user of soil water and is well adapted to different types of soils and dry weather conditions. Therefore, the present was undertaken to evaluate the growth and physiological parameters, role of antioxidants activities in Panicum miliaceum L. under drought deficit condition.

MATERIALS AND METHODS

Collection of plant materials

The seeds of *Panicum miliaceum* (L.) were collected from Kolimalai, of Salem district, Tamilnadu, India and were identified by Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The experiment was laid out in a completely randomized block design (CRBD). Quality seeds were disinfected by 0.2% $HgCl_2$ solution for 2minutes with frequent shaking and rinsed with deionised water to remove traces of $HgCl_2$. Then, seeds were soaked in distilled water for 6hour and the hydrated seeds were sown in plastic pots containing sand, red soil and farmyard manure in the ratio of 1:1:1. For each treatment three replicates were maintained (each replicate is one pot containing four plants). After 15 days, drought stress treatment was given by altering the irrigation schedule at different day intervals as 3, 5 and 7 days (DID). While control (0 DID) plants were routinely irrigated daily with tap water. Samples were collected on 30, 45 and 60 DAS (days after sowing) for further analysis.

Estimation of Morphological parameters

Proso-millets were randomly uprooted carefully from each treated plant and brought to laboratory immediately, then rinsed with tap water followed by distilled water and blot dried with tissue papers for

morpho-physiological and biochemical estimation.

Estimation of Root and Stem length, Fresh weight (FW) and Dry weight (DW)

The root length and the shoot length were expressed in cm plant^{-1}. The FW and DW were taken in electronic balance and expressed in g plant^{-1}.

Estimation of Photosynthetic Pigments

Chlorophyll concentration was determined using Arnon's method (1949). Fresh leaf tissue (0.5 g) was extracted with 80 % acetone (v/v) and centrifuged at 2,500 rpm for 10 min at 4^{0}C. The extract was made up to 10 ml with 80 % acetone. The optical density (O.D.) of the extract (3ml) was measured against a blank of pure 80% aqueous acetone at wavelengths of 645 and 663nm using Hitachi U-2000 spectrophotometer and was expressed in terms of mg g^{-1} fresh weight.

Determination of Antioxidant Enzyme Activities

For extraction of enzymes, fresh samples (0.5g) were homogenized with 1.5 cm^3 of 100mM potassium phosphate buffer solution (pH 7.0) containing 2mM phenylmethylsulfonyl fluoride (PMSF), and centrifuged at 14,0009g at 40C for 20 min.

SOD (EC 1.15.1.1) activity assayed was described by Beauchamp and Fridovich (1971). The reaction mixture contained 1.17 9 10-6 M riboflavin, 0.1 M methionine, 2 × 10^{-5} M potassium cyanide (KCN), and 5.6 × 10^{-5} M nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8) 3 ml of the reaction medium was added to 1 ml of enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30^{0}C for 1 h, identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity was expressed in units. One unit (U) is defined as the amount of change in the absorbance by 0.1 h^{-1} mg^{-1} protein.

CAT (EC 1.11.1.6) was determined according to the method of Chandlee and Scandalios (1984) with slight modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 is followed by the decline in absorbance at 240 nm. The enzyme activity is expressed in U mg-1 protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Peroxidase (EC 1.11.1.7) activity was estimated by the method followed by Reddy *et al.* (1995). 20 % homogenate was prepared in 0.1 M phosphate buffer (pH 6.5). The reaction mixture contained 3.0 ml of pyrogallol solution and 0.1 ml of the enzyme extract. To the test cuvette, 0.5 ml of H_2O_2 was added and mixed. The change in absorbance was recorded at 430 nm every 30s up to 3min. One unit of peroxidase is defined as the change in absorbance/minute at 430 nm.

Estimation of non-enzymatic antioxidants

Ascorbic acid content was estimated as described by Omay *et al.* (1979) and the results were expressed in mg/g DW. Alpha -tocopherol (α-toc) content was determined according to Backer *et al.* (1980) and result was expressed in mg/g FW.

Statistical analysis

Data were analyzed using statistical program SPSS v16.0 and presented as the means ± SD of three replicates; each replicate consisted of four plants.

RESULTS

Root and shoot length

In the present study, a significant decrease was found in the shoot length of *P. miliaceum* with the increase in drought period. The highest decreased rate (48.67cm) was found on 7 DID at 60 DAS as compared to control (68.67cm). However, root length of *P. miliaceum* was found to be increased at all growth stage with the duration of drought stress and the highest value (42.00 cm) was found on 7 DID as compared to control (29.27cm) (Table 1).

Fresh weight and dry weight

Drought stress decreased the whole plant fresh weight and dry weight at all growth stages of *P. miliaceum* significantly than control *ones*. Whole plant fresh weight and dry weight at 5DID and 7DID drought treatments was more affected than at mild stress (3DID). When compared with control, fresh weight and dry weight were noted as 29.25 g plant^{-1} and 12.41 g plant^{-1} respectively, on 7DID at 60DAS (Table 2).

Chlorophyll Pigments

With increasing severity and period of drought stress there was a significant decrease in the Chlorophyll 'a' and Chlorophyll 'b' content. As compared to Chl *'a*' there was a significant decrease in Chlorophyll *'b'* content of *P. miliaceum*. The decreased rate recorded was 0.06 and 0.05mg g^{-1}FW respectively compared to control. Moreover, total chlorophyll content (0.11 mg g^{-1}FW) was found to be decreased with the severity of drought, compared to control (Table 3).

Antioxidants

SOD activity both in the leaves and roots increased with increased drought treatment in all stages of growth. SOD activity varied in different parts of the plant. The highest activity was recorded in leaves 3.33 (U. mg^{-1} protein h^{-1}) than roots 2.57 (U. mg^{-1} protein h^{-1}) compared to control having less SOD content on 7DID at 60 DAS (Table 4).

Catalase activity has been increased progressively under drought period in both the root and leaves of *P. miliaceum* in all the treatments as compared to control. The highest level recorded was 0.52 (U. mg^{-1} protein min^{-1}) in leaves and 0.31(U. mg^{-1} protein min^{-1}) in roots at 7DID of 60 DAS respectively (Table 5).

Drought treated plants shown increased POX activity with increased duration of drought stress when compared to the control plants. Highest POX activity (2.46 U. mg^{-1} protein min^{-1}) and 2.03(U. mg^{-1} protein min^{-1}) was recorded in both leaves and roots of *P. miliaceum* at 7DID on 60DAS (Table 6).

The AsA content increased with the age of the plant in control and drought treated plants in both the leaves and root samples. Among them, the leaves recorded the highest AA content than roots and overall increase than the control. The higher AA content recorded was 3.11mg/g DW and 2.07 mg/g DW respectively, in the leaves and roots samples of *P. miliaceum* at 7DID on 60DAS (Table 7).

Similarly, α-Tocopherol content found in drought treated plants was higher (Table 8) than that of control on 30^{th}, 45^{th} and 60^{th} DAS. The highest α-toc content recorded was 0.98 mgg^{-1} FW and 0.83 mgg^{-1}FW in leaves and roots on 60^{th} DAS in 7DID treated plants.

Table 1: Effect of drought stress on shoot length and root length of *P. miliaceum* L. (Values are expressed in cm $plant^{-1}$)

		Shoot Length (cm)		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	56.00±0.58	53.27±1.12	46.54±1.53	40.31±0.93
45	63.22±0.27	56.17±0.09	52.17±0.30	45.81±0.37
60	68.67±0.29	61.11±0.58	54.75±0.53	48.67±0.76
		Root Length(cm)		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	19.00±0.22	23.00±0.40	25.60±0.66	28.00±0.30
45	24.21±0.25	29.00±0.34	33.34±0.30	35.24±0.20
60	29.27±0.25	33.25±0.20	38.10±0.42	42.00±0.27

Values represented above are mean of 3 replicates; '±' standard deviation.

Table 2: Effect of drought stress on fresh weight and dry weight of *P. miliaceum* L. (Values are expressed in g $plant^{-1}$)

		Fresh Weight		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	29.17±0.15	27.10±0.34	23.30±0.17	18.34±0.24
45	34.04±0.23	30.84±0.35	28.42±0.20	25.50±0.53
60	39.07±0.34	36.02±0.13	33.40±0.28	29.25±0.54
		Dry Weight		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	13.02±0.61	11.12±0.25	9.10±0.23	6.70±0.44
45	17.01±0.22	15.14±0.45	11.06±0.62	8.39±0.66
60	19.30±0.16	16.00±0.60	14.60±0.50	12.41±0.30

Values represented above are mean of 3 replicates; '±' standard deviation

Table 3: Effect of drought stress on chlorophyll '*a*' chlorophyll '*b*' and Total Chlorophyll (a+b) of *P. miliaceum* L. **(Values are expressed in mg g^{-1} FW)**

		Chlorophyll "a"		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.06±0.12	0.05±0.13	0.04±0.09	0.03±0.06
45	0.08±0.14	0.06±0.05	0.05±0.08	0.04±0.07
60	0.16±0.23	0.13±1.00	0.09±1.01	0.06±1.03
		Chlorophyll "b"		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.05±0.03	0.04±0.03	0.03±0.01	0.02±0.05
45	0.08±0.02	0.07±0.03	0.05±0.02	0.04±0.08
60	0.11±0.12	0.09±0.04	0.08±0.03	0.05±0.10
		Total Chlorophyll(a+b)		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.11±0.08	0.09±0.07	0.07±0.05	0.05±0.02
45	0.16±0.17	0.13±0.14	0.10±0.11	0.08±0.07
60	0.27±0.23	0.22±0.16	0.17±0.12	0.11±0.06

Values represented above are mean of 3 replicates; '±' standard deviation

Table 4: Effect of drought stress on superoxide dismutase activiy in shoot and root of *P. miliaceum* L.**(Values are expressed in U. mg^{-1} protein h^{-1})**

		Shoot		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.65±0.97	1.31±0.59	1.74± 1.02	2.01±1.22
45	1.54±0.82	2.01±0.91	2.12±1.19	2.53±0.83
60	2.06±0.27	2.62±1.52	3.05±1.72	3.33±1.62
		Root		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.62±0.83	0.87±0.05	1.03±0.82	1.47±0.98
45	1.21±0.93	1.59±0.81	1.82±0.95	2.01±1.01
60	1.45±1.06	2.06±1.05	2.37±0.82	2.57±0.96

Values represented above are mean of 3 replicates; '±' standard deviation

Table 5: Effect of drought stress on catalase activity in shoot and root of *P. miliaceum* L. (**Values are expressed in U. mg^{-1} protein min^{-1}**)

		Shoot		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.23±0.06	0.28±0.06	0.33±0.11	0.40±0.12
45	0.31±0.07	0.36±0.08	0.40±0.11	0.46±0.10
60	0.39±0.10	0.43±0.11	0.47±0.13	0.52±0.13
		Root		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.14±0.04	0.17±0.04	0.21±0.05	0.25±0.07
45	0.22±0.06	0.24±0.06	0.26±0.07	0.29±0.08
60	0.25±0.07	0.26±0.08	0.28±0.13	0.31±0.19

Values represented above are mean of 3 replicates; '±' standard deviation.

Table 6: Effect of drought stress on peroxidase activity in shoot and root of *P.miliaceum* L. (**Values are expressed in U. mg^{-1} protein min^{-1}**).

		Shoot		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.52±0.14	0.74±0.32	0.81±0.52	1.04±0.59
45	0.85±0.29	1.31±0.34	1.46±0.53	1.80±0.65
60	1.14±0.37	1.62±0.43	2.13±0.55	2.46±0.72
		Root		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.32±0.23	0.57±0.24	0.66±0.35	0.81±0.45
45	0.84±0.48	1.07±0.49	1.46±0.48	1.64±0.51
60	1.03±0.81	1.43±0.74	1.68±0.70	2.03±0.71

Values represented above are mean of 3 replicates; '±' standard deviation.

Table 7: Effect of drought stress on ascorbic acid activity in shoot and root of *P.miliaceum* L. (**Values are expressed in mg/g DW**)

		Shoot		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	1.28±0.22	1.58±0.39	1.72±0.56	2.41±0.54
45	1.90±0.26	2.18±0.49	2.46±0.59	2.73±0.58
60	2.21±0.45	2.43±0.60	2.65±0.61	3.11±0.63
		Root		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.66±0.02	0.87±0.03	0.99±0.04	1.21±0.05
45	1.14±0.08	1.25±0.10	1.36±0.13	1.48±0.15
60	1.43±0.14	1.72±0.16	1.85±0.19	2.07±0.23

Values represented above are mean of 3 replicates; '±' standard deviation.

Table 8: Effect of drought stress on α-tocopherol activity in shoot and root of *P. miliaceum* L. (**Values are expressed in mg/g FW**)

		Shoot		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.34±0.04	0.47±0.05	0.63±0.07	0.85±0.09
45	0.51±0.16	0.62±0.19	0.78±0.21	0.98±0.23
60	0.63±0.35	0.74±0.36	1.02±0.41	1.36±0.53
		Root		
DAS	**Control**	**3DID**	**5DID**	**7DID**
30	0.31±0.03	0.42±0.04	0.54±0.06	0.71±0.08
45	0.48±0.12	0.57±0.16	0.68±0.19	0.82±0.22
60	0.56±0.18	0.63±0.19	0.71±0.34	0.83±0.44

Values represented above are mean of 3 replicates; '±' standard deviation.

DISCUSSION

Drought stress occurs when the plants are subjected to water shortage, which reduces photosynthesis, cell number and growth. In each of these processes, large number of genes, enzymes, hormones and metabolites are involved (Skirycz and Inze, 2010). Water deficit causes an increase in ROS production in plants, and leads to an oxidative stress. However, there is an internal ROS scavenging system in plants enzymatic and non-enzymatic antioxidants such as Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), Ascorbic acid (AsA) and alpha-tocopherol that alleviates oxidative damages, maintains intracellular redox state homeostasis, thus ensuring normal cellular function. (Langebartels *et al.* 2002; Foyer and Noctor 2005, Fang and Xiong 2015, Abass *et al.*, 2017).

The root length of *P. miliaceum* increased with increased drought stress treatment. Increasing root length due to water stress was reported in Pearl millet (Kusaka *et al.*, 2005), *Catharanthus roseus* (Jaleel *et al.*, 2008), *Triticum aestivum* (Dickin and Wright, 2008) and *Panicum sumatrense* (Ajithkumar and Panneerselvam, 2013). It is obvious from the results that with increasing severity and period of drought, there was significant decrease in stem length of *P. miliaceum*. Similarly, significant decrease in stem length had been found in soybean (Specht *et al.*, 2001) and stressed citrus seedlings under water deficit condition (Wu *et al.*, 2008). Such decreased in shoot length may be due to impaired mitosis, cell elongation and expansion resulted from drought and causes reduced growth and yield traits (Hussain *et al.*, 2008).

The whole plant fresh weight and dry weight of *P. miliaceum* was significantly declined with increased drought stress as compared to control. This decline was directly proportional to severity and duration of drought stress. Kobashi *et al.* (2000) found that the degree of drought controls the fresh weight such that it increases with moderate drought but decreases with severe drought. They attributed that decline in fresh weight to the decrease in the water content of stressed plant cells and tissues which lose their turgor and thus shrink.

Total dry weight decreased may be due to the considerable decrease in plant growth, photosynthesis and canopy structure as indicated by leaf senescence during drought stress in wheat (Gong *et al.*, 2003).

Severe water stress may result in arrest of photosynthesis, disturbance of metabolism and finally drying (Liang *et al.*, 2006). Mild water stress affected the shoot dry weight, while shoot dry weight was greater than root dry weight loss under severe stress in sugar beet genotypes (Mohammadian *et al.*, 2005). Pekcan *et al.*, (2016) also found similar results of reduction in both dry and fresh biomasses under the drought stress applications in sunflower genotypes.

The decrease in Chlorophyll content is a commonly observed phenomenon under drought stress (Mafakheri *et al.*, 2010; Din *et al.*, 2011). The decrease in chlorophyll content during drought stress may be due to chlorophyll degrading enzyme chlorophyllase. Increased activity of chlorophyllase and peroxidase enzymes under severe drought results decreased chlorophyll content (Abaaszsadeh *et al.*, 2007). Moreover, decrease in net photosynthetic rate under water stress is also related to disturbances in biochemical processes of a non-stomatal nature, caused by oxidation of chloroplast lipids, changes in the structure of pigments and proteins (Marcinska *et al.*, 2013). Similarly, Cao *et al.*, (2015) reported that drought stress inhibited biosynthesis of the precursor of chlorophyll in tomato leaves which ultimately reduced the chlorophyll content.

A progressive increase in superoxide dismutase activity in both the shoots and roots of *P. miliaceum* was observed with the increasing severity of drought. Our results are in line with the results noted earlier in three cultivars of rice (Sharma *et al.*, 2005) and *Phaseolus vulgaris* (Zlatev *et al.*, 2006). Salekjalali *et al.* (2012) and Naderi *et al.*, (2014) also reported that drought stress increased SOD activity in barley and wheat respectively. Increased SOD activity might be due to production of ROS under drought that has negative impacts on photosynthesis.

A positive correlation has been noticed between increased rate of catalase activity and severity of drought in both the shoots and roots of *P. miliaceum* L. Our results correlated with the findings of Simova-Stoilova *et al.* (2010), who reported increased CAT activity in sensitive varieties of wheat under drought stress. Catalase activity increased under drought stress in *Zea mays* (Jiang and Zhang, 2002), wheat (Dalmia and Sawhney, 2004) and *P. acutifolius* (Turkan *et al.*, 2005). The increase in catalase activity might be useful in disproportionating H_2O_2 that is the key product in reducing senescence under extreme moisture stress.

Similar case was found with peroxidase activity in the shoots and roots of *P. miliaceum with* increased drought period. Water stress could increase the accumulation of peroxidase substrate, which in turn is scavenger of activated oxygen species (Winston 1990). The important role of peroxidase in relation to oxidative tolerance has been reported in *Arabidopsis thaliana* (O'Kane *et al.*, 1996) and cucumber (Lee and Lee, 2000). Increased peroxidase activity under water deficit condition was reported previously in soybean and wheat genotypes (Zhang *et al.*, 2006; Shao *et al.*, 2007).

Ascorbic acid can donate electrons to a wide range of enzymatic and non enzymatic reactions. Water stress resulted in significant increases in antioxidant ascorbic acid concentration in turf grass (Zhang and Schmidt, 2000) and *Picea asperata* seedlings (Yang *et al.,* 2008). In *Withania somnifera* roots and leaves, the ascorbic acid content increased with age in drought stressed plants than normal one (Jaleel *et al.*, 2009).

A direct proportional relationship was also observed between α-tocopherol content and duration of drought period in the shoots and roots of *Panicum miliaceum* i.e., with increased drought period α-tocopherol content also increased. It has been reported that water deficiency may result in an increase of tocopherol concentration in plant tissue (Wu *et al.*, 2007; Shao *et al.*, 2007). Synthesis of low-molecular weight antioxidants, such as, α-tocopherol has been reported in drought stressed wheat plants (Zhao *et al.*, 2008). Some evidences implied that content of tocopherol was increased in wheat and spinach leaves as the amount of rainfall decreased or subjecting to water deficit (Shao *et al.*, 2006, 2007).

CONCLUSION

From the above study it can be concluded that drought a kind of abiotic stress invites accelerated ROS that have deleterious effects on plant metabolism. However, *P. miliaceum* overcomes on decreased rate of chlorophyll contents and biomass content upto an

optimal level by increasing a series of antioxidants that possess potential of scavenging ROS with increasing duration of drought. Thus, from these observations we are able to say that *P. miliaceum* may be drought tolerant with adequate ROS scavenging system. Hence, in view of considerable variations in the protective mechanisms against ROS, *P. miliaceum* can be used to improve drought tolerance in sensitive crops by genetic engineering to get rid off from the drought and overcome food crisis.

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