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ALTERATIONS IN ANTIOXIDANT METABOLISM AND GROWTH IN *PASPALUM SCROBICULATUM* L. VARIETIES SUBJECTED TO DROUGHT STRESS

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ABSTRACT

Drought is a major factor limiting crop productivity worldwide. Plants have different mechanism to cope with water scarcity. Hence, the aim of the present study was to understand the effect of drought stress on growth and anti-oxidant activities of two varieties of *Paspalum scrobiculatum.* The experiments were carried out in a pot culture. Two varieties of *P scrobiculatum* L. were selected for the study. Three regimes of drought stress (3, 5 and 7 DID) were imposed from 20 to 40 DAS. The samples were collected in 40, 60 and 80 DAS and were analyzed for estimating growth parameters and antioxidant enzymes. The data clearly showed increased drought stress declines shoot length, total leaf area and shoot fresh and dry weight whereas, root length, root fresh and dry weight increased. The enzyme activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), ascorbic acid (AA) and α-tocopherol (α-TOC) increased in both root and shoot of *P. scrobiculatum* under drought stress, supports its drought tolerance adaptations. In addition, both the varieties showed a tendency of recovery when the drought stresses were removed.

KEYWORDS: Antioxidant enzymes, water potential, adaptation, drought stress.

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INTRODUCTION

Environmental factors influence the characters, composition, growth and development of individual plants and plant communities. When any of these environmental factors exceed the optimum tolerance of a plant, the result is stress to the plant [1]. Plants in nature are continuously exposed to many biotic and abiotic stresses. Among these stresses, drought stress is one of the most adverse factors of plant growth and productivity and considered a severe threat for sustainable crop production in the conditions of changing climate. Future climate scenarios suggest that global warming may be beneficial for the millet crop in some regions, but could reduce productivity in zones where optimal temperatures already exist. Drought triggers a wide variety of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Understanding the biochemical and molecular responses to drought is essential for a holistic of perception plant resistance mechanisms to water-limited conditions [2]. Relative water content (RWC), leaf water potential, stomatal resistance, the rate of transpiration, leaf temperature and canopy temperature are important characteristics that influence plant water relations. Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC is related to water uptake by the roots as well as water loss by transpiration [2]. During optimal conditions, the balance between reactive oxygen species (ROS) formation and consumption is tightly controlled by antioxidant enzymes and redox metabolites [3]. These include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and redox metabolites such as ascorbic acid and glutathione. Most environmental stress, including drought induce enhanced production of ROS [4]. Antioxidant enzymes are important components in the mechanism of drought and desiccation tolerance [5, 6]. Drought stress leads also to increased accumulation of reactive oxygen species (ROS) in plants. Various sub-cellular organelles such as chloroplast, mitochondrion and peroxisome are the common sites of ROS production. Increased levels of ROS cause damage to various cellular mechanisms, such as enzyme inhibition, protein degradation, DNA and RNA damage, and membrane lipid per-oxidation, which ultimately culminate in cell death [7, 8]. Oxidative stress can lead to inhibition of the photosynthesis and respiration processes and, thus, plant growth. As the key process of primary metabolism, photosynthesis plays a central role in plant performance under drought, via decreased CO_2 diffusion to the chloroplast and metabolic constraints [9]. Modulation in the activities of antioxidant enzymes may be one of the important factors intolerance of various plants to environmental stress [10]. When molecular O_2 undergoes reduction, it gives rise of ROS such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$). Singlet oxygen ($^1O_2$), which may arise due to reaction of O_2 with exciting chlorophyll molecules, is also considered as one of the potential ROS.

SOD (EC 1.15.1.1) is an enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, H_2O_2. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. CAT (EC 1.11.1.6) is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second [11]. POD (EC 1.11.1.7) can be coupled to other proteins via its amino groups, as well as its carbohydrate moiety. POD also protects the cells against the destructive influence of H_2O_2 by catalyzing its decomposition through oxidation of phenolic and endiolic co-substrates [12]. APX (EC 1.11.1.11) is an enzyme that detoxifies peroxides such as hydrogen peroxide using ascorbate as a substrate. The reaction they catalyse is the transfer of electrons from

ascorbate to peroxide, producing dehydroascorbate and water as products [13]. GR (EC 1.8.1.7) is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant [14]. The peroxidases then scavenged the produced H_2O_2 by and by oxidation of substrates such as phenolics or other antioxidants [15]. Coarse cereals (millets) have the potential to provide food and nutrition as well as ensure sustainability of poor farmers in fragile ecosystems. Paspalum scrobiculatum L. (Kodo millet), belongs to family *Poaceae,* is an important millet crop cultivated almost throughout India. It is recommended for diabetic persons as a substitute for rice and has medicinal and insecticidal properties, which are uncommon and relatively unknown to modern societies [16]. *Paspalum scrobiculatum* is still a traditional food plant in Africa. This little-known grain has potential to improve nutrition, boost food security, foster rural development and support sustainable landcare [17, 18, 19]. *P. scrobiculatum* forms an important component of dry land, tribal and hilly agriculture, mostly cultivated in rain-fed areas. So, it is important to understand its drought adaptation mechanism for better yield. Therefore, this experiment was conducted by considering the importance of kodo millet in rain-fed areas and hence the aim of the present study was to investigate the effects of drought stress on growth, relative water content and changes in enzymatic and non-enzymatic antioxidants of two varieties of kodo millet (*Paspalum scrobiculatum* L.).

MATERIALS AND METHODS

The seeds of *Paspalum scrobiculatum* L. were collected from local farmers of Kovilpatty, Trichy, Kollimalai, Vadalur and Virudhunagar areas of Tamil Nadu and were identified by Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India. The research work was conducted in the Botanical Garden and Stress – Physiology Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India (11°23′59″N, 79°41′37″E). Two varieties of *Paspalum scrobiculatum* L., CO-1 and CO-2, were selected for the study. Earthen pots of 30 cm diameter and 40 cm height size were used for the study. The pots were filled with 10 kg of soil mixture containing red soil, sand and farm yard manure in the ratio 1:1:1 and 120 pots were arranged in a completely randomized block design. One set of 15 pots was kept as control and the other 3 sets of 45 pots were used for drought stress treatments for each variety. The seeds were sown and the seedlings were thinned to 05 per pot on 10th DAS (days after sowing). The plants were allowed to grow up to 20 DAS and were watered regularly. From 20th day onwards, all the potted plants were grown under the poly – house to avoid any type of atmospheric precipitation. The control plants were irrigated every day. Mild stress (irrigation once in 3 days), moderate stress (irrigation once in 5 days) and severe stress (irrigation once in 7 days) from 20th to 40th DAS were maintained as drought treatments, after that all plants were irrigated in alternate days. The samples were collected in 40, 60 and 80 DAS for analysis. Plants were uprooted randomly, washed carefully and separated into root and shoot for estimating growth parameters and antioxidant enzymes.

Plant Growth

The growth parameters like shoot length, root length, total leaf area, fresh weight and dry weight of root and shoot were carried out in 40, 60 and 80 DAS respectively. The length between shoot tip and point of root-shoot transition region was taken as shoot length. Below the point of root – shoot transition to the fibrous root tips were taken as total root length. The root and the stem length were expressed in centimeters per plant. The total leaf area of the plants was measured using LICOR Photo Electric Area Meter (Model LI-3100, Lincoln, USA) and expressed in cm^2 per plant. After washing the plants in the tap water, fresh weight was determined by using an electronic balance (Model – XK3190-A7M). After taking fresh weight, the plants were dried at 60 (C in hot air oven for 24 hours. After drying, the weight was measured and the values were expressed in grams per plant.

Leaf water relations

Leaf water relations were measured only for the drought period in comparison with daily irrigated control plants at 40th DAS. To determine relative water content (RWC), six

plants from each treatment were randomly selected and the method described by Turner [20] was followed. About 0.1 g leaf sample was cut into smaller pieces and weighed to determine initial weight (W_i). The leaf samples were then floated in freshly de-ionized water for 12 h and weighed thereafter to determine fully turgid weight (W_f). The sample was oven-dried at 80°C for 3 days and the dry weight was obtained (W_d). The relative water content (RWC) was determined using the following formula: $RWC = (W_i - W_d)(W_f - W_d)^{-1} \times 100$. The leaf water potential (Ψ_W) was measured in the 3 upper fully expanded leaves 2 h after the beginning of the light period using a pressure chamber (model C52-SF, WESCOR, Inc.) [21].

Antioxidant content extraction and assay

The ascorbic acid content was assayed as described by Omaye et al. [22]. One gram of fresh material was ground in a pestle and mortar with 5 ml of 10 per cent TCA (Tricarboxylic Acid), the extract was centrifuged at 3500 rpm for 20 minutes. The pellet was re-extracted twice with 10 percent TCA and supernatant was made to 10 ml and used for estimation. With 0.5 ml of extract, 1 ml of DTC reagent (2, 4-Dinitrophenyl hydrazine-Thiourea-$CuSO_4$ reagent) was added and mixed thoroughly. The tubes were incubated at 37 (C for 3 hours and to this 0.75 ml of ice cold 65 percent H_2SO_4 was added. The tubes were then allowed to stand at 30 (C for 30 minutes. The resulting color was read at 520 nm in spectrophotometer (U-2001-Hitachi). The ascorbic acid content was determined using a standard curve prepared with ascorbic acid and the results were expressed in milligrams per gram dry weight.

α – Tocopherol activity was assayed as described by Backer et al. [23]. Five hundred milligrams of fresh tissue was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for estimation of α – Tocopherol. To one ml of extract, 0.2 ml of 2 percent 2, 2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 minutes. The resulting red color was diluted with 4 ml of distilled water and mixed well. The resulting color in the aqueous layer was measured at 520 nm. α– tocopherol content was calculated by using a standard graph made with known amount of α– tocopherol.

Extraction and determination of antioxidative enzymes

Fresh leaf tissues of control and treated plants were used for determination of antioxidative enzymes. Fresh leaves (0.2 g) were sliced and ground to a fine powder in liquid nitrogen using a pestle and mortar. The powder was homogenized in 0.5 ml extraction buffer containing 50 mM Na-phosphate (pH 7.0), 0.25 mM EDTA, 2% (w/v) polyvinylpyrrolidone-25, 10% (w/v) glycerol, and 1 mM ascorbic acid. The homogenate was then centrifuged at 15, 000g for 20 min at 0°C. The supernatant (soluble fraction) was used as the crude extract for the superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) assay [24]. Superoxide dismutase (SOD, EC:1.15.1.1) was assayed on the basis of its ability to inhibit the photochemical reduction of Nitro blue tetrazolium (NBT), according to the methods of Beauchamp and Fridovich [25] and Beyer and Fridovich [26]. The reaction mixture contained 50 mM phosphate buffer (pH = 7.8), 13 mM methionine, 75 mM NBT, 100 μM EDTA, 200 mL of enzyme extract and 2 mM riboflavin. The reaction mixture was read at 560 nm. The increase in absorbance in the absence of the enzyme was taken as 100 and 50% initially was taken an equivalent to 1 unit of SOD activity.

Ascorbate peroxidase (APX; E.C.1.11.1.11) activity was measured according to Panchuk [27]. Reaction mixture contained 25 mM Na-phosphate (pH 7.0), 0.1 mM EDTA, 1 mM H2O2, 0.25 mM ascorbic acid (AsA), and protein extract in a total volume 1 ml. The oxidation rate of AsA was assayed photometrically using a UV/Visible Spectrophotometer by monitoring the decrease in A290 after 1 minute of incubation following the addition of protein extract. A fall in absorbance at 290 nm was measured as ascorbate was oxidized. APX activity (unit/g FW) was calculated using an extinction coefficient of 2.8 mmol/l/cm for ascorbate at 290 nm [28]. Catalase (CAT, EC 1.11.1.6) activity was measured by the method of Tan *et al*., [29]. Enzyme extract and 4 ml of 50 mM phosphate buffer (pH 7.0) were mixed and

incubated at 30°C for 10 min. The reaction was started by adding 1 ml of 50 µM H_2O_2, and terminated after 1 minute by adding 2 ml of 10% H_2SO_4. CAT activity was then determined by estimating the residual H_2O_2 in the reaction solution using 10 mM $KMnO_4$ titration to pink. CAT activity was expressed as U mg^{-1} FW.

Statistical analysis

The data represent means calculated from six replicates. Variance analysis of mean values was performed with Duncan Multiple Comparison test (one-way ANOVA) using SPSS software for Microsoft Windows (Ver. 16.0, SPSS Inc., USA) and significance level were determined at the 5% (P□0. 05) level.

RESULTS

Plant growth

Drought stress treatments affected plant growth in both varieties of *P. scrobiculatum* as compared to unstressed plants (control). Drought stress reduced shoot length, total leaf area, shoot fresh and dry weight, but increased root length and root dry weight (Table 1, 2 & 3). The reduction in shoot length and leaf area was found higher in CO-2 as compared to CO-1 under drought stress.

Table 1

Drought stress induced changes in root length, shoot length and total leaf area of P. scrobiculatum L. Varieties at different growth stages.

Growth Stages	Control		3 DID		5 DID		7 DID	
	CO-1	CO-2	CO-1	CO-2	CO-1	CO-2	CO-1	CO-2
Root length (cm/plant)								
40 DAS	11.9 ± 0.45	13 ± 0.50	12.6 ± 0.46	13.9 ± 0.51	14.2 ± 0.50	15.7 ± 0.56	15.8 ± 0.54	17.6 ± 0.60
60 DAS	12.6 ± 0.48	14.5 ± 0.55	14.7 ± 0.54	17 ± 0.60	16 ± 0.57	18 ± 0.66	17.4 ± 0.60	20.2 ± 0.69
80 DAS	17.2 ± 0.66	19.2 ± 0.73	18.3 ± 0.67	22.5 ± 0.83	18.8 ± 0.67	23.5 ± 0.83	19.3 ± 0.66	24.8 ± 0.85
Shoot length (cm/plant)								
40 DAS	41.3 ± 1.42	52.3 ± 1.86	35.8 ± 1.27	45.2 ± 1.61	33 ± 1.22	31.9 ± 1.18	28.7 ± 1.10	30 ± 1.15
60 DAS	58.2 ± 2.08	59 ± 2.03	52.5 ± 1.87	49 ± 1.75	48.2 ± 1.78	43 ± 1.59	41 ± 1.57	40 ± 1.53
80 DAS	61 ± 2.10	63 ± 2.17	58.5 ± 2.07	58 ± 2.07	50.7 ± 1.87	49.5 ± 1.83	48.7 ± 1.87	45.3 ± 1.74
Total leaf area (cm^2/plant)								
40 DAS	119 ± 4.10	155 ± 5.53	110 ± 3.92	134 ± 4.96	98 ± 3.62	109 ± 4.03	89 ± 3.42	76 ± 0.29
60 DAS	141 ± 4.86	198 ± 6.82	135 ± 4.82	156 ± 5.57	123 ± 4.55	143 ± 5.29	116 ± 4.46	131 ± 5.03
80 DAS	262 ± 9.03	354 ± 13.6	244 ± 8.71	301 ± 10.7	218 ± 8.07	276 ± 10.2	198 ± 7.61	255 ± 9.80

(values are the mean ± S.D of six replicates expressed in cm/plant and cm^2/plant)

Table 2
Drought stress induced changes in root and shoot fresh weight of P. Scrobiculatum L varieties at different growth stages.

Growth Stages	Control		3 DID		5 DID		7 DID	
	CO-1	CO-2	CO-1	CO-2	CO-1	CO-2	CO-1	CO-2
Root (g/plant)								
40 DAS	0.52 ± 0.20	0.78 ± 0.03	0.71 ± 0.02	0.94 ± 0.03	0.73 ± 0.02	0.97 ± 0.03	0.75 ± 0.02	1.00 ± 0.03
60 DAS	0.98 ± 0.03	1.15 ± 0.04	1.10 ± 0.04	1.25 ± 0.04	1.13 ± 0.04	1.31 ± 0.04	1.15 ± 0.04	1.36 ± 0.05
80 DAS	1.89 ± 0.06	2.95 ± 0.11	1.94 ± 0.07	3.14 ± 0.11	1.97 ± 0.07	3.28 ± 0.11	1.99 ± 0.07	3.37 ± 0.11
Shoot (g/plant)								
40 DAS	4.13 ± 0.14	7.54 ± 0.26	3.12 ± 0.11	5.94 ± 0.21	2.25 ± 0.08	4.87 ± 0.18	1.75 ± 0.06	3.90 ± 0.15
60 DAS	7.55 ± 0.26	13.17 ± 0.45	5.35 ± 0.19	10.44 ± 0.37	4.33 ± 0.16	9.09 ± 0.33	3.53 ± 0.13	7.80 ± 0.30
80 DAS	15.07 ± 0.51	20.92 ± 0.80	13.44 ± 0.48	16.57 ± 0.59	12.11 ± 0.44	15.30 ± 0.56	10.32 ± 0.39	14.91 ± 0.57

(values are the mean ± S.D of six replicates expressed in g/plant)

Plant water relations

The decrease in leaf relative water content (RWC) was found to be lowest, 63.25% under the7 DID stress treatment. RWC has been affected in all water deficit levels compared to the control. RWC was found more affected in CO-1 compared to CO-2 under all stress treatments. Drought stress treated plants showed marked decrease in leaf water potential when compared with control plants (Table 4). CO-1 had the highest potentiality of ψ_w decrease (1.8-fold greater than the control plants) as compared to CO-2 (1.6-fold) under 7 DID treatment.

Table 3
Drought stress induced changes in the root and shoot dry weight of P. scrobiculatum L. varieties at different growth stages.

Growth Stages	Control		3 DID		5 DID		7 DID	
	CO-1	CO-2	CO-1	CO-2	CO-1	CO-2	CO-1	CO-2
Root (g/plant)								
40 DAS	0.08 ±0.02	0.11 ±0.03	0.10 ±0.03	0.13 ±0.04	0.11 ±0.03	0.14 ±0.05	0.12 ±0.05	0.15 ±0.06
60 DAS	0.14 ±0.06	0.17 ±0.07	0.15 ±0.05	0.19 ±0.07	0.16 ±0.06	0.20 ±0.08	0.17 ±0.07	0.21 ±0.09
80 DAS	0.27 ±0.11	0.41 ±0.14	0.29 ±0.11	0.42 ±0.15	0.30 ±0.12	0.43 ±0.16	0.32 ±0.13	0.45 ±0.17
Shoot (g/plant)								
40 DAS	0.63 ±0.21	1.15 ±0.39	0.46 ±0.16	0.91 ±0.32	0.34 ±0.12	0.74 ±0.27	0.26 ±0.10	0.60 ±0.23
60 DAS	1.16 ±0.40	2.02 ±0.69	0.92 ±0.34	1.76 ±0.62	0.66 ±0.24	1.39 ±0.51	0.54 ±0.20	1.20 ±0.46
80 DAS	2.31 ±0.79	3.21 ±1.10	2.06 ±0.73	2.74 ±0.97	1.86 ±0.68	2.35 ±0.87	1.58 ±0.60	2.17 ±0.83

(values are the mean ± SD of six replicates and expressed in g/plant)

Table 4
Effect of drought stress on leaf relative water content (RWC) and leaf water potential of P. scrobiculatum L. Varieties at 40th DAS.

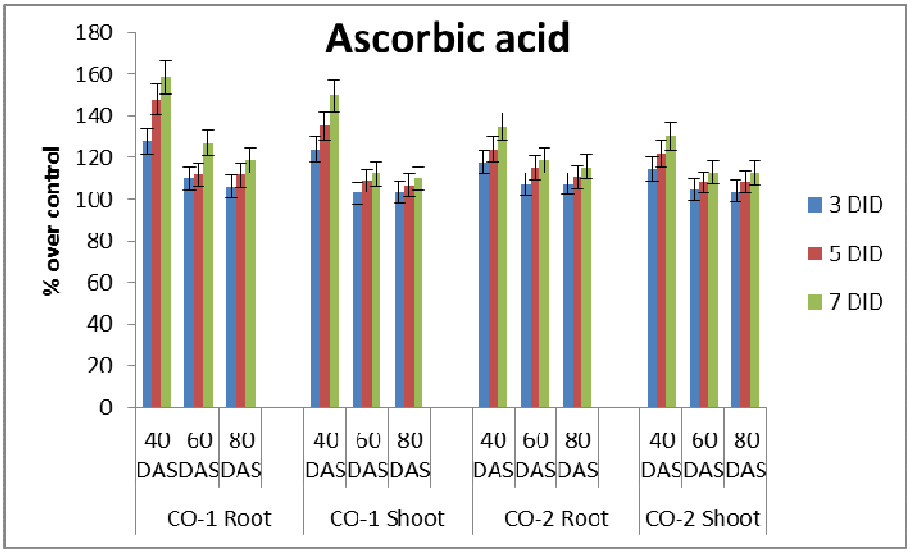
Parameters	Control	3 DID	5 DID	7 DID
Relative Water Content (%)				
CO-1	90.05 ± 1.32	79.32 ± 1.76	71.15 ± 2.04	63.25 ± 1.52
CO-2	92.31 ± 2.31	81.47 ± 1.87	74.91 ± 1.08	68.40 ± 2.11
Leaf Water Potential (MPa)				
CO-1	-0.66 ± 0.03	-0.89 ± 0.05	-1.02 ± 0.06	-1.25 ± 0.02
CO-2	-0.65 ± 0.04	-0.82 ± 0.02	-0.93 ± 0.05	-1.08 ± 0.03

(values are the mean ± SD of six replicates)

Antioxidant content

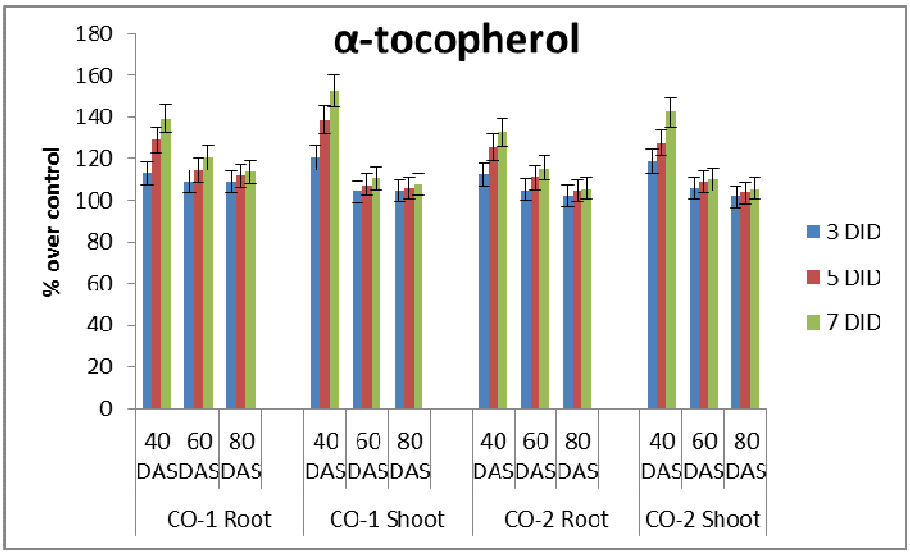
Both the varieties showed increased ascorbic acid content under drought stress treatments in both root and shoot when compared with control (Fig. 1). CO-1 showed better ascorbic acid accumulation as compared to CO-2 under all drought stress treatments. Both the varieties showed a tendency of recovery after stresses were removed.

Figure 1
Effect of drought stress on ascorbic acid content in roots and shoots of P. scrobiculatum varieties at different growth stages.



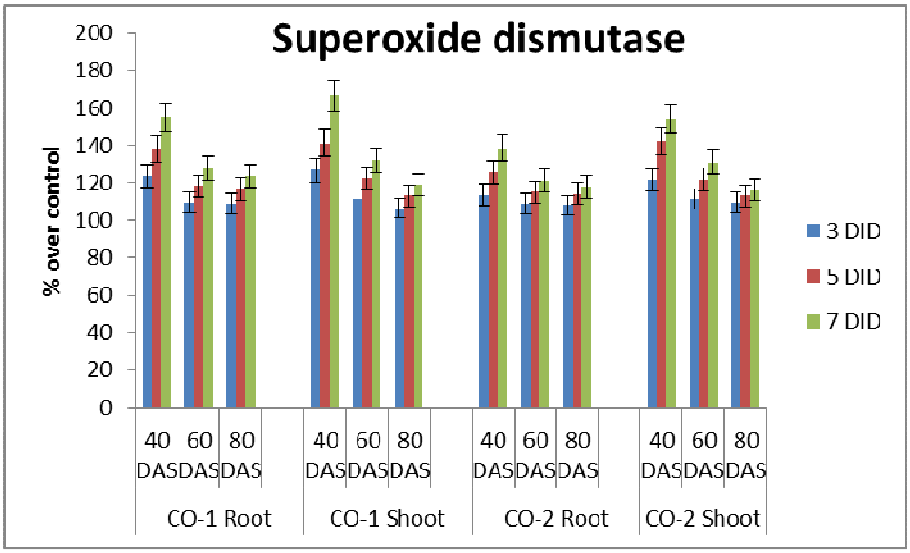
The α-tocopherol content increased under drought stress in the roots as well as shoots of both the varieties when compared with control (Fig. 2). The 7 DID treatment resulted very much effective than other drought stress treatments. The α-tocopherol content was found higher in CO-1 than CO-2 under drought stress treatments.

Figure 2
Effect of drought stress on α-tocopherol content in roots and shoots of P. scrobiculatum varieties at different growth stages.



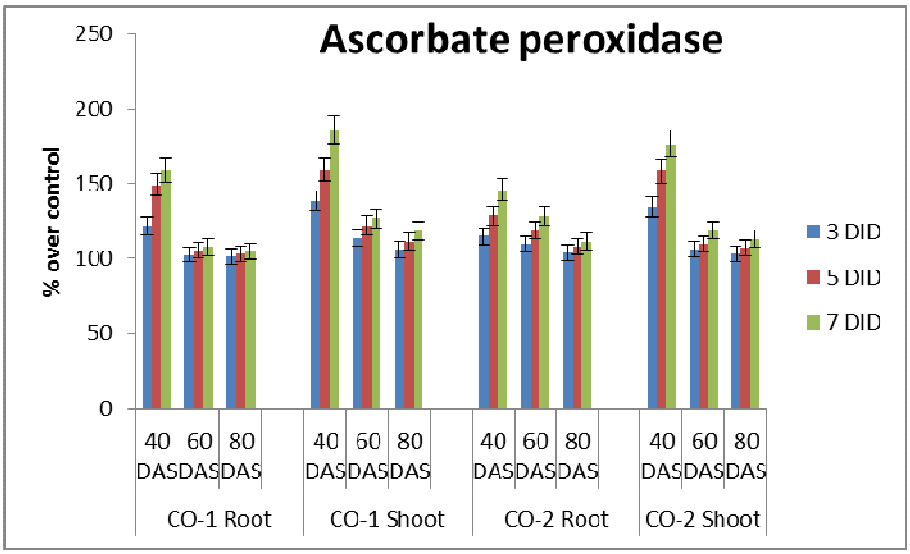
Antioxidant enzymes

Figure 3
Effect of drought stress on superoxide dismutase activity in roots and shoots of P. scrobiculatum varieties at different growth stages.



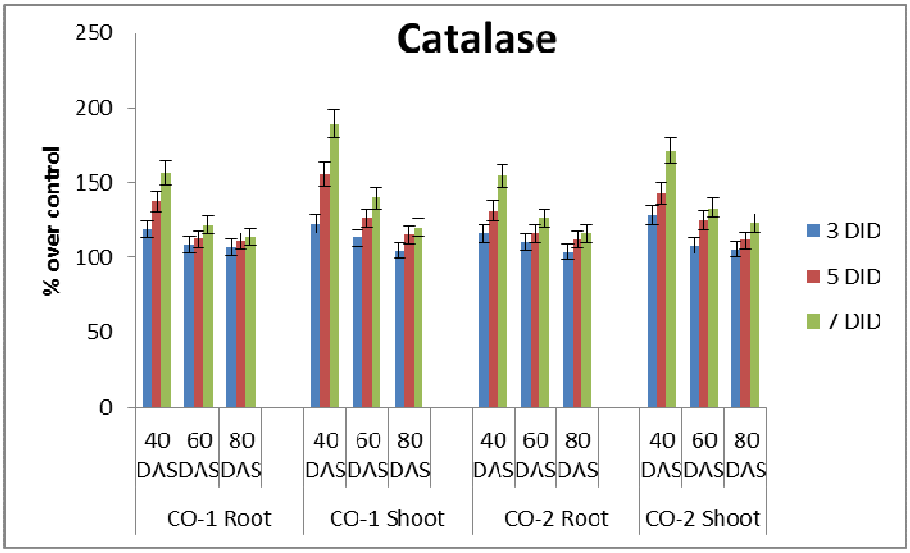
The superoxide dismutase activity increased in the roots of both the varieties under drought stress when compared with control (Fig. 3). However, the SOD activity was found to increase significantly on 40 DAS than 60 and 80 DAS when compared with their respective controls under stress treatments. The total SOD activity was found higher in CO-1 as compared to CO-2 under drought stress. The Ascorbate peroxidase activity increased under drought stress in the roots and shoots of both the varieties when compared with control (Fig. 4). The peroxidase activity was found higher under 7 DID than 3 and 5 DID treatments. CO-1 showed higher peroxidase activity as compared to CO-2 under drought stress on 40 DAS. Furthermore peroxidase activity was higher in the shoot than root in both the varieties.

Figure 4
Effect of drought stress on ascorbate peroxidase activity in roots and shoots of P. scrobiculatum varieties at different growth stages.



The catalase activity was found increased to a large extent under drought stress in both roots and shoots of both the varieties when compared with control (Fig. 5). The rate of catalase activity was higher under 7 DID than 3 and 5 DID treatments. However, the rate of catalase activity was higher in both the varieties on 40 DAS than 60 and 80 DAS when compared with their respective controls. CO-1 showed higher catalase activity under drought stress as compared to CO-2 on all the growth stages. In present investigation it was observed that after removal of drought stress, the differences in both antioxidant content as well as an antioxidant enzyme activity under stress treatments and control were found less distinctive on 60th and 80th DAS in both root and shoot of *P. scrobiculatum* varieties. In other words, we may say that plant showed a tendency to recover towards the normal condition on re-watering.

Figure 5
Effect of drought stress on catalase activity in roots and shoots of P. scrobiculatum varieties at different growth stages.



DISCUSSION

Drought stress limits plant growth by adversely affecting various physiological and metabolic processes, such as photosynthesis, nitrogen metabolism, antioxidant metabolism, proline metabolism and osmolyte accumulation [30]. From the data of present investigation, it is clear that drought stress significantly decreased plant growth of *P. scrobiculatum* from 30 to 60 DAS (days after sowing). It is also evident from our results that drought stress markedly reduced shoot length and total leaf area, which may result in shoot fresh and dry weight reduction. Similar results were found under drought stress in soybean [31] and *Populus* species [32]. The plant height was reduced up to 25% in water stressed *Sorghum* [33], cowpea [34], maize [35] and Olive [36]. Leaf area and shoot length is an important morphological marker for drought stress. Such decrease in shoot length and leaf area in response to drought may be either due to decline in cell elongation resulting from water shortage, which led to a decrease in cell turgor, cell volume and eventually the cell growth [37]. Decreased total dry weight may be due to the considerable decrease in plant growth, due to decline in cell enlargement, photosynthesis and canopy structure and increase in leaf senescence during drought stress in *Abelmoschus esculentum* [38]. Severe water stress may result in arrest of photosynthesis, disturbance of metabolism, and finally drying [39]. The reduction in shoot length and leaf area may decrease the transpiration by decreasing the number of stomata, which may prevent the water loss, hence helps the plant to survive under extreme water deficit conditions. By increasing the severity and duration of drought from three to five days, the root length and root fresh and dry weight of drought stress treated plants significantly increased as compared to control. The root length was found higher in CO-2 when compared with CO-1 under drought stress condition. Previous studies mention that drought stress increased the root length in *Eucalyptus microthea* seedlings [40], *Populus* species [32], Pearl millet [41], Olive [36], *Triticum aestivum* [42] which coincide with those of our study. Zhu et al. [43] proposed an interesting hypothesis for enhanced root growth and root-length density. They hypothesized that root cortical aerenchyma (RCA) reduces root respiration in maize by converting living cortical tissue to air volume. This should reduce root metabolic cost and release more energy for root growth. Their data for maize lines of low and high RCA show that high RCA was associated with an appreciable increase in root-length density and depth.

Measuring plant water status is an important physiological index in identification of plant response to drought stress. RWC and leaf water potential were decreased by increasing drought intensity (Table 4) it is in line with the results of Khadem et al. [44]. Also, Saneoka *et al*. [45] reported that RWC in lenti decreased by drought stress as compared with non-stress conditions. Reduction in RWC of the leaf due to drought stress is related to the reduction in soil humidity; under these conditions the stomata should be closed to avoid more water waste. The reason of stomata closure is abscisic acid that is made in the root in drought stress conditions and accumulates in stomata cells [46]. Our findings are also supported by a decrease in the RWC in response to drought stress in a wide variety of plants [8, 47] that when the leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. The utilization of leaf RWC as an indicator of plant water status is usual [48]. In several legume cultivars of *Phaselus vulgaris* [49], *Vigna glabrescens* [50] and *Lupinus albus* [51] the close relationship between RWC and predawn water potential observed during progressive water deficit supports its utilization as an indicator of plant water status. The concentration of ascorbic acid was increased under drought stress in both the varieties when compared to control. The ascorbic acid concentration was higher in shoots as compared to roots in both the varieties under drought stress. Ascorbic acid concentration was found higher in CO-1 as compared to CO-2. Water stress resulted in a significant increase in antioxidant ascorbic acid concentration in turf grass [52]. Similar results were observed in *sorghum* seedlings

[53], *Triticum aestivum* [54] and maize [55]. Ascorbic acid is a very important reducing substrate for H_2O_2 detoxification in photosynthetic organisms [56]. Ascorbic acid participates in the removal of H_2O_2 as a substrate of ascorbate peroxidase. A continuous oxidative assault on plants during drought stress has led to the presence of an arsenal enzymatic and non-enzymatic plant antioxidant defenses to counter the phenomenon of oxidative stress in plants [57, 58].

Drought stress increased the α-tocopherol levels in both the varieties when compared to control. α-tocopherol concentration was higher in shoots as compared to roots in both the varieties. CO-1 showed higher α-tocopherol levels as compared to CO-2 under drought stress condition. Zhang and Schmidt [52] reported a two-fold increase in α-tocopherol in turf grass under water stress. Synthesis of low-molecular-weight antioxidants, such as α-tocopherol, has been reported in drought-stressed *sorghum* seedlings [53], wheat [54], maize [55], rice [59] and apple tree [60]. Similar results were found in rosemary species and lemon balm under drought stress [61]. α–tocopherols interaction with the polyunsaturated Acyl groups of lipids, stabilize membranes, scavenge and quench various reactive oxygen species (ROS) and lipid soluble byproducts of oxidative stress [40, 52]. Oxidative stress activates the expression of genes responsible for the synthesis of tocopherols in plants [62]. The present investigation showed increased activity of SOD, APX and CAT under drought stress. Similar results were found under water deficit stress in wheat [53] and *Hordeum vulgare* [63]. The generation of reactive oxygen species (ROS) is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses. The production of ROS in plants, known as the oxidative burst, is an early event of plant defense responses to water-stress and acts as a secondary message to trigger subsequent defense reaction in plants. The leading role in protecting the plants from ROS belongs to antioxidant enzymes. The expressions of the antioxidant enzyme-related genes were up-regulated by water stress treatments. SOD activity directly modulates the amount of ROS. This suggests that drought adaptation improved the antioxidant capacity, which may effectively decrease ROS injury during heat stress. Similar results were seen in the case of CAT activity also. SOD and CAT activities have been reported to be negatively correlated with the degree of damage of plasma membrane, chloroplast, and mitochondrial membrane systems, and positively related to the indices of stress resistance [64]. It is reported that the APX found in organelles is believed to scavenge H_2O_2 produced from the organelles, whereas the function of cytosolic APX is probably to eliminate H_2O_2 that is produced in the cytosol or apoplast and that which has diffused from organelles. In the chloroplast, H_2O_2 can be detoxified by the ASA-GSH-NAPDH system that has been catalyzed by APX [65].

CONCLUSION

From the above results, it may be concluded that drought stress reduced growth to a large extent in *Paspalum scrobiculatum*. Both the varieties of *P. scrobiculatum* L. showed reduced growth and altered enzymatic and non-enzymatic activities under drought stress conditions. CO-1 showed higher rates of enzyme activity than CO-2 under drought stress. CO-1 showed better growth under drought condition. In addition, both the varieties showed a tendency to recover when the drought stresses were removed. So it may be concluded that the plant, *Paspalum scrobiculatum*, is a drought tolerant, because it showed the adaptations for survival against drought stress.

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