



Effect of water stress on the physiological and biochemical responses of two different *Coleus* (*Plectranthus*) species

Research Article

Cite this article: Prathyusha IVSN and Chaitanya KV. 2019. Effect of water stress on the physiological and biochemical responses of two different *Coleus* (*Plectranthus*) species. *Biol. Fut.* 70, 312–322.

Received: 1 October 2018

Accepted: 11 September 2019

Keywords:

Coleus forskholii (*Plectranthus barbatus*), *Coleus* (*Plectranthus amboinicus*), drought stress, antioxidants, osmolyte accumulation, leaf water potential

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DOI: [10.1556/019.70.2019.35](https://doi.org/10.1556/019.70.2019.35)

Introduction: Effect of water stress on the physiology and biochemistry of two different *Coleus* species, *Coleus forskholii* and *Coleus amboinicus*, was studied. **Materials and methods:** Drought stress was imposed by withholding the water supply until leaf water potentials reached -0.4 , -0.8 , and -1.2 MPa. Physiological parameters such as relative water content and water uptake capacity were studied along with lipid peroxidation, superoxide, H_2O_2 , and $\cdot OH$ accumulation-, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assays. Antioxidant defense system in *Coleus* under drought stress was studied by quantifying the Trolox equivalent antioxidant capacity, ascorbic acid, reduced glutathione-, and α -tocopherol content as well as activities of superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, and glutathione reductase. Accumulation of osmolytes proline, glycine betaine, and phytohormone abscisic acid was also used as key parameters for assessing their performance. **Results:** There was a marked variation in the antioxidative defense system and osmolyte accumulation in these two species under drought stress. Relative water content was reduced and water uptake capacity was increased. **Discussion:** A comparative study in the perspectives of osmolyte accumulation, antioxidant, and physiological responses inferred *C. amboinicus* as a drought stress-tolerant species when compared to *C. forskholii*.

INTRODUCTION

Drought stress is one of the most important abiotic stress factors that are limiting plant growth and productivity globally (Michaletti et al., 2018). Plants being sessile cannot abscond from prevailing drought stress, ultimately affecting their growth and productivity (Lanari et al., 2018). With increasing global warming and erratic nature of rainfall, proportion of drought stricken lands is on a constant rise across the globe. Drought usually occurs at extended periods of lowered rainfall when there is reduced water availability accompanied with continuous loss of water by evaporation or transpiration to the environment (Mishra & Cherkauer, 2010). Plants cope up with this situation by maximizing water uptake capacity (WUC), osmotic adjustments, changes in cell wall elasticity, accumulating the abscisic acid (ABA) in their guard cells, and shutting off their stomatal system to regulate transpiration, which effects the net carbon assimilation (Saha et al., 2016).

Reduction in photosynthesis during drought increases oxidative load on tissues mainly because of enhanced metabolic flux through photorespiratory pathway leading to oxidative stress at cellular level (Ebadzad et al., 2015). Generation of reactive oxygen species (ROS) including superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) is an important response to the oxidative stress. Increased ROS concentrations in plant cells can disrupt the cellular metabolism by damaging lipids, proteins, chlorophyll, and nucleic acids. They are capable of damaging membrane phospholipids and increasing lipid peroxidation leading to electrolytic leakage and ultimately cell death (Jafarnia et al., 2018). To control the ROS production under drought stress, plants developed antioxidative defense system, comprising of different enzymatic and non-enzymatic components.

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Vital antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidases, glutathione reductase (GR), and ascorbate peroxidase (APX) well supported by non-enzymatic antioxidants ascorbate, glutathione, and α -tocopherol, whose coordinated function prevents cell damage. Accumulation of small molecular weight organic molecules during extended periods of water deficit is believed to have a positive effect on cell turgor over a wide range of plant species. The accumulated osmolytes function as energy source, free radical scavengers, and protein stabilizers without interfering with the biochemical properties of surrounding molecules. Proline and glycine betaine are the most extensively studied osmolytes in plants during drought stress. The survival and recovery of plant from drought depends on the strategies adopted against oxidative stress and the efficiency of osmolyte accumulation.

Genus *Coleus* (recently named as *Plectranthus*) popularly known for its ethnobotanical importance belongs to *Lamiaceae* family with around 300 species distributed in tropical parts of Africa, Asia, and Australia. Many species of this genus are widely used in Ayurveda to treat heart diseases; abdominal and respiratory disorders like insomnia, convulsions, asthma, bronchitis; intestinal disorders; burning sensation; constipation; and epilepsy (Ramana & Chaitanya, 2015). *Coleus forskholii* (renamed as *Plectranthus barbatus*) is an aromatic herb cultivated for its tuberous root containing a diterpenoid called forskolin, used to treat hypertension, insomnia, convulsions, eczema, respiratory disorders, and congestive heart failure. It also possesses therapeutic features of curing asthma, psoriasis, and cancer. Forskolin is well known for its properties in preventing blood clotting, helps in nerve regeneration, activates adenylate cyclase enzyme, and reduces the intra-ocular pressure in glaucoma (Chowdhary & Sharma, 1998). *Coleus amboinicus* (renamed as *Plectranthus amboinicus*) is considered as carminative, lactagogue, analgesic, anti-septic, and anti-pyretic. The leaf extracts of this plant are used to treat headache, toothache, bites, burns, and also effective against malarial parasite. These two species are well studied for their bioactive constituents relating to medicinal properties and their products are extensively used by pharmaceutical industries. There is an increase in the global demand for cultivation of medicinal plants in modern era of medicine. However, environmental factors have a substantial effect on plant growth and survival finally impacting the economic concerns. Not much information is available relating to abiotic stress such as drought in genus *Coleus*. Considering the medicinal importance of this genus and need to understand its tolerance levels to water deficit, this study was conducted aiming a clear understanding of physiological, antioxidant, and osmotic responses to drought stress in two different *Coleus* species.

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazolium (NBT), 2,4-dinitrophenyl hydrazine (DNPH), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), dithiothreitol (DTT), 1-diphenyl-2-picrylhydrazyl (DPPH)

radical, polyvinyl pyrrolidone, nicotinamide adenine dinucleotide phosphate reduced (NADPH), bovine serum albumin, and methionine were provided by Sisco Research Laboratories Pvt., Ltd. (Mumbai, India). Thiobarbituric acid (TBA) was purchased from Molychem (Mumbai, India). Antioxidant assay kit and Sephadex G-25 column were purchased from Cayman Chemical Company (MI, USA) and Sigma-Aldrich (MO, USA), respectively. Ethanol and α -tocopherol were obtained from Changshu Yangyuan Chemical (Jiangsu, China) and Acros Organics (NJ, USA), respectively. All the other chemicals employed were of standard analytical grade and provided by Thermo Fisher Scientific India Pvt., Ltd. (Mumbai, India).

Plant growth conditions and drought stress treatment

Two *Coleus* species, *C. forskholii* and *C. amboinicus* plants, were propagated in the GITAM University botanical garden in 12-in. plastic pots arranged 1 m apart following completely randomized design with five replications containing soil, sand, and peat in equal volumes under natural photoperiod. Plants were allowed to acclimate for a 4-week period before application of drought stress. All pots were irrigated daily with normal tap water and periodically fertilized with Hoagland nutrient solution. Drought stress was imposed by restraining the water supply until the leaf water potentials (LWP) reached -0.4 , -0.8 , and -1.2 MPa, respectively, as determined by Scholander pressure bomb technique using a pressure chamber (SKPM 1400, Skye Instruments Ltd., UK). Control plants were irrigated normally. Fully expanded third and fourth leaves from shoot apex were used for all experiments.

Soil moisture content

The moisture content of the soil in the pots was measured by gravimetric method. Soil sample was collected near the roots of the plants in an air-tight aluminum container. Samples were weighed and were dried in an oven at 105°C for 24 hr. The samples were removed from the oven, cooled to the room temperature, and were weighed again to identify the difference in the amount of moisture in the soil, calculated using the following formula: $100 \times (\text{wet weight} - \text{dry weight}) / \text{dry weight}$. The soil moisture levels (%) were decreased in the pots subjected to drought stress from 48% to 35.8% at -0.4 MPa, 22.4% at -0.8 MPa, and 12.1% at -1.2 MPa, respectively.

Relative water content (RWC), WUC, and water saturation deficiency (WSD)

Fully expanded mature leaves were excised at base of lamina, weighed immediately to obtain the fresh weight (FW) and floated over distilled water for 12 hr at room temperature. The turgid leaves were blotted using paper towel to remove surface moisture and weight was recorded as turgid weight (TW). Finally, dry weight (DW) was obtained after oven drying the saturated leaves at 60°C . RWC was calculated using formula $\text{RWC} = 100 \times (\text{FW} - \text{DW}) / (\text{TW} - \text{DW})$ according to Barrs (Barrs & Weatherly, 1962) and WUC using formula, $\text{WUC} = (\text{TW} - \text{FW}) / \text{DW}$ according to Sangakkara et al. (1996). WSD

was calculated by the formula, $WSD = 100 \times (TW - FW) / (TW - DW)$, according to Slatyer (1961).

Membrane Stability Index (MSI)

MSI was calculated according to Premachandra et al. (1990) and was modified by Sairam (1994). Leaf disks (0.1 g) were washed thoroughly in tap water followed by washing in distilled water. The disks were incubated in double distilled water at 40 °C for 30 min and the electrical conductivity (C1) was recorded. Furthermore, the leaf disks were transferred into a boiling water bath (100 °C) and were incubated for 10 min and the electrical conductivity (C2) was recorded. The MSI was determined according to the following formula:

$$MSI\% = 100 \times [1 - (C1/C2)]. \quad (1)$$

Determination of lipid peroxidation

The extent of lipid peroxidation in leaves was measured according to method of Dhindsa et al. (1981).

Free radical scavenging assays

$O_2^{\cdot-}$ scavenging activity was assayed according to Liu et al. (1997). An amount of 16 mM of Tris-HCl buffer (pH 8.0), NBT (50 μ M), NADH (78 μ M), and dried leaf methanolic extract (20 mg/ml) were added to 10 μ M of phenazine methosulfate (PMS) in a tube to final volume of 3 ml, shaken well, and incubated for 5 min at room temperature. $O_2^{\cdot-}$ radicals generated through non-enzymatic PMS–NADH system were quantified by measuring the purple-colored formazan at 560 nm against blank that is devoid of PMS.

H_2O_2 scavenging ability of dried leaf methanolic extract was determined according to method of Dehpour et al. (2009). An amount of 0.1 mg/ml dried leaf methanolic extract was added to 40 mM of H_2O_2 solution prepared in phosphate buffer (pH 7.4) and incubated for 10 min. Absorbance was read at 230 nm against blank containing phosphate buffer without H_2O_2 .

The ability of dried leaf methanolic extract in scavenging $\cdot OH$ radicals generated by Fenton reaction through Fe^{3+} -EDTA-ascorbate- H_2O_2 system was determined according to Halliwell et al. (1987). Reaction mixture contained 28 mM of 2-deoxy D-ribose, 20 mM KH_2PO_4 (pH 7.4), 1.04 mM EDTA, ferric chloride (1:1 v/v), 1.0 mM H_2O_2 , 1.0mM ascorbic acid, and dried leaf methanolic extract (500 μ g/ml). Simultaneously, a blank was set containing 2-deoxy D-ribose and buffer. Tubes were shaken well and incubated at 37 °C for 1 hr. About 1% TBA and 2.8% TCA were added and incubated for 20 min at 100 °C. Tubes were cooled and yellow-colored product formed by condensation of malonaldehyde (degradation product of 2-deoxy2-ribose) with TBA was read at 532 nm.

The DPPH radical scavenging ability of *Coleus* foliar extract was assayed according to modified method of Blois (1958). Methanolic leaf extract at different concentrations (5–300 μ g/ml) was prepared in 1 ml aliquots, and 5 ml DPPH (33 mg/L in methanol) was added. Tubes

were incubated in dark for 30 min and absorbance was measured at 517 nm against methanol blank. Ascorbic acid equivalent antioxidant capacity (AEAC) according to Lim et al. (2007) was calculated using the following formula:

$$AEAC \text{ (mg/100 g)} = \frac{IC_{50}(\text{ascorbic acid})}{IC_{50}(\text{sample})} \times 10^5. \quad (3)$$

Estimation of non-enzymatic antioxidants

Total antioxidant content in *Coleus* foliar extracts was assayed according to Miller et al. (1993) using antioxidant assay kit obtained from Cayman Chemical Company. Antioxidant activity in terms of Trolox equivalents was calculated using the following equation:

$$TEAC \text{ (mM)} = \frac{\text{(average of sample absorbance)} - y\text{-intercept}}{\text{slope}} \times \text{dilution}. \quad (4)$$

Ascorbic acid content was determined according to Roe and Kuether (1943). An amount of 1 g of sample was homogenized with 5 ml of 10 % TCA, centrifuged at $3,500 \times g$ for 20 min, reextracted twice, and supernatant was made up to 10 ml. About 0.5 ml of supernatant was made to 2.0 ml in 4% TCA. A portion of 0.5 ml of 2% DNPH in 9N of H_2SO_4 followed by two drops of 10% thiourea were added and incubated for 3 hr at 37 °C. Osazone crystals formed were dissolved in 2.5 ml of 85% H_2SO_4 in cold and absorbance was read at 540nm. Ascorbic acid content in foliar extracts was calculated by standard ascorbic acid curve (10–100 μ g/ml) and reported as mg ascorbic acid/ g fresh weight.

Reduced glutathione content was estimated by homogenizing 0.5 g of leaf tissue with 2.5 ml of 5% TCA and centrifuged at $1,000 \times g$ for 10 min. An amount of 100 μ l of supernatant was made up to 1 ml using 0.2 M sodium phosphate buffer (pH 8.0) and 2 ml of 0.6 mM DTNB solution was added. After incubation for 10 min at 37 °C, absorbance was determined at 412 nm (Moron et al., 1979).

α -Tocopherol was quantified according to Rosenberg (1942). An amount of 2.5 g fresh leaf was crushed in 0.1 N of H_2SO_4 and volume was made to 50 ml. Homogenate was allowed to stand overnight, shaken vigorously, and filtered using Whatman No.1 filter paper. A portion of 1.5 ml each of filtrate, α -tocopherol standard (10 mg/L in absolute alcohol), and double distilled water were pipetted into three separate stoppered tubes. About 1.5 ml of ethanol and xylene were added and shaken vigorously. Tubes were centrifuged and 1.0 ml xylene layer was aspirated into another stoppered tube. 1.0 ml of 2,2'-dipyridyl reagent (1.2 g/L in *n*-propanol) was added and the absorbance was recorded at 460 nm. An amount of 0.33 ml ferric chloride (1.2 g/L in ethanol) was added to all tubes and mixed well. The red color developed was read at 520 nm after 15 min. Tocopherol content was calculated using the following formula:

Tocopherol ($\mu\text{g/g}$ leaf tissue) =

$$\frac{[(\text{sample}_{\text{abs}520\text{nm}} - \text{sample}_{\text{abs}460\text{nm}}) / \text{standard}_{\text{abs}520\text{nm}}] \times 0.29 \times 0.15}{(5)}$$

Extraction of antioxidant enzymes

Mature and fully expanded leaves were washed in tap water and distilled water, surface sterilized with 0.001% of HgCl_2 , and blotted over paper towel to remove surface moisture. An amount of 10 g of fresh leaf was crushed in 50 volumes of extraction buffer [10mM Tris-HCl (pH 7.5) containing 5 mM of DTT, 10 mM of MgCl_2 , 1 mM of EDTA, 5 mM of magnesium acetate, and 1.5% PVP]. Homogenate was squeezed in four-layered cheesecloth and centrifuged at $10,000 \times g$ for 10 min. Using 75% (w/v) ammonium sulfate, the protein was precipitated and separated by centrifuging at $30,000 \times g$ for 25 min. Precipitate obtained was dissolved in 50 mM Tris-HCl (pH 7.8) containing 1 mM of DTT and 2 mM of EDTA. Preparation obtained was applied onto Sephadex G-25 column (Sigma-Aldrich) that was pre-equilibrated with 10 mM of Tris-HCl buffer (pH 8.0) containing 1mM of DTT, 10 mM of sodium bicarbonate, 20 mM of MgCl_2 , and 0.2 mM of NADPH.

SOD was assayed according to Beyer and Fridovich (1987). Reaction mixture contained 50 mM of potassium phosphate buffer (pH 7.8), 0.1 mM of EDTA, 12 mM of L-methionine, 75 μM of NBT, 2 μM of riboflavin, and 0.1 ml of enzyme extract in a total volume of 3.0 ml. Reaction was initiated by adding riboflavin and exposing the tubes to fluorescent light. The reaction mixture devoid of enzyme served as control and that incubated in dark was considered as blank. Absorbance was recorded for all tubes at 560 nm after 15 min of incubation period. One enzyme unit is the amount of SOD producing 50% inhibition of NBT reduction under assay conditions.

CAT activity was measured according to Chandless and Scandalios (1984). Reaction was initiated by adding 15 mM of H_2O_2 into 50 mM of potassium phosphate buffer (pH 7.0), and enzyme extract to total volume of 3.0 ml of reaction mixture. CAT activity was monitored with decreasing absorbance for 3 min at 240 nm. One enzyme unit is the amount of enzyme required to decompose 1 μM of H_2O_2 in unit time under assay conditions.

APX enzyme was assayed according to Nakano and Asada (1981). Reaction was initiated by adding 0.25 mM of H_2O_2 to tube containing 0.1 ml of enzyme extract, 50 mM of potassium phosphate buffer (pH 7.0), 0.2 mM of EDTA, and 0.5 mM of ascorbate in 3 ml volume. Enzyme activity was monitored with the gradual decline in ascorbate concentration at 290 nm and one unit is APX that can oxidize micromole ascorbate per unit time.

GR activity was determined according to David and Richard (1983). The assay mixture contained 0.1 mL crude enzyme extract in 0.12 M OF potassium phosphate buffer (pH 7.2), 15 mM of EDTA, 10 mM of sodium azide, and 6.3 mM of oxidized glutathione. Volume was made to 2.0 ml with double distilled water in a test tube and incubated for 3 min. An amount of 0.1 ml of 6.3 mM NADPH was added and decline in absorbance was recorded

for 3 min with 15-s interval at 340 nm. For all enzyme activity calculations, protein content was determined spectrophotometrically at 595 nm by method of Bradford (1976) using bovine serum albumin as standard.

Quantification of proline and glycine betaine

Proline concentrations in the *Coleus* leaf sample were assayed according to Bates et al. (1973). Glycine betaine content was measured according to the method of Grieve and Grattan (1983).

Determination of ABA content

One gram of *Coleus* leaf tissue was homogenized in 15 ml of extraction medium containing 80% methanol, 100 mg/L of butylated hydroxytoluene, and 0.5g/L of citric acid monohydrate and the suspension was centrifuged at $1,000 \times g$ for 20 min at 4 °C. The supernatant was passed through Sep-Pak-C-18 column and the pooled washings were evaporated under vacuum. The residue was partitioned thrice into equal volume of ethyl acetate (pH 3.0). Resulting organic phase was evaporated and the residue was redissolved in 2 ml of TBS buffer (pH 7.5) and was subjected to ELISA using ABA immunoassay kit (Labex Corporation, New Delhi, India).

Statistical analysis

The results mentioned are reported as the mean \pm standard error (SE) values of five independent experiments, conducted on five different plants in each experiment. SE values were calculated directly from the data according to standard methods (Taylor, 1982). Data analyses were carried out using the SPSS (Chicago, IL, USA) package. Mean values were compared by Duncan's multiple range test and *p* values, which are less or equal to 0.05 were considered as statistically significant.

RESULTS

Drought stress-induced physiological changes

RWC gradually declined with decreasing LWP values in both *C. forskholii* and *C. amboinicus*. However, *C. amboinicus* maintained relatively higher RWC (60.57%) at LWP of -1.2 MPa, whereas in *C. forskholii* it was 54.21% (Table 1). With increasing drought stress, WUC was found to be increased in *C. forskholii* (19.2%–81.2%) and *C. amboinicus* (22.2%–86.3%) at a LWP of -1.2 MPa (Table 1). Water saturation deficit (WSD) increased and MSI decreased in both *Coleus* species with decreasing LWPs (Table 1). MSI was found to be decreasing from 61% to 31% in *C. forskholii* and from 69% to 44% in *C. amboinicus* (Table 1).

Drought stress-induced membrane damage and radical scavenging activities

Increased generation of ROS leads to a rapid membrane damage as the membrane lipid peroxidation is a common

Table 1. Relative water content, water uptake capacity, water saturation deficit, Membrane Stability Index, and relative membrane injury in two different *Coleus* species when subjected to drought

<i>Coleus</i> species	<i>C. forskholii</i>	<i>C. amboinicus</i>
<i>Relative water content (%)</i>		
Control	94.8 ± 1.09 ^a	97.89 ± 1.68 ^a
–0.4 MPa	90.9 ± 0.88 ^c	97.12 ± 0.94 ^c
–0.8 MPa	61.79 ± 1.02 ^b	80.54 ± 1.16 ^b
–1.2 MPa	54.21 ± 0.86 ^c	60.57 ± 0.94 ^c
<i>Water uptake capacity (%)</i>		
Control	19.2 ± 0.38 ^b	22.2 ± 0.34 ^b
–0.4 MPa	30.7 ± 0.22 ^a	25.8 ± 0.48 ^a
–0.8 MPa	54.5 ± 0.46 ^c	72.4 ± 0.28 ^c
–1.2 MPa	81.2 ± 0.28 ^d	86.3 ± 0.81 ^d
<i>Water saturation deficit (%)</i>		
Control	24.2 ± 1.62 ^a	31.7 ± 4.92 ^a
–0.4 MPa	38.1 ± 3.80 ^b	44.6 ± 3.32 ^b
–0.8 MPa	54.2 ± 2.28 ^c	61.5 ± 2.72 ^c
–1.2 MPa	66.8 ± 1.19 ^d	78.4 ± 1.52 ^d
<i>Membrane Stability Index (%)</i>		
Control	61.29 ± 1.30 ^b	69.82 ± 1.40 ^b
–0.4 MPa	53.64 ± 1.82 ^c	63.10 ± 1.11 ^a
–0.8 MPa	47.94 ± 1.61 ^a	56.59 ± 1.10 ^a
–1.2 MPa	31.17 ± 1.50 ^d	44.07 ± 1.80 ^c

Note. Each value is the mean ± SE of five independent determinations [$t_{(4)}=6.1$, $p < .05$] for relative water content, [$t_{(4)}=2.1$, $p < .05$] for water uptake capacity, [$t_{(4)}=3.8$, $p < .05$] for water saturation deficit, and [$t_{(4)}=1.8$, $p < .05$] for Membrane Stability Index, respectively. Data with different letters indicate significant differences at $p < .05$.

circumstance of oxidative stress triggered during drought. Membrane lipid peroxidation quantified in terms of malondialdehyde (MDA) accumulation was measured in *C. forskholii* and *C. amboinicus* subjected to drought stress (Fig. 1). In *C. forskholii*, MDA content increased from 2.29 to 11.16 nM/ml, particularly between the LWP –0.4 and –0.8 MPa, the MDA levels were doubled indicating the level of membrane damage occurred in this plant during drought. In *C. amboinicus*, MDA content was increased gradually from 2.18 to 6.52 nM/ml with decreasing LWPs.

An enhancement of ROS scavenging activity with the increasing intensity of drought is often considered as a marker of stress tolerance in plants. In this study, both *Coleus* species have upregulated their ROS scavenging activities under drought. $O_2^{\cdot-}$ scavenging activity has shown a maximum value of 81.1% in *C. forskholii* and 92.3% in *C. amboinicus* at –1.2 MPa (Table 2). H_2O_2 scavenging activity was increased from 36.5% to 78.3% in *C. forskholii* and from 42.7% to 89.6% in *C. amboinicus* (Table 2). The $\cdot OH$ scavenging activity increased from 15.1% to 59.5% in *C. forskholii*, and from 8.2% to 14.2% in *C. amboinicus*. DPPH scavenging activity was measured as ascorbic acid equivalents per 100 g dry weight has shown an increase from 31.8 to 69.2 g ascorbic acid 100 g⁻¹ in *C. forskholii* and 37.2 to 77.4 g ascorbic acid 100 g⁻¹ in *C. amboinicus* (Table 2). The results revealed an efficient superoxide and H_2O_2 scavenging activity and a comparatively poor $\cdot OH$ scavenging activity in the *Coleus*

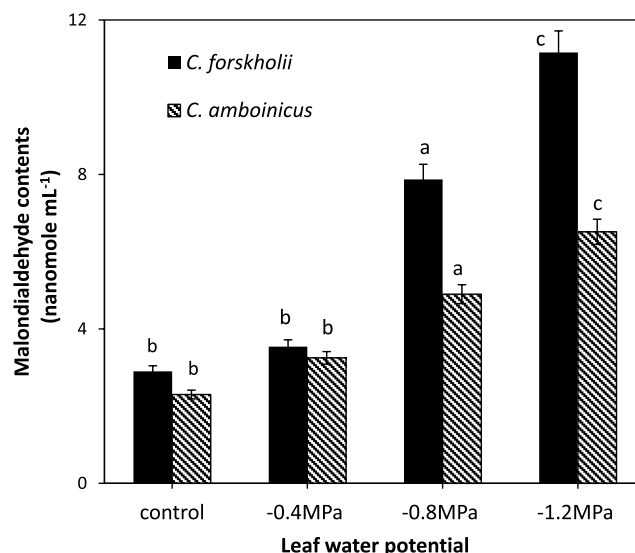


Fig. 1. Lipid peroxidation rates in two different *Coleus* species when subjected to drought stress. Each value is the mean ± SE of five independent determinations [$t_{(4)}=2.8$, $p < .05$]. Data with different letters indicate significant differences at $p < .05$

species with increasing intensity of drought stress. However, ROS scavenging activity was high in *C. amboinicus* compared with *C. forskholii*.

Non-enzymatic antioxidant levels

Levels of ascorbic acid reduced glutathione and α -tocopherol were increased in *C. forskholii* and *C. amboinicus* with concomitant increase of drought stress (Table 3). Ascorbic acid level in *C. forskholii* was 125 mg/g FW and it was 130 mg/g FW in *C. amboinicus* at –1.2 MPa LWP. Levels of reduced glutathione are essential for homeostasis of the cellular redox environment. Although the reduced glutathione content remained nearly similar in control, mild, and moderate stages of drought, a significant elevation in the levels of reduced glutathione was observed at –1.2 MPa LWP (Table 3). α -Tocopherol contents were found to be significantly increased from 1.983 to 2.907 μ g/g FW in *C. forskholii* and from 0.984 to 5.214 μ g/g FW in *C. amboinicus* with increasing duration of drought. There was a fivefold increase of α -tocopherol in *C. amboinicus* subjected to drought stress (Table 3). Total antioxidant capacity of *Coleus* leaf extracts measured as Trolox equivalent antioxidant capacity (TEAC) has shown a gradual increase with decreasing LWPs (Table 3).

Enzymatic antioxidant activities

SOD activity of *C. forskholii* and *C. amboinicus* was found to be increased with decreasing LWPs (Fig. 2). The effect of drought stress on CAT enzyme activity in both *Coleus* species was depicted in Fig. 3. There was a threefold increase in the CAT activity of *Coleus* plants subjected to drought. A gradual increase in the APX activity was noticed in the *Coleus* leaf extracts subjected to drought stress (Fig. 4). However, the increase was high in *C. amboinicus*. GR activity was increased in both the *Coleus* species during drought stress (Fig. 5). The increase of GR in *C. amboinicus* was prominent with increase by twofolds compared with control plants.

Table 2. Superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), and 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activities in two different *Coleus* species under drought stress

<i>Coleus</i> species	Control	−0.4 MPa	−0.8 MPa	−1.2 MPa
$O_2^{\bullet-}$ scavenging activity (%)				
<i>C. forskholii</i>	41.2 ± 1.84 ^a	56.0 ± 1.26 ^b	67.5 ± 0.74 ^c	81.1 ± 1.26 ^b
<i>C. amboinicus</i>	46.4 ± 0.92 ^a	69.5 ± 1.78 ^b	80.5 ± 1.10 ^c	92.3 ± 1.38 ^b
H_2O_2 scavenging activity (%)				
<i>C. forskholii</i>	36.5 ± 0.42 ^a	49.4 ± 0.22 ^b	68.9 ± 0.64 ^c	78.3 ± 0.81 ^c
<i>C. amboinicus</i>	42.7 ± 0.78 ^a	60.7 ± 0.86 ^b	72.6 ± 0.78 ^c	89.6 ± 0.92 ^c
$\bullet OH$ scavenging activity (%)				
<i>C. forskholii</i>	15.1 ± 0.08 ^a	28.6 ± 0.01 ^b	49.6 ± 0.03 ^c	59.5 ± 0.09 ^c
<i>C. amboinicus</i>	18.2 ± 0.04 ^a	32.3 ± 0.04 ^b	51.6 ± 0.08 ^c	64.2 ± 0.06 ^c
DPPH scavenging activity (g ascorbic acid 100 g ^{−1})				
<i>C. forskholii</i>	31.8 ± 0.10 ^a	47.8 ± 0.09 ^b	59.1 ± 0.38 ^c	69.2 ± 0.46 ^a
<i>C. amboinicus</i>	37.2 ± 0.18 ^a	53.9 ± 0.10 ^b	65.5 ± 0.82 ^c	77.4 ± 0.18 ^a

Note. Each value is the mean ± SE of five independent determinations [$t_{(4)} = 1.4$, $p < .05$] for $O_2^{\bullet-}$, [$t_{(4)} = 3.7$, $p < .05$] for H_2O_2 , [$t_{(4)} = 1.2$, $p < .05$] for $\bullet OH$, and [$t_{(4)} = 4.2$, $p < .05$] for DPPH, respectively. Data with different letters indicate significant differences at $p < .05$.

Table 3. Trolox equivalent antioxidant capacity (TEAC), ascorbic acid, reduced glutathione, and α -tocopherol levels in two different *Coleus* species subjected to drought stress

<i>Coleus</i> species	Control	−0.4 MPa	−0.8 MPa	−1.2 MPa
TEAC ($\mu M/g$ FW)				
<i>C. forskholii</i>	13.02 ± 0.09 ^a	28.3 ± 0.18 ^b	29.8 ± 0.38 ^c	39.71 ± 0.81 ^d
<i>C. amboinicus</i>	41.5 ± 0.86 ^a	46.6 ± 0.22 ^b	52.5 ± 0.48 ^c	55.4 ± 0.43 ^d
Ascorbic acid (mg/g FW)				
<i>C. forskholii</i>	0.72 ± 0.04 ^a	1.12 ± 0.02 ^b	1.64 ± 0.06 ^c	2.18 ± 0.06 ^d
<i>C. amboinicus</i>	0.94 ± 0.07 ^a	1.45 ± 0.08 ^b	1.98 ± 0.02 ^c	2.59 ± 0.05 ^d
Reduced glutathione (mg/g FW)				
<i>C. forskholii</i>	0.42 ± 0.03 ^a	0.74 ± 0.02 ^b	1.06 ± 0.06 ^c	1.42 ± 0.09 ^d
<i>C. amboinicus</i>	0.66 ± 0.02 ^a	0.92 ± 0.01 ^b	1.35 ± 0.02 ^c	1.67 ± 0.08 ^d
α -Tocopherol (mg/g FW)				
<i>C. forskholii</i>	0.98 ± 0.04 ^a	2.08 ± 0.06 ^b	3.33 ± 0.06 ^b	5.90 ± 0.01 ^c
<i>C. amboinicus</i>	1.18 ± 0.02 ^a	3.66 ± 0.02 ^b	5.05 ± 0.08 ^b	7.21 ± 0.07 ^c

Note. Each value is the mean ± SE of five independent determinations [$t_{(4)} = 5.2$, $p < .05$] for TEAC, [$t_{(4)} = 1.8$, $p < .05$] for ascorbic acid, [$t_{(4)} = 3.6$, $p < .05$] for reduced glutathione, and [$t_{(4)} = 4.2$, $p < .05$] for α -tocopherol, respectively. Data with different letters indicate significant differences at $p < .05$. FW: fresh weight.

Drought stress-induced changes in osmoprotectants

Drought stress-induced proline levels in both of the *Coleus* species were represented in Fig. 6. There was a fourfold increase in the proline levels in the *Coleus* leaves subjected to drought stress when compared with the respective control plants. However, the proline content was relatively high in *C. amboinicus* compared with *C. forskholii* at all stages of drought stress (Fig. 6). The glycine betaine levels in foliar extracts of both *Coleus* species, when subjected to drought stress, were represented in Fig. 7. In *C. forskholii*, glycine betaine levels increased from 1.2 to 4.5 $\mu g/ml$ in *C. forskholii*, whereas in *C. amboinicus*, the increase was more prominent, ranging from 1.6 to 5.6 $\mu g/ml$.

ABA content

Accumulation of phytohormone ABA was measured in the *Coleus* leaves, exposed to drought stress (Fig. 8). There was approximately two- and half-fold increase in the ABA concentrations in the *Coleus* leaves subjected to drought stress.

However, *C. amboinicus* has shown to possess significantly high amounts of ABA (621 ng/g FW) compared with *C. forskholii* (510 ng/g FW) during water deficit.

DISCUSSION

Plant water relations

Drought stress is a complicated and catastrophic threat to plants because of its diverse and destructive effects. The osmotic imbalance during drought is characterized by the disturbance of several metabolic and physiological processes. To withstand the drought stress, plants have adapted mechanisms, such as stress avoidance, escape, and tolerance. Understanding the mechanisms by which the plants respond to drought stress will help in identification of their drought tolerance (Juenger, 2013). Leaf RWC is considered as an important indicator of the plant water status, which reflects the balance between water absorbed by the plant and its transpiration rate. Drought stress induced

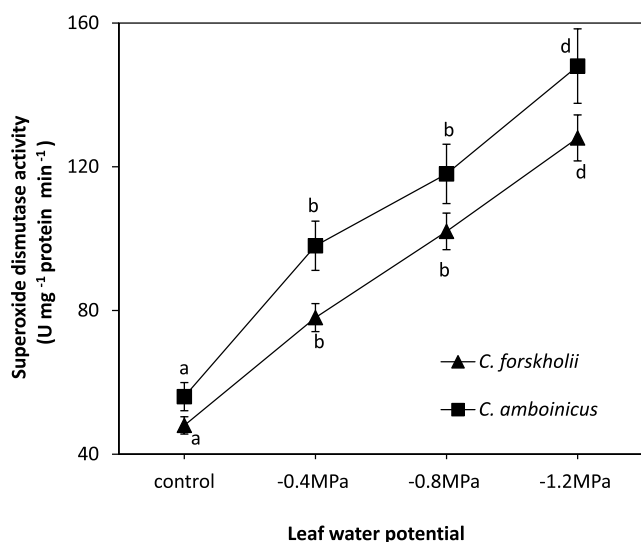


Fig. 2. Superoxide dismutase enzyme activity in two different *Coleus* species when subjected to drought stress. Each value is the mean \pm SE of five independent determinations [$t_{(4)} = 4.2$, $p < .05$]. Data with different letters indicate significant differences at $p < .05$.

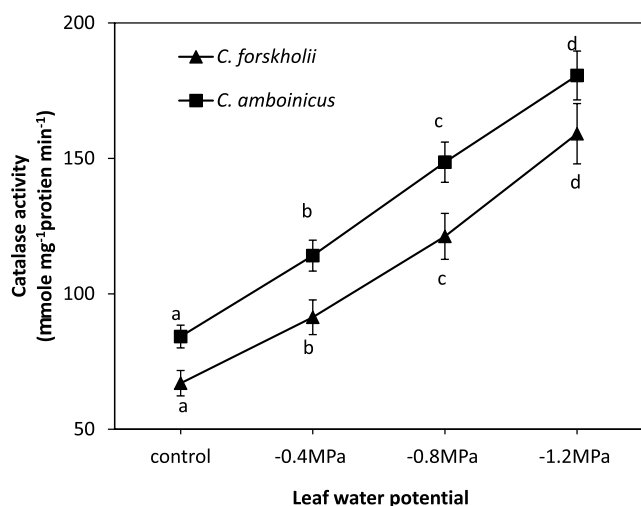


Fig. 3. Catalase enzyme activity in two different *Coleus* species when exposed to drought stress. Each value is the mean \pm SE of five independent determinations [$t_{(4)} = 5.9$, $p < .05$]. Data with different letters indicate significant differences at $p < .05$.

a notable decrease in RWC of *C. forskholii* and *C. amboinicus*; however, *C. amboinicus* maintained a relatively higher RWC than *C. forskholii*. Plants that maintain higher RWC are resistant to drought and the decline of RWC in *Coleus* species can be understood as a consequence of disturbed equilibrium between water intake and loss by the evapotranspiration. Decline in RWC during the period of stress was reported in *Plantaginaceae* members (Rahimi et al., 2010). Regulating RWC during severe drought was considered as stress tolerance factor in cowpea (Hayatu et al., 2014). An increase in WUC observed in both *C. forskholii* and *C. amboinicus* with corresponding increase in the intensity of drought can be accounted for the accumulated small molecular weight molecules in the cellular interior. Accumulation of small molecular weight molecules

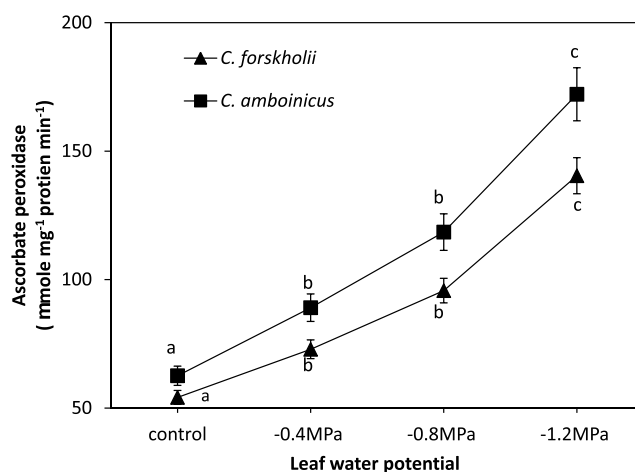


Fig. 4. Ascorbate peroxidase activity levels in two different *Coleus* species when exposed to drought stress. Each value is the mean \pm SE of five independent determinations [$t_{(4)} = 7.8$, $p < .05$]. Data with different letters indicate significant differences at $p < .05$.

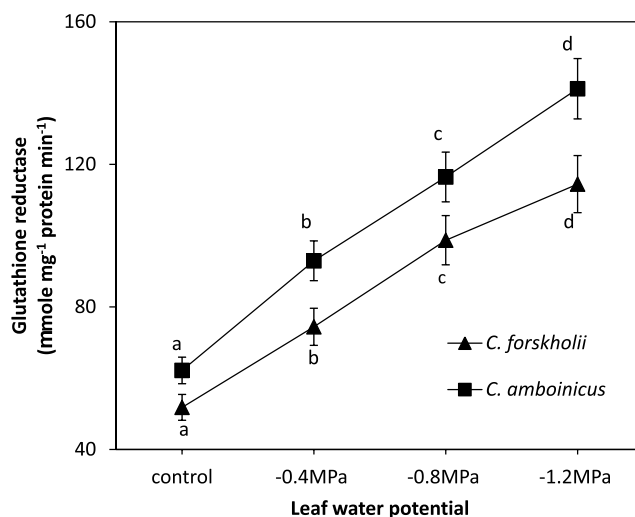


Fig. 5. Glutathione reductase activity in the leaf extracts of two different *Coleus* species exposed to drought stress. Each value is the mean \pm SE of five independent determinations [$t_{(4)} = 6.4$, $p < .05$]. Data with different letters indicate significant differences at $p < .05$.

in the cells maintains a balance in turgor, thus ensuring a continuance of normal metabolic processes within the cells during low water regimes (Bodner et al., 2015). An increase in WUC has been reported as a stress tolerance feature in *Catharanthus* species at severe stages of drought (Jaleel et al., 2008). WSD indicates the amount of water deviated from the saturated leaf, which defines the exact amount of the water in it. An increase in the WSD was noticed in the *C. forskholii* and *C. amboinicus* leaves when subjected to drought stress indicating the level of water deficit in these two *Coleus* species.

Cell membrane injury

Survival of a plant under environmental stress is mediated by the integrity of cell membrane, as the cell membrane is

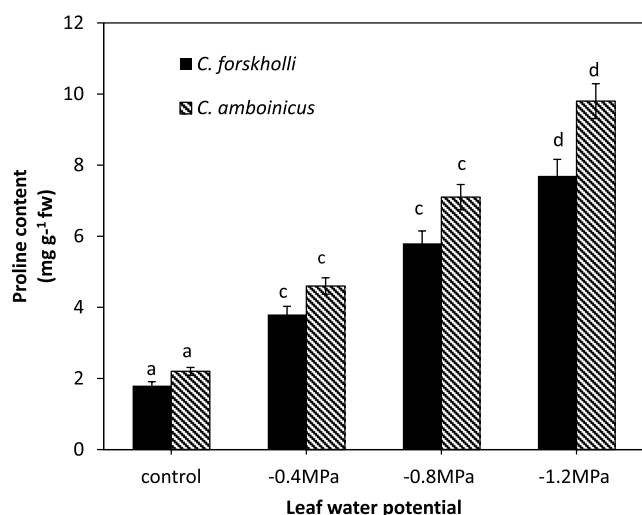


Fig. 6. Influence of drought stress on proline content in two different *Coleus* species. Each value is the mean \pm SE of five independent determinations [$t_{(4)} = 6.1, p < .05$]. Data with different letters indicate significant differences at $p < .05$

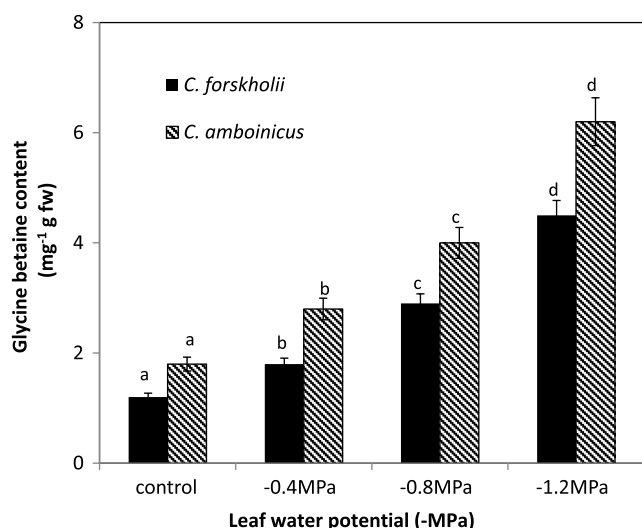


Fig. 7. Effect of drought stress on glycine betaine content in two different *Coleus* species. Each value is the mean \pm SE of five independent determinations [$t_{(4)} = 2.3, p < .05$]. Data with different letters indicate significant differences at $p < .05$

considered as the initial site of injury due to the ongoing stress. Thus, evaluation of the cell membrane integrity during drought stress is considered as an important criterion for screening the plants with tolerance. In this study, the integrity of the *Coleus* cell membrane during drought stress was examined by measuring the MSI of the leaf cells. MSI in the *Coleus* leaves was decreased with decreasing LWP. However, the cell membrane was less damaged in *C. amboinicus*.

Drought stress is characterized by the imprudent generation of ROS as a consequence of constraints in stomatal gaseous exchange and electron diversion in electron transport chains and other energy-dissipating pathways. Enhanced production of ROS leads to the rapid membrane damage followed by leakage due to peroxidation of membrane lipids (Guo et al., 2018). In this study,

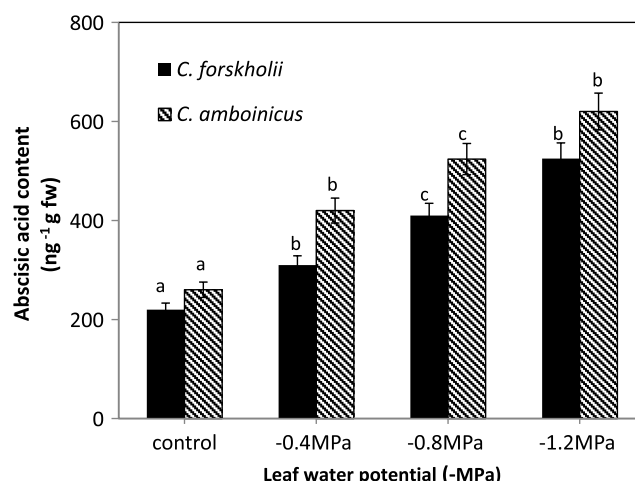


Fig. 8. Effect of drought stress on abscisic acid content in two different *Coleus* species. Each value is the mean \pm SE of five independent determinations [$t_{(4)} = 3.1, p < .05$]. Data with different letters indicate significant differences at $p < .05$

a prominent elevation in lipid peroxidation rates quantified as MDA accumulated in both *Coleus* species is a clear inference of drought stress-induced ROS proliferation. However, MDA levels were less in *C. amboinicus* indicating this species is better protected from oxidative stress than *C. forskholii*.

ROS accumulation impeded by an efficient ROS scavenging system is considered as a marker for the abiotic stress tolerance in plants (Guan et al., 2015). The scavenging abilities of superoxide ($O_2^{\bullet-}$), H_2O_2 , and $\cdot OH$ in *Coleus* leaves were increased progressively with increasing drought stress. *C. forskholii* have shown a relatively lower ROS scavenging ability, which could have accounted for higher rates of lipid peroxidation. DPPH scavenging activity considered as a measure of antioxidant ability of plant extract has shown an increase in the leaf extracts of both *Coleus* species with increasing intensity of drought. An enhanced DPPH scavenging activity during drought treatment was also identified in *Salvia* (Bettaieb et al., 2011) and *Fraxinus* (Štajner et al., 2011).

ROS accumulation is minimized by an array of small molecular weight non-enzymatic antioxidant compounds that can serve as electron donors in a wide range of defense systems. Ascorbic acid, reduced glutathione, and α -tocopherol are the principal non-enzymatic antioxidant molecules, involved in a number of ROS scavenging reactions. In this study, levels of ascorbic acid, reduced glutathione, and α -tocopherol were found to be increased with increasing drought stress in the leaves of both *C. forskholii* and *C. amboinicus*. Ascorbic acid is a H_2O_2 scavenger and a principal metabolite playing multitude of roles chiefly concerned with the cellular growth and survival (Behairy et al., 2012). It is an efficient antioxidant with high potentiality in donating electrons to a wide spectrum of reactive species. The results are consistent with findings in *Vigna* species (Nair et al., 2008) and *Triticum* cultivars (Farooq et al., 2013) where a considerable elevation in ascorbic acid content was reported with increasing severity of drought. Reduced glutathione levels increased to twofold at severe stages of drought in both

Coleus species. Upregulation in reduced glutathione levels was reported in *Brassica* species with the gradual increase of drought (Alam et al., 2013). α -Tocopherol is a predominant ROS scavenging molecule found in the photosynthetic tissues involved in the reduction of lipid peroxyl radicals to hydroperoxides, quinines, and epoxides and maintaining the redox equilibrium (Li et al., 2000). Even though an increase in α -tocopherol levels with increasing drought stress was observed in *Coleus*, the pattern of their elevation differed consistently among both species. Foliar extracts of tobacco, rosemary, and lemon balm were reported to show a similar increase in α -tocopherol content at severe stages of drought (Espinoza et al., 2013).

The association of non-enzymatic antioxidants with enzymatic systems comprising SOD, CAT, APX, and GR will help in maintaining the redox homeostasis within the cells (Gill & Tuteja, 2010). SOD is a ubiquitous enzyme modulating the cellular quantities of $O_2^{\bullet-}$ and H_2O_2 and is considered as first line of defense with respect to abiotic stress (Jaleel et al., 2008; Hasanuzzaman et al., 2011). Upregulation of SOD was reported in canola (Tohidi-Moghaddam et al., 2009) and horse gram cultivars (Bhardwaj & Yadav, 2012). H_2O_2 is a by-product of SOD activity on the $O_2^{\bullet-}$ radical (Sheng et al., 2014). Apart from this, H_2O_2 is also produced by the photosynthetic electron transport chain and peroxisomes through photorespiration when RUBISCO accepts molecular oxygen. Although CAT and APX are spatially compartmentalized, they play a crucial part in maintaining the cellular H_2O_2 levels at balance during oxidative stress. APX requires an ascorbate and glutathione regeneration system, whereas CAT directly transforms H_2O_2 into H_2O and O_2 . Even though the pattern of elevation differed among the *Coleus* species, activities of SOD, CAT, and APX were increased significantly under drought stress when compared with control plants. Increase in CAT and APX activities in *Coleus* during drought stress were in line with the studies conducted in *Brassica napus* (Hosseini et al., 2015) and *Beta vulgaris* (Sayfzadeh & Rashidi, 2010). Reduced glutathione is utilized as reductant in ascorbate–glutathione cycle, and GR is chiefly concerned with its regeneration (Naderi et al., 2014). Both GR and glutathione are physiologically interlinked through ascorbate–glutathione cycle where GR is an enzymatic antioxidant catalyzing the NAD(P)H-dependent reaction thus efficiently maintaining the reduced glutathione pool. From this study, a significant positive correlation can be made with respect to ROS generation and a harmonized enhancement in the activity of antioxidant scavenging enzymes in *Coleus* species subjected to drought stress. Upregulation of antioxidant enzyme activity in *Coleus* suggests the existence of a strong antioxidant defense system in these two species with respect to drought stress. However, *C. amboinicus* has shown to possess a better antioxidative defense system than *C. forskholii* during drought stress.

Plants undergo multitude of adaptations to withstand the prevailing drought stress conditions by accumulating low molecular weight osmotic solutes (Obidiegwu et al., 2015). The osmolyte accumulation results in net increase in the cellular solute concentration, aiding hydration thereby maintaining the cell turgor. Proline is an efficient

osmoprotectant (Solanki & Sarangi, 2014) and is a radical scavenger, protecting the cellular machinery from reactive oxygen-mediated damage (Sairam, 1994). Drought-induced proline accumulation in both *Coleus* species is found to be in agreement with the previous investigations on *Helianthus* (Ünyayar et al., 2004) and *Abelmoschus* (Sankar et al., 2007). Accumulation of proline is understood as stress tolerance symptom, since it participates in stabilizing the membrane-associated macromolecules and function as an antioxidant in drought-effected cellular environment (Ashraf & Harris, 2013). Glycine betaine is accumulated in plants during abiotic stress and is known to protect the plants from the ongoing stress conditions. Plants that accumulated GB naturally are reported to perform well under drought stress (Chen & Murata, 2008). Both the *Coleus* species considered for this study have accumulated glycine betaine when subjected to drought stress. However, the accumulation was higher in the leaves of *C. amboinicus* than in *C. forskholii*. Accumulation of GB in transgenic apple expressing stress regulator gene *Osmyb4* enhanced the tolerance to drought. Exogenous application of GB has improved the growth and survival rate of plants under drought stress (Giri, 2011).

Phytohormone ABA is a plant hormone, which accumulates during drought stress and triggers a plethora of responses concerned with the protection of plants. ABA acts as an endogenous messenger in the regulation of plant water status. It is involved in several physiological processes, such as stomatal closure, synthesis of storage proteins and lipids, leaf senescence, and triggering of the stress-responsive genes through ABA-dependent pathway (Kuromori et al., 2018). This study on the ABA accumulation in *Coleus* leaves subjected to drought stress revealed that ABA levels were increased with decreasing LWPs. There was a threefold increase in the ABA levels of *C. amboinicus* leaves under drought stress.

CONCLUSIONS

This study had clearly demonstrated the effect of drought stress on the physiology of two *Coleus* species, with their decline in the LWPs and RWC leading to an increase in WUC and WSD. The intensity of the membrane damage was studied by measuring the MSI and it was found that a relative membrane injury occurred due to oxidative damage-induced lipid peroxidation. An enhancement in the radical scavenging abilities of the foliar extracts indicates an efficient antioxidative system in the foliar extracts, supported by the increased enzymatic and non-enzymatic antioxidant levels. Increase in the levels of osmolytes proline and glycine betaine with increasing drought intensity showed that both species have efficient drought tolerance mechanisms with a notable accumulation of phytohormone ABA. Based on the evident differences in responses adapted by two *Coleus* species with respect to various physiological as well as biochemical parameters, *C. amboinicus* possesses significantly better tolerance to drought stress when compared with *C. forskholii*.

Acknowledgments: The authors acknowledge University Grants Commission, Govt. of India for funding. IVSNP acknowledges GITAM University for the fellowship.

Funding Statement: This work is an output of research funded by the grants from the University Grants Commission, Govt. of India (no. 42-197/2013) to Dr. KVC.

Ethical Statement: The authors testify that this article submitted to *Biologia Futura* journal has not been published in whole or in part elsewhere and is not being considered for the publication in any other journal.

Data Accessibility: The authors confirm that the data supporting the findings of this study are available within the article.

Competing Interests: The authors declare no financial interest or financial conflict with the subject matter or the materials discussed in this manuscript and no conflict of interest.

Authors' Contributions: All authors have actively and equally contributed to the substantive work leading to the manuscript.

REFERENCES

- Alam, M. M., Hasanuzzaman, M., Nahar, K., Fujita, M. (2013) Exogenous salicylic acid ameliorates short-term drought stress in mustard (*Brassica juncea* L.) seedlings by up regulating the antioxidant defense and glyoxalase system. *Aust. J. Crop Sci.* 7, 1053–1063.
- Ashraf, M., Harris, P. J. C. (2013) Photosynthesis under stressful environments: an overview. *Photosynthetica* 51, 163–190.
- Barrs, H. D., Weatherly, P. E. (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* 24, 519–570.
- Bates, L., Waldren, R. P., Teari, D. (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39, 205–207.
- Behairy, R. T., Mohamed, E. I. D., Lyle, C. (2012) Impact of ascorbic acid on seed germination, seedling growth, and enzyme activity of salt-stressed fenugreek. *J. Med. Active Plants* 1, 106–113.
- Bettaieb, I., Hamrouni-Sellami, I., Bourgou, S. (2011) Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. *Acta Physiol. Plantarum* 33, 1103–1111.
- Beyer, W. F., Fridovich, I. (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Ann. Biochem.* 161, 559–566.
- Bhardwaj, J., Yadav, S. K. (2012) Comparative study on biochemical parameters and antioxidant enzymes in drought tolerant and sensitive variety of horse gram (*Macrotyloma uniflorum*) under drought stress. *Am. J. Plant Physiol.* 7, 17–29.
- Blois, M. S. (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 181, 1199–1200.
- Bodner, G., Nakhforoosh, A., Kaul, H. P. (2015) Management of crop water under drought: a review. *Agron. Sustain. Dev., Springer* 35, 401–442.
- Bradford, M. M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Chandlee, J. M., Scandalios, J. G. (1984) Analysis of variants affecting the catalase development program in maize scutellum. *Theor. Appl. Genet.* 69, 71–77.
- Chen, T. H., Murata, N. (2008) Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends Plant Sci.* 13, 499–505.
- Chowdhary, A. R., Sharma, M. L. (1998) GC-MS investigations on the essential oil from *Coleus forskohlii* Briq. *Indian Perfumer* 42, 15–16.
- David, M., Richard, J. S. (1983) Glutathione reductase. In: Bergmeyer, H. U. (ed.) *Methods of Enzymatic Analysis*. Academic Press, New York, pp. 258–265.
- Dehpour, A. A., Ebrahimzadeh, M. A., Nabavi, S. M., Nabavi, S. F. (2009) Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. *Grasas Y Aceities* 60, 405–412.
- Dhindsa, R. A., Plumb-Dhindsa, P., Thorpe, P. A. (1981) Leaf senescence: with increased permeability and lipid peroxidation and decreases levels of superoxide dismutase and catalase. *J. Exp. Bot.* 126, 93–101.
- Ebadzad, G., Medeira, C., Maia, I., Martins, J., Cravador, A. (2015) Induction of defense responses by cinnamons against *Phytophthora cinnamomi* in *Quercus suber* and *Quercus ilex* subs. *rotundifolia*. *Eur. J. Plant Pathol.* 143, 705–723.
- Espinoza, A., Martín, A. S., López-Climent, M. F., Ruiz-Lara, S., Gómez-Cadenas, A., Casaretto, J. A. (2013) Engineered drought-induced biosynthesis of α -tocopherol alleviates stress-induced leaf damage in tobacco. *J. Plant Physiol.* 170, 1285–1294.
- Farooq, M., Irfan, M., Aziz, T., Ahmad, I., Cheema, S. A. (2013) Seed priming with ascorbic acid improves drought resistance of wheat. *J. Agron. Crop Sci.* 199, 12–22.
- Gill, S. S., Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48, 909–930.
- Giri, J. (2011) Glycine betaine and abiotic stress tolerance in plants. *Plant Signal. Behav.* 6, 1746–1751.
- Grieve, C. M., Grattan, S. R. (1983) Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil* 70, 303–307.
- Guan, G. F., Wang, Y. S., Cheng, H., Jiang, Z. Y., Fei, J. (2015) Physiological and biochemical response to drought stress in the leaves of *Aegicerascorniculatum* and *Kandeliaobovata*. *Ecotoxicology* 24, 1668–1676.
- Guo, Y. Y., Yu, H. Y., Yang, M. M., Kong, D. S., Zhang, Y. J. (2018) Effect of drought stress on lipid peroxidation, osmotic adjustment and antioxidant enzyme activity of leaves and roots of *Lycium ruthenicum* Murr. seedling. *Russ. J. Plant Physiol.* 65, 244–250.
- Halliwell, B., Gutteridge, J. M. C., Arouma, O. I. (1987) The deoxyribose method: a simple test tube assay for the determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.* 165, 215–219.

- Hasanuzzaman, M., Hossain, M. A., Fujita, M. (2011) Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnol. Rep.* 5, 353–365.
- Hayatu, M., Muhammad, S.Y., Habibu, U. A. (2014) Effect of water stress on the leaf relative water content and yield of some cowpea (*Vigna unguiculata* (L) Walp.). *Genotype Int J. Sci. Technol. Res.* 3, 148–152.
- Hosseini, S. M., Hasanloo, T., Mohammadi, S. (2015) Physiological characteristics, antioxidant enzyme activities and gene expression in 2 spring canola (*Brassica napus* L.) cultivars under drought stress conditions. *Turk. J. Agric. For.* 39, 413–420.
- Jafarnia, S., Akbarinia, M., Hosseinpour, B., Modarres, S. A. M., Salami, S. A. (2018) Effect of drought stress on some growth, morphological, physiological, and biochemical parameters of two different populations of *Quercus brantii*. *iForest* 11, 212–220.
- Jaleel, C. A., Gopi, R., Sankar, B., Gomathinayagam, M., Panneerselvam, R. (2008) Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *C. R. Biol.* 331, 42–47.
- Juenger, T. E. (2013) Natural variation and genetic constraints on drought tolerance. *Curr. Opin. Plant Biol.* 16, 274–281.
- Kuromori, T., Seo, M., Shinozaki, K. (2018) ABA transport and plant water stress responses. *Trends Plant Sci.* 23, 513–522.
- Lanari, V., Silvestroni, O., Palliotti, A., Sabbatini, P. (2018) Plant and leaf responses to cycles of water stress and re-watering of ‘Sangiovese’ grapevine. *Folia Horticolt.* 30, 27–38.
- Li, C., Berninger, F., Koskela, J., Sonninen, E. (2000) Drought responses of *Eucalyptus microtheca* F. Muell. Provenances depend on seasonality of rainfall in their place of origin. *Aust. J. Plant Physiol.* 27, 231–238.
- Lim, Y. Y., Lim, T. T., Tee, J. J. (2007) Antioxidant properties of several tropical fruits: a comparative study. *Food Chem.* 103, 1003–1008.
- Liu, F., Ooi, V. E., Chang, S. T. (1997) Free radical scavenging activity of mushroom polysaccharides extract. *Life Sci.* 60, 763–771.
- Michaletti, A., Naghavi, M. R., Toorchi, M., Zolla, L., Rinalducci, S. (2018) Metabolomics and proteomics reveal drought-stress responses of leaf tissues from spring-wheat. *Nat. Sci. Rep.* 8, 5710–5718.
- Miller, N. J., Rice-Evans, C., Davies, M. J. (1993) A new method for measuring antioxidant activity. *Biochem. Soc. Trans.* 21, 95S.
- Mishra, V., Cherkauer, K. A. (2010) Retrospective droughts in the crop growing season: implications to corn and soybean yield in the Midwestern United States. *Agric. For. Meteorol.* 150, 1030–1045.
- Moron, M. S., De Pierre, J. W., Vik, B. M. (1979) Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat and lung liver. *Biochim. Biophys. Acta* 582, 3170–3185.
- Naderi, R., Valizadeh, M., Toorchi, M., Shakiba, M. R. (2014) Antioxidant enzyme changes in response to osmotic stress in wheat (*Triticum aestivum* L.) seedling. *Acta Biol. Szegediensis* 58, 95–101.
- Nair, A. S., Abraham, T. K., Jaya, D. S. (2008) Studies on the changes in lipid peroxidation and antioxidant in drought stress induced cowpea (*Vigna unguiculata* L.) varieties. *J. Environ. Biol.* 29, 689–691.
- Nakano, Y., Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22, 867–880.
- Obidiegwu, J. E., Bryan, G. J., Jones, H. G., Prashar, A. (2015) Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Front. Plant Sci.* 6, 542.
- Premchandra, G. S., Sanoeka, H., Ogata, S. (1990) Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. *J. Agric. Sci. (Camb.)* 115, 6–66.
- Rahimi, A., Madah Hosseini, S., Pooryousef, M., Fateh, I. (2010) Variation of leaf water potential, relative water content and SPAD under gradual drought stress and stress recovery in two medicinal species of *Plantago ovata* and *P. psyllium*. *J. Plant Ecophysiol.* 2, 53–60.
- Ramana, G. V., Chaitanya, K. V. (2015) Variations in $\delta^{13}\text{C}$ rates and Crassulacean acid metabolism of six *Coleus* species. *Br. J. Appl. Sci. Technol.* 6, 295–303.
- Roe, J. H., Keuther, C. A. (1943) The determination of ascorbic acid in whole blood and urine through 2,4-dinitrophenyl hydrazine derivative of dehydroascorbic acid. *J. Biol. Chem.* 147, 399–407.
- Rosenberg, H. R. (1942) *Chemistry and Physiology of the Vitamin*. Inter Science Publishers Incorporated, New York.
- Saha, B., Borovskii, G., Panda, S. K. (2016) Alternative oxidase and plant stress tolerance. *Plant Signal. Behav.* 11, e1256530.
- Sairam, R. K. (1994) Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian J. Exp. Biol.* 32, 594–597.
- Sangakkara, U. R., Hartwig, U. A., Nosberger, J. (1996) Responses of root branching and shoot water potentials of french bean (*Phaseolus vulgaris* L.) of soil moisture and fertilizer potassium. *J. Agron. Crop Sci.* 177, 165–173.
- Sankar, B., Jaleel, C. A., Manivannan, P., Kishore Kumar, A., Soma Sundaram, R., Panneer Selvam, R. (2007) Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench. *Acta Bot. Croat.* 66, 43–56.
- Sayfzadeh, S., Rashidi, M. (2010) Effect of drought stress on antioxidant enzyme activities and root yield of sugar beet (*Beta vulgaris*). *Am.-Eurasian J. Agric. Environ. Sci.* 9, 223–230.
- Sheng, Y., Abreu, I. A., Cabelli, D. E., Maroney, M. J., Miller, A. F., Teixeira, M., Valentine, J. S. (2014) Superoxide dismutases and superoxide reductases. *Chem. Rev.* 114, 3854–3918.
- Slatyer, R. O. (1961) Effect of several osmotic substrates on the water relationships of tomato. *Aust. J. Biol. Sci.* 14, 519–540.
- Solanki, J. K., Sarangi, S. K. (2014) Effect of drought stress on proline accumulation in peanut genotypes. *Int. J. Adv. Res.* 2, 301–309.
- Štajner, D., Orlović, S., Popović, B. M., Kebert, M., Galić, Z. (2011) Screening of drought oxidative stress tolerance in Serbian melliferous plant species. *Afr. J. Biotechnol.* 10, 1609–1614.
- Taylor, J. R. (1982) *An Introduction to Error Analysis*. University Science Books, Mill Valley, CA.
- Tohidi-Moghaddam, H. R., Shirani-Rad, A. R., Noor-mohammadi, G., Habibi, D., Boojar, M. M. A. (2009) Effect of super absorbent application on antioxidant enzyme activities in canola (*Brassica napus* L.) cultivars under water stress conditions. *Am. J. Agric. Biol. Sci.* 4, 215–223.
- Ünyayar, S., Yüksel, K., Ünal, E. (2004) Proline and ABA levels in two sunflower genotypes subjected to water stress. *Bulgarian J. Plant Physiol.* 30, 34–47.